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Keynote Lecture

World Buffalo Production: Challenges in Meat and Milk Production, and Mitigation of Methane Emission

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ABSTRACT
Buffalo (Bubalus bubalis), the long-time ruminant animal contributing to the integrated farming systems, as a source of draft power, transportation, on-farm manure, meat, milk and livelihood of the farmers. The increase in meat consumption is quite dramatic, due to the linearly increase in the world population especially in the developing countries. As buffaloes have been raised by the rural farmers, well-adapted to harsh environment and are capable of utilizing low quality roughages especially the agricultural crop-residues and by-products, hence their potential are therefore remarkable in terms of meat and milk production using locally available feed resources. Furthermore, the quality of buffalo meat has been found high in iron and conjugated linoleic acid (CLA), which are essential for good health. Global warming has been attributed by various sources including animal agriculture. Rumen fermentation can be manipulated by many ways in order to increase fermentation efficiency and to mitigate methane production. Although the world buffalo population has been slightly increasing, their vital role are accountable for the demand of meat and milk. Manipulation of the rumen in reducing methane using chemicals, feed additives, roughage and concentrate utilization, use of plants containing secondary compound, oils have been reported. However, among many approaches, nutritional manipulation by using feeding management and especially the use of plant extracts or plant containing secondary compounds (condensed tannins and saponins) have been receiving more attention and most promising. At the current stage, more research concerning this hot issue with the role of livestock on global warming warrants further research undertakings. It is therefore highly recommended for those engage in buffalo production to do more research and to expand the world buffalo production in order to meet the increasing demand of meat and milk. In addition, infrastructure and human resources development require indicate and continuous implementation as well as higher input contribution.

Keywords: buffalo production, global warming, methane production, nutritional manipulation, rumen fermentation
INTRODUCTION

Currently, livestock systems have both positive and negative effects on the natural resource base, public health, social equity and economic growth (World Bank, 2009). Animal agriculture has been an important component in the integrated farming systems in the crop-livestock farming systems in developing countries. It serves in a paramount diversified role in producing animal protein food, draft power, farm manure as well as ensuring social status-quo and enriching livelihood. As the world population is expected to increase from 6 billion to about 8.3 billion in the year 2030 with the average growth of 1.1 per annum, it is essential and vital to be prepared to produce sufficient protein animal food for the increased population especially in the developing countries. It has been reported that consumption of animal food was 10 kg per annum in 1960s and increased to 26 kg per annum in the year 2000 and is expected to be 37 kg per annum in the year 2030, respectively (FAO, 2008; 2009).

The world buffalo population is estimated to be approximately 177.25 million, spreading in some 42 countries of which 171 million (97%) of them are found in Asia, while approximately 5.38 million (3%) are found in the rest of the world (FAO, 2008). The major source of the buffalo meat, especially, in Asia where meat from ruminants constitute only about 21.0% of the total meat production, buffalo meat is about 11.5% of the total ruminant meat, and about 2.7% of all meat produced in the region (Cruz, 2010). In the past century, the buffalo meat in the world was not accepted by the consumers and there was no market. However, the actual trends in consumption require meat with low fat content, therefore, nontraditional meat become an important source. Among them, buffalo meat has high protein level, low fat and cholesterol content compared to beef (Murthy & Devadason, 2003). Hence, researchers are trying to improve buffalo of potential breeds and meat quality for human consumption. According to Heintz (2001) it was found that when compared to meat from young buffalo and young cattle, has clearly shown that buffalo meat is indeed as good as cattle meat. Moreover, buffalo meat is required with the population increased, requirements of food, in developing countries, particularly, trend of the market in the world, the price of buffalo milk is twice that of cows’ milk, buffalo skin is used in the leather industry and buffalo feces is used for manures and fuel in rural areas.

Global warming is a hot issue which affects environment and livestock production. Total emissions of greenhouse gases (GHGs) from agriculture, including livestock, are estimated to be between 25 – 32%, depending on the source (USEPA, 2006; IPCC, 2007) and on the proportion of land conversion that is ascribed to livestock activities. Moreover, Goodland and Anhang (2009) reported that livestock production and its by-products are responsible for at least 51 percent of global warming gases or account for at least 32.6 billion tons of carbon dioxide per year. While, CO₂ is the largest green house gases at 55-60% and methane are the second green house gases at 15-20%. Therefore, livestock is the one sector of methane producer from the rumen. It has been estimated that global anthropogenic greenhouse gas (GHG) emissions from the livestock sector approximate to between 4.1 and 7.1 billion tonnes of CO₂ equivalents per year, equating to 15-24% of total global anthropogenic GHG emissions (Steinfeld et al., 2006). Currently, researchers
try to reduce methane production in the rumen by using many feed additives to inhibit methanogenesis. Meanwhile, plants produce a diverse array of plant secondary metabolites to protect against microbial and insects attacks (Wallace, 2004). These natural plant ecochemicals such as essential oils (EO), saponins, tannins and organosulphur compounds have been shown to selectively modulate the rumen microbial populations (Wallace, 2004; Patra & Saxena, 2009a), resulting in an improvement of rumen fermentation and nitrogen metabolism, and a decrease in methane production and nutritional stress such as bloat or acidosis, thus improving the productivity and health of animals (Wallace et al., 2002; Kamra et al., 2006; Rochfort et al., 2008). Recently, a number of studies have discussed the potential of plant bioactives as modifiers of rumen microbial fermentation and ruminant production (Wallace et al., 2002; Wallace, 2004; Hart et al., 2008; Calsamiglia et al., 2007; Patra & Saxena, 2009b).

BUFFALO POPULATION AND VITAL ROLE IN FARMING SYSTEM

The world population of buffalo (*Bubalus bubalis*) has been estimated at over 140 million head (FAO, 1991; Borgese, 2005). Therefore, these are more than 97% found in Asia and the Pacific region, mainly in India (75 million), China (21 million), Pakistan (14 million) and Thailand (6 million and decrease to about 2 millions in 2010). Moreover, the world buffalo population were increased and estimated to be approximately 177.24 million, spreading in some 42 countries of which 171 million (97%) of them are found in Asia, while approximately 5.38 million (3%) are found in rest of the world (FAO, 2008). In fact, buffalo population were be distributions in major buffalo producing countries in Asia, therefore, in the Asian buffaloes dominate the world population, representing 96.4% of the worldwide population of 180.70 million as of 2008 (Table 1; FAO, 2010). Within the Asian region, about 74.8% of buffaloes are in the South, 12.8% in East Asia, and only 8.4% are found in South-East Asia. Moreover, buffalo productions are found some of minimum, about 0.626% in America, 0.22% in Europe, 2.895% in Africa (Cruz, 2010). In fact, buffalo population will be popular in the world market with the good quality of meat and still play important role in some certain regions, especially in developing countries. These are slightly increases of buffalo population in many regions, except those in South East Asia with slight decrease (Table 1).

Buffaloes produce meat, milk, saving bank, draft power, transportation, and other purpose for human and on-farm manure to crop farming. Therefore, feed utilization of buffaloes is more effective than cattle when cattle and buffaloes were kept under similar conditions, particularly well-adapted to harsh environment and are capable of utilizing low quality roughages especially the agricultural crop-residues and by-products, hence their potential are therefore remarkable in terms of meat and milk production using locally available feed resources. However, decrease in the number of buffaloes has been occurring in some countries in the world due to several factors: holsteinization, mechanization, and the poor market demand for buffalo products (Borghese, 2010) and others like high rate of formal slaughtering and insufficiency input for research and development by government agencies (Wanapat, 1999). However, some countries, buffalo numbers have increased due to the demand for particular products obtained from buffalo milk and meat to both on
the national and international market.

**BUFFALO PRODUCTION SYSTEMS**

Feeding of ruminants in the tropical area, could be separated depending on seasons, particularly, dry and rainy season. However, these ruminants often encounter low productivity because of deficiencies in feed supply, in both quality and quantity (Wanapat & Devendra, 1992). The use of rice straw as a feed in the dry season, in spite of its low nutritive value, has been a common feeding system, generally practiced by smallholder farmers when green forages are often scarce (Wanapat, 1999). Available local feed resources have been recommended for uses under smallholder farming (Wanapat, 2009). However, buffalo in the tropical area for feeding systems are based on unrestricted grazing, tethering or stall-feeding and free grazing, sometimes under the control of herders, is common in countries with native grasslands and fallows. Tethering and stall-feeding are practiced in areas where there is limited land and with cropping. In many situations, there appeared to be roughage limitations for animals in the stall-feeding and tethering systems (Wanapat & Chanthakhoun, 2009). Currently, Singh & Barwal (2010) reported that in India, due to the better animal husbandry practices significant improvement has taken place in the buffalo production system in rural areas. The awareness in the farmers about the feeding, breeding and health management of live-stock considerably increased. The buffalo production system in rural areas may be classified as: 1). Extensive: small farm, with a maximum of 2 buffaloes, kept on natural grasses, in communal paddocks during the rainy season. Agricultural by-products are used for feeding, marginal land, family labour and minimum investment, with simple, traditional technology; 2). Semi Intensive: animals are kept in irrigated areas, with cultivated fodders, crop by-products and concentrates. Buffaloes are confined in adequate buildings; and 3). Intensive: Herd strength ranges from 5 to 100 buffaloes, kept for milk production as in Haryana, Punjab, Uttar Pradesh, Rajasthan, Gujarat and close to the large populated areas in India. The herds are fed on cultivated fodders and concentrate.

In many of these systems, the livestock element is interwoven with crop production, as in the rice/buffalo or cereal/cattle systems of Asia (Devendra, 2007). Animal manure is often essential for maintaining soil fertility, and the role of animals in nutrient cycling is often an important motivation for keeping animals, particularly where this involves a transfer of nutrients from common property resources to private land. In other cases, such as the seminomadic pastoral systems of the world’s natural grassland regions, environmentally stable balances of human society, animal population and vegetative biomass have been maintained for centuries. On the contrary, livestock production currently supports and sustains the livelihoods of an estimated 675 million rural poor (Steinfeld et al., 2006). These people fully or partially depend on livestock for income and/or subsistence. Livestock can provide a steady stream of food and revenues and help to raise whole farm productivity; livestock are often the only livelihood option available to the landless because they allow the exploitation of common-property resources for private gain (Chantalakhana & Skumun, 2002; Wanapat, 2009; Nardone et al., 2010). Buffalo production systems are variable between developed and developing regions, depending on local conditions and practices.
regions, by far the largest variation in intensity of production is found within the system, which is the largest producer of buffalo products countries, though the developing regions are dependable on the system’s production worldwide.

Buffalo meat production

Buffalo meat production, it was reported that about 88% of world buffalo meat is in Asia and over 21 million buffaloes slaughtered annually in Asia, 48% are slaughtered in India and about 18% each in Pakistan and China. Pakistan annually produces 0.68m tones of buffalo meat valued at 102 billion ($ 1.3 billion). Therefore, in developing countries of Asia where meat from ruminants constitute only about 21.0% of the total meat production, buffalo meat is about 11.52% of the total ruminant meat, and about 2.7% of all meat produced in the region. The average annual growth rate in production was about 1.3%. Undoubtedly, the majority of world's buffalo meat is in Asia, representing 91.89% and with volume of 3.08M tons in 2008 (FAO, 2010). Neath et al. (2007) reported that feeding trials comparing buffalo and cattle of similar age, feeding regime and carcass treatment, clearly demonstrated that there is ample scientific evidence to show that buffalo meat are more tender than beef. Anjaneyulu et al. (2007) reported that buffalo meat has been recognized as one of the healthiest meats for human consumption. It has outstanding attributes such as: lower intramuscular fat, lower cholesterol and calories, higher units of essential amino acids, higher biological value, and higher mineral content. The quality of buffalo meat has been markedly improved with crossbreds and in Australia it is hoped to be the future standard in the “Tender Buff Program”, which has gained much popularity. Compared to beef, buffalo meat contains one % less intramuscular fat, 92% less saturated fat, 25% less calories, 67% less cholesterol, 11 to 30% more protein, 10% more minerals, low cholesterol in buff meat has been re-emphasized by Lazar (2001) (Table 2).

Buffalo milk production

Recently, buffalo milk production in Asia represents 96.78% of the total volumes of world's buffalo milk of 89.2 Million tons. Production in South and Southwest Asia, primarily from India and Pakistan contributed a hefty 93.17% (FAO, 2010). Buffaloes are significant sources of milk in this sub-region contributing as high as 68.35% of the total milk yield in Pakistan, and 56.85% in total milk production in India. Average annual growth rate in buffalo milk production between the years 1998 to 2008 was 4.39% in the whole of Asia. Evidently, this growth level can readily be associated with the consistent good growth in milk production in India and Pakistan, with combined growth rate of 4.52%. Buffalo milk production in China has not been as robust as in the South Asia, with average annual growth of 1.6%. What is notable is the 8.18% average annual growth in SEA, though in relative volume, such increases have not been very significant. Among the SEA countries, Myanmar registered the highest buffalo milk production, apparently because it has the highest population of dairy buffalo of about 40,000 hd as of 2000 (Hlaing, 2001). Buffalo milk production from the other SEA countries are coming mainly from smaller population of introduced riverine buffaloes and the resulting crossbreds of the dairy breed with the existing swamp
buffalo population. In this sub region where farmers tend their animals primarily for work, only a small %age of crossbreds produced are fully utilized for milk production. Comparative milk composition in buffalo and cow as present in Table 5 and was shown that buffalo milk is healthy as it is richer in saturated fatty acids. The Indian diet is mainly vegetarian and people relish the hot thick creamy milk for their breakfast associated with higher fat content (Table 3). Swamp buffalo milk has even higher fat (7.9%), protein (4.2%), Calcium (264.0 mg/100 g) and Cholesterol (0.65 mg/g).

**Potential buffalo production on various markets**

Currently, buffalo markets in the world, especially, in Asia have been increasingly emerging to produces good quality meat and milk and with high acceptability by consumers. As shown by the increase in buffalo meat exports of about 159,703 MT in 1995-96 to as much as 456,907 MT in 2008-2009. Export of Buffalo meat from India rose 2 folds in volume from 234,355M tons in 2001 to 456,907M tons in 2009, and by more than 400% in value from US$243.4M to US$1.043B during the same period. Interestingly, Vietnam, Malaysia and the Philippines get about 43.13% of the total Indian buffalo export 2008-2009, the rest went to 10 other countries in the Middle East (Borghese, 2010). In the Philippines, practically all brands of processed corned beef are derived from imported Indian buffalo meat constituting more than 60.0% of the total buffalo supply in the Philippines. This has allowed significant growth in the local meat processing industry in the country (Borghese, 2010). Intensive feeding of male buffaloes in commercial feedlot for quality meat production started in 1999. Male calves at the age of 8-10 months are purchased from farmers and are fed high protein/high energy diet to put on additional weight of 120 kg in 4 months. Murrah yearling grow by 0.9 to 1.0 kg/day and would have high dressing %age (Ranjhan, 2004). Buffalo meat and mike consumer demand and of market possibilities, the modern applied technologies on milk and meat processing and industry, could be a serious basis to increase the availability of quality products for a positive trend of buffalo development and of higher profits for farmers and linked companies (Borghese, 2010). Meat export is desired to effectively utilize the available livestock resources and improve returns to the farmers by popularizing buffalo meat (Kadeephan et al., 2009). Buffalo meat is the major item of Indian animal product export comprising 48.76% of the total animal products exported. The major destinations of buffalo meat include Malaysia, USA, Jordan, Oman and UAE. Buffalo meat is exported to the tune of 306,970.81 MT amounting 1,615.59 cores which is the highest among all animal products exported from India.

There are 22.72 million buffalos in China in 2007, representing 17.37% of all cattle in the whole country (BingZhuang et al., 2010). According to statistical data (FAO, 2008), the output of buffalo meat in China was 0.306 million tons. Most of the meats were directly sold to consumers, only few meats were processed to byproducts such as dried beef, sausages and hams. The output of buffalo hide in 2007 is 92 thousand tons (FAO, 2008), the hides were made into various products, which were sold well on the market. The crossbreeding of buffalos has been performed for many decades, while the number of crossbred buffalos for milking is
not large. According to statistics of Cao et al., there were 30000 milking buffalos in China in 2006, 61.5% of them were crossbreds, while 38.5% of them were local buffalos. The buffalo milk production was 0.29 million tons in China in 2008. The buffalo dairy industry in China is a newly emerging industry, with great potential and promising prospect. Chinese government has attached great importance and increased input to the exploitation of buffalo industry during recent years, the government has also programmed the medium and long term development of buffalo industry. In the coming 10 years, the population of buffalo for milking will achieve 0.5 million, it brings opportunities to development of buffalo dairy industry.

According to Borgese (2005), in the Philippines there were 3.2 million Carabao buffaloes, 99 percent belong to small farmers that have limited resources, low income and little access to other economic opportunities. The Carabao Development Programme is a massive programme started in 1993 to improve the native Swamp buffalo locally known as the Carabao to develop their meat, milk and draught potential. An elite herd of Riverine buffalo has now been established at the Philippine Carabao Center, Science City of Muñoz, by importing about 3,000 Murrah buffaloes with pedigree performance records from Bulgaria. Each female crossbred when raised for milk can produce about 1,350 kg of milk per lactation (Cruz, 2003). In the past Thailand had the second largest number of Swamp buffalo in the world. However this buffalo population drastically declined from 4.7 million in 1990 to 1.9 million in 1998. The number of buffaloes has decreased yearly and the present number is about 2.0 million and is tending to decrease gradually.

In Italy particularly the increasing demand for buffalo mozzarella cheese both on the national and international markets, the Denomination of Controlled Origin (DOP) as "Mozzarella di Bufala Campana" for this cheese registered in Italy and in Brussels for the European Union (EU), and the milk quotas on surplus bovine milk imposed by the EU, led to an increase in the buffalo population of about 142 percent from 1993 to 2001 (compared with a 7.8 percent increase in the world population in the same period) and to an increase of 1600 percent (16 times) from 1957 to 2002 (Borgese, 2005). In Italy this increase in the number of buffaloes is not only remarkable for this percentage increase but also when compared with the trends in other species, which have all decreased over the last 50 years particularly for cattle, dairy cows and horses. In South America, although the herd size of buffaloes have been small, but have been increasing interest and development of buffalo population in various countries including Brazil, Venezuela, Argentina and others to increase the herds and production efficiency in meat and milk, especially for cheese production (Borghese, 2005).

GREENHOUSE GAS AND METHANE EMISSION BY LIVESTOCK

Livestock are already well-known to contribute to GHG emissions. Livestock’s Long Shadow, the widely-cited report by the United Nations Food and Agriculture Organization (FAO), estimates that 7,516 million metric tons per year of CO₂ equivalents (CO₂), or 18 percent of annual worldwide GHG emissions, are attributable to cattle, buffalo, sheep, goats, camels, horses, pigs, and poultry. Livestock contribute about 9% of total carbon dioxide (CO₂) emissions, but 37% of methane (CH₄), and 65% of nitrous oxide (N₂O) (Steinfeld et al., 2006). That
amount would easily qualify livestock for a hard look indeed in the search for ways to address climate change. But new analysis shows that livestock and their byproducts actually account for at least 32,564 million tons of CO₂ per year, or 51 percent of annual (Goodland & Anhang, 2009). This report has brought an alarming call and has created high interest and lots of debate on the statistics and how it has been estimated. Figure 1, depicts the estimation of methane gas emission from various sources especially from the livestock enteric fermentation, which accounts for 28% of the total methane emission.

Animal production plays four important roles in the release of gases into the atmosphere, as reported by Leng (2011), as follows;

- directly through production of methane in fermentative digestion of ruminants
- indirectly when a proportion of the fecal materials decompose anaerobically
- indirectly through CO₂ production from fossil fuels to provide the production and marketing infrastructure and inputs such as motorized transport, fertilizers, herbicides and insecticides
- through the clearing of forests and range lands, the timber on which was a natural sink for carbon dioxide.

**METHANOGENS**

Rumen ecology is an unique environment where anaerobic fermentation process occurs by the reaction of the rumen microorganisms namely bacteria, protozoa and fungi. The main fermentation end-products are those of volatile fatty acids, ammonia nitrogen (used for microbial protein synthesis), and methane production. All feeds especially, roughages will be degraded by the microorganisms by cellulolytic bacteria via the Embden Myerhof Parnas pathway from glucose to pyruvate and further to the synthesis of the short chain volatile fatty acids (VFAs); acetate (C2), propionate (C3), butyrate (C4), valerate (C5), caproate (C6). These VFAs are used as major sources of energy for the ruminants. In addition, hydrogen produced in the rumen will be trapped by methanogens to produce methane gas and later be eructated by the ruminants into atmosphere. Fermentation efficiency can be manipulated by many possible ways including the nutritional feeding strategies (Figure 2; Wanapat, 2012).

As reported by Boadi et al. (2004) that methanogens represent a unique group of microorganisms. They possess three coenzymes which have not been found in other microorganisms. The three coenzymes are: coenzyme 420, involved in electron transfer in place of ferredoxin, coenzyme M, involved in methyl transfer, and factor B, a low molecular weight, oxygen-sensitive, heat-stable coenzyme involved in the enzymatic formation of CH₄ from methyl coenzyme M. Five species of methanogens were reported to have been isolated in the rumen. These include *Methanobrevibacter ruminantium*, *Methanosarcina barkeri*, *Methanosarcina mazei*, *Methanobacterium formicicum* and *Methanomicrobium mobile*. Only *Methanobrevibacter ruminantium* and *Methanosarcina barkeri* have been found in the rumen at populations greater than 106 mL⁻¹, and are assumed to play a major role in ruminal methanogenesis. Methanogens are hydrophobic and therefore stick to feed particles as well as onto the surface of protozoa. The number of
methanogens associated with protozoa reached a maximum (10 to 100 times pre-feeding levels) after feeding, when the rate of fermentation is the highest. It was shown that the symbiotic relationship of methanogens and protozoa might generate 37% of rumen CH₄ emissions.

**Dietary manipulation in reducing rumen methane**

There are several factors which can have great impacts on rumen methane production namely level of intake, frequency of feeding, type of roughages, ratio of roughage to concentrate, type and concentration of non-structural carbohydrates etc. All of these factors can play important roles on rumen pH, volatile fatty acids production, ammonia nitrogen and microbial protein synthesis and the consequences on rumen methanogens and methane production, protozoa and cellulolytic bacteria. Boadi et al (2004) and Hook et al. (2010) have proposed numerous potential ways as how to mitigate the rumen methane production. The main approaches are as follows: improving animal productivity, nutritional and management strategies (type of carbohydrates, level of intake, forages type and quality, feeding frequency, roughage treatment/processing, grazing management,), management of rumen fermentation (propionate enhancers, use of fats and essential oils, use of plant secondary compounds: condensed tannins and saponins etc (Table 6).

**Plant secondary compounds**

Plant secondary compounds (tannins and saponins) are more important as ruminant feed additives, particularly on CH₄ mitigation strategy because of their natural origin in opposition to chemicals additives. Tannins containing plants, the antimethanogenic activity has been attributed mainly to condensed tannins. There are two modes of action of tannins on methanogenesis: a direct effect on ruminal methanogens and an indirect effect on hydrogen production due to lower feed degradation. Also, there is evidence that some condensed tannins (CT) can reduce CH₄ emissions as well as reducing bloat and increasing amino acid absorption in small intestine. Methane emissions are also commonly lower with higher proportions of forage legumes in the diet, partly due to lower fibre contact, faster rate of passage and in some case the presence of condensed tannins (Beauchemin et al., 2008). Supplementation of PCH at 600 g/ha/d was beneficial in swamp buffaloes fed rice straw as a basal roughage, as it resulted in increased DM intake, reduced protozoal and methane gas production in the rumen, increased N retention as well as efficiency of rumen microbial CP synthesis (Chanthakhoun et al., 2011). Legumes containing condensed tannin (e.g., Lotuses) are able to lower methane (g kg⁻¹ DM intake) by 12-15% (Beauchemin et al., 2008; Rowlinson et al., 2008). Also, some authors reported that condensed tannins to reduce CH₄ production by 13 to 16% (DMI basis) (Grainger et al., 2009; Woodward et al., 2004), mainly through a direct toxic effect on methanogens. More recently Woodward et al. (2004) carried out a similar trial with cows fed Lotus corniculatus, on methane was 24.2, 24.7, 19.9 and 22.9 g kg⁻¹ DMI for the respective treatments. The CT in lotus reduced methane kg⁻¹ DMI by 13% and the cows fed lotus produced 32% less methane kg⁻¹ milk solids (fat+protein) compared to those fed good quality ryegrass. It was reported that extracts from plants such as rhubarb and garlic could decrease CH₄ emissions.
However, there is only limited information on the effect of different saponins on rumen bacteria. Saponins are natural detergents found in many plants. There have been increased interests in saponin-containing plants as possible means of suppressing or eliminating protozoa in the rumen. A decrease in protozoa numbers has been reported in the rumen of sheep infused with saponins or fed on saponin-containing plants. Decreased numbers of ruminal ciliate protozoa may enhance the flow of microbial protein from the rumen, increase efficiency of feed utilization and decrease methanogenesis. Saponins are also known to influence both ruminal bacterial species composition and number through specific inhibition, or selective enhancement, of growth of individual species. Saponins have been shown to possess strong defaunating properties both in vitro and in vivo which could reduce CH$_4$ emissions (Rowlinson et al., 2008). Beauchemin et al. (2008) recently reviewed literature related to their effect on CH$_4$ and concluded that there is evidence for a reduction in CH$_4$ from at least some sources of saponins, but that not all are effective (Rowlinson et al., 2008). While extracts of CT and saponins may be commercially available, their cost is currently prohibitive for routine use in ruminant production systems. However, still required on the optimum sources, level of CT astringency (chemical composition), plus the feeding methods and dose rates required to reduce CH$_4$ and stimulate production. Moreover, there have been reports of decreased methane emission by ruminants consuming plant secondary compounds (Carulla et al., 2005; Puchala et al., 2005). Supplementation of pellets containing condensed tannins and saponins (MP and soapberry fruit) influenced rumen ecology by significantly lowering methane concentration in rumen atmosphere and reduced methanogen population (Pourghompu et al., 2009). However, high CT concentrations (>55 g CT/kg DM) may reduce voluntary feed intake and digestibility (Beauchemin et al., 2008; Grainger et al., 2009). Waghorn et al. (2002) reported a 16% depression in CH$_4$ emissions kg$^{-1}$ DMI (13.8 to 11.5 g kg$^{-1}$ DMI) due to the presence of CT in a diet of Lotus pedunculatus fed to sheep housed indoors.

**Processing and preservation of feeds**

Forage processing and preservation affect enteric CH$_4$ production but limited information with regard to these effects is available in the literature. Methanogenesis tends to be lower when forages are ensiled than when they are dried and when they are finely ground or pelleted than when coarsely chopped (Martin et al., 2010). Grinding or pelleting of forages to improve the utilization by ruminants has been shown to decrease CH$_4$ losses per unit of feed intake by 20-40% when fed at high intakes.

**Roughage and concentrate**

The forage to concentrate ratio of the ration has an impact on the rumen fermentation and hence the acetate: propionate ratio (declines with F: C ratio). The CH$_4$ reduction is well in line with the observations of Bannink et al. (1997) that concentrate rich diets showed lower and higher coefficients of conversion of substrate into acetate and propionate, respectively. However, many experimental databases suggest that a higher proportion of concentrate in the diet leads to a
reduction in CH$_4$ emissions as a proportion of energy intake (Blaxter & Clapperton, 1965; Yan et al., 2000) due mainly to an increased proportion of propionate in ruminal VFA. The scope for reductions in CH$_4$ emissions depends on the starting level of concentrates, as there are dietary limitations and there are large differences in current usage of concentrates in different regions of the world (Rowlinson et al., 2008). The poor tolerance to low pH by protozoa and cellulolytic bacteria decreases further hydrogen production. A positive correlation between cellulolytic and methanogens in the rumen of different animal species (cattle, sheep, deer) has been shown (Rowlinson et al., 2008), except in the buffalo. This exception was explained by the fact that F. succinogenes, a non-hydrogen-producing cellulolytic species, was the major cellulolytic bacteria of this animal. On the contrary to other researchers, Sejian et al. (2011) reported that higher proportion of forage to concentrate resulted in decreasing ruminal methane production. They are stated that lower CH$_4$ production from high forage: grain diet can be attributed to the effect of the high content of fat in the diet which could potentially reduce fiber degradation and amount of feed that is fermentable as well as forage grinding effects. Yurtseven & Ozturk (2009) observed that amount of ruminal methane produced from corn was lower than that of barley grain in ruminant. This may be due to higher starch content and slow starch degradability of corn vs. barley grain. With regard to the ingredient composition of concentrates, selecting carefully defined carbohydrate fractions, such as more starch of a higher rumen resistance and less soluble sugars could significantly contribute to a reduction in CH$_4$ emission (Tamminga et al., 2007). Sejian et al. (2011) reported that Total mixed ration (TMR) feeding leads to decrease methane production vs. separate forage-concentrate feeding.

**Plant oils**

There are five possible mechanisms by which lipid supplementation reduces CH$_4$: reducing fibre digestion (mainly in long chain fatty acids); lowering DMI (if total dietary fat exceeds 6-7%); suppression of methanogens (mainly in medium chain fatty acids); suppression of rumen protozoa and to a limited extent through biohydrogenation (McGinn et al., 2004; Beauchemin et al., 2008; Johnson and Johnson, 1995). Oils offer a practical approach to reducing methane in situations where animals can be given daily feed supplements, but excess oil is detrimental to fibre digestion and productions. Oils may act as hydrogen sinks but medium chain length oils appear to act directly on methanogens and reduce numbers of ciliate protozoa (Machmuller et al., 2000). However, Kongmun et al. (2010) reported that supplementation of coconut with garlic powder could improve *in vitro* ruminal fluid fermentation in terms of volatile fatty acid profile, reduced methane losses and reduced protozoal population. In contrast, Johnson et al. (2002) and (2008) found no response to diets containing 2.3, 4.0 and 5.6% fat (cottonseed and canola) fed over an entire lactation. Beauchemin et al. (2008) recently reviewed the effect of level of dietary lipid on CH$_4$ emissions over 17 studies and reported that with beef cattle, dairy cows and lambs, there was a proportional reduction of 0.056 in CH$_4$ (g kg$^{-1}$ DM intake) for each 10 g kg$^{-1}$ DM addition of supplemental fat. While this is encouraging, many factors need to be considered such as the type of oil, the form of the oil (whole crushed oilseeds vs. pure oils), handling issues (e.g., coconut oil has a
melting point of 25°C) and the cost of oils which has increased dramatically in recent years due to increased demand for food and industrial use. In addition, there are few reports of the effect of oil supplementation on CH₄ emissions of dairy cows, where the impact on milk fatty acid composition and overall milk fat content would need to be carefully studied. Strategies based on processed linseed turned out to be very promising in both respects recently. Most importantly, a comprehensive whole system analysis needs to be carried out to assess the overall impact on global GHG emissions (Rowlinson et al., 2008).

BUFFALO RESEARCH DEVELOPMENT AND EDUCATION

Despite the important and vital role of buffalos in the world, but there have been relatively limited and at low input contributed by the various governments and agencies across the countries regarding inputs and emphasis on species development, nutrition and feeding, production and management technology, disease prevention and control etc. as compared with others ruminant species. Furthermore, the lack of input structure and improvement standard human resources development, curriculum development and net-working of buffalo forum have been at low profile and moving at slow pace. In order to move ahead in the near future to increased capability of buffalo production, it is imperative and highly recommended to take up the above mentioned aspects at the policy level and implement such activities at short medium and long terms. All available existing know-how and strategies implementation should warrant high priorities for immediate undertakings.

CONCLUSIONS

As presented, the buffalo population are distributed widely around the world especially with their vital role to provide animal production (meat and milk) to support increasing human demand and for others essential functions. There buffaloes both meat and milk could efficiency utilized available feed resources particularly those locally available and the agricultural crop-residues. Manipulation of dietary fermentation and rumen enhancement would result in improved rumen fermentation end-products and reduced methane emission, thus enhancing productively. Improvement of infra-structure for research and development as well as know-how technology for buffalo production are highly encouraged and widely disseminated. Furthermore, emphasis on buffalo production curriculum net-working ad forum developing the young scientists warrant immediate attention and action-undertakings to cope up with the challenges of increasing meat and milk demand of global population.

ACKNOWLEDGEMENTS

The authors wish to express sincere gratitude to TROFREC, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand and the 10th World Buffalo Congress Organizing Committee for their kind invitation and financial support for participating in the Conference. Assistances in preparing the paper from the graduate students are highly appreciated.
REFERENCES


Table 1. Total buffalo population in the world and in Asia 1998-2008.

<table>
<thead>
<tr>
<th>Year</th>
<th>World</th>
<th>Asia</th>
<th>South Asia</th>
<th>East Asia</th>
<th>South-East Asia</th>
</tr>
</thead>
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<tr>
<td>1998</td>
<td>160,715,087</td>
<td>156,335,297</td>
<td>117,706,250</td>
<td>22,553,806</td>
<td>15,450,089</td>
</tr>
<tr>
<td>2004</td>
<td>172,651,049</td>
<td>167,386,406</td>
<td>129,551,154</td>
<td>22,287,212</td>
<td>14,955,766</td>
</tr>
<tr>
<td>2005</td>
<td>174,526,286</td>
<td>169,182,246</td>
<td>131,256,213</td>
<td>22,365,381</td>
<td>14,873,479</td>
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<td>2006</td>
<td>176,188,724</td>
<td>170,845,267</td>
<td>132,418,951</td>
<td>22,498,838</td>
<td>15,059,327</td>
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<td>2007</td>
<td>177,376,972</td>
<td>171,863,188</td>
<td>133,382,123</td>
<td>22,720,762</td>
<td>15,191,439</td>
</tr>
<tr>
<td>2008</td>
<td>180,702,923</td>
<td>174,208,357</td>
<td>135,187,037</td>
<td>23,271,909</td>
<td>15,197,734</td>
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</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Growth, %</th>
</tr>
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<table>
<thead>
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<table>
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<table>
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</tr>
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</table>

<table>
<thead>
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<th>Year</th>
<th>Growth, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

Source: FAO (2010)

Table 2. Certain nutritional components of buffalo and cow meat (100 g).

<table>
<thead>
<tr>
<th>Contents</th>
<th>Buffalo</th>
<th>Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories, Kcal</td>
<td>131.00</td>
<td>289.00</td>
</tr>
<tr>
<td>Protein, g</td>
<td>26.83</td>
<td>24.07</td>
</tr>
<tr>
<td>Fat, g</td>
<td>1.80</td>
<td>20.69</td>
</tr>
<tr>
<td>Fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated</td>
<td>0.60</td>
<td>8.13</td>
</tr>
<tr>
<td>Monosaturated</td>
<td>0.53</td>
<td>9.06</td>
</tr>
<tr>
<td>Polysaturated</td>
<td>0.36</td>
<td>0.77</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>61.00</td>
<td>90.00</td>
</tr>
<tr>
<td>Minerals, mg</td>
<td>641.80</td>
<td>583.70</td>
</tr>
<tr>
<td>Vitamins, mg</td>
<td>20.95</td>
<td>18.52</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>61.00</td>
<td>90.00</td>
</tr>
<tr>
<td>Minerals, mg</td>
<td>641.80</td>
<td>583.70</td>
</tr>
<tr>
<td>Vitamins, mg</td>
<td>20.95</td>
<td>18.52</td>
</tr>
</tbody>
</table>

Table 3. Typical composition of buffalo milk and cow milk.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Cow</th>
<th>Buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids, %</td>
<td>13.10</td>
<td>16.30</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.30</td>
<td>7.90</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.60</td>
<td>4.20</td>
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<tr>
<td>Lactose, %</td>
<td>4.80</td>
<td>5.00</td>
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<tr>
<td>Tocopherol, mg/g</td>
<td>0.31</td>
<td>0.33</td>
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<tr>
<td>Cholesterol, mg/g</td>
<td>3.14</td>
<td>0.65</td>
</tr>
<tr>
<td>Calcium, mg/100 g</td>
<td>165.00</td>
<td>264.00</td>
</tr>
<tr>
<td>Phosphorus, mg/100 g</td>
<td>213.00</td>
<td>268.00</td>
</tr>
<tr>
<td>Magnesium, mg/100 g</td>
<td>23.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Potassium, mg/100 mg</td>
<td>185.00</td>
<td>107.00</td>
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<tr>
<td>Sodium, mg/100 mg</td>
<td>73.00</td>
<td>65.00</td>
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<tr>
<td>Vitamin A (incl. Carotene) IU.</td>
<td>30.30</td>
<td>33.00</td>
</tr>
<tr>
<td>Vitamin C, mg/100 g</td>
<td>1.90</td>
<td>6.70</td>
</tr>
</tbody>
</table>

Source: Anonymous (1995)

Table 4. Comparison of milk performance in different buffalo breeds (kg/day).

<table>
<thead>
<tr>
<th>Breed</th>
<th>Lactations (n)</th>
<th>Lactation length (days)</th>
<th>Milk yield (kg)</th>
<th>Average milk yield per day (kg)</th>
<th>Highest daily milk yield (kg)</th>
</tr>
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<tbody>
<tr>
<td>L</td>
<td>70</td>
<td>280.4±20.2</td>
<td>1092.8±207.4</td>
<td>3.79</td>
<td>6.6</td>
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<tr>
<td>M</td>
<td>237</td>
<td>324.7±73.9</td>
<td>2132.9±78.3</td>
<td>6.57</td>
<td>17.40</td>
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<tr>
<td>N</td>
<td>164</td>
<td>316.8±83.6</td>
<td>2262±663.9</td>
<td>7.14</td>
<td>18.40</td>
</tr>
<tr>
<td>MLF1</td>
<td>157</td>
<td>313.7±96.7</td>
<td>1240.5±479.8</td>
<td>3.95</td>
<td>7.57</td>
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<tr>
<td>MLF2</td>
<td>118</td>
<td>313.9±90.1</td>
<td>1423.3±534.5</td>
<td>4.53</td>
<td>8.30</td>
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<tr>
<td>NLF1</td>
<td>45</td>
<td>326.7±96.4</td>
<td>2041.2±540.9</td>
<td>6.25</td>
<td>16.65</td>
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<tr>
<td>NLF2</td>
<td>55</td>
<td>321.4±118</td>
<td>2325.6±994.4</td>
<td>7.22</td>
<td>19.35</td>
</tr>
<tr>
<td>N.MLF2</td>
<td>168</td>
<td>317.6±78.4</td>
<td>2294.6±772.1</td>
<td>7.22</td>
<td>18.80</td>
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<tr>
<td>N.MLG1</td>
<td>70</td>
<td>329.1±89.8</td>
<td>1994.9±635.0</td>
<td>6.06</td>
<td>18.50</td>
</tr>
</tbody>
</table>

L=local, M=Murrah, N=Nili-Ravi, G=Santa Gertrudis
Source: Borghese (2005) and Bingzhuang et al. (2003)
Table 5. Global methane emissions by livestock production system.

<table>
<thead>
<tr>
<th>Livestock production system</th>
<th>Dairy cattle</th>
<th>Other cattle</th>
<th>Buffalo</th>
<th>Sheep and goat</th>
<th>Pigs</th>
<th>Poultry</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global methane emission from manure management</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazing</td>
<td>4.73</td>
<td>21.98</td>
<td>0.00</td>
<td>2.95</td>
<td>0.00</td>
<td>-</td>
<td>29.58</td>
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<tr>
<td>Mixed</td>
<td>10.96</td>
<td>27.53</td>
<td>9.23</td>
<td>6.50</td>
<td>0.80</td>
<td>-</td>
<td>55.02</td>
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<tr>
<td>Industrial</td>
<td>0.00</td>
<td>0.73</td>
<td>0.00</td>
<td>0.00</td>
<td>0.31</td>
<td>-</td>
<td>1.04</td>
</tr>
<tr>
<td>Subtotal</td>
<td>15.69</td>
<td>50.16</td>
<td>9.23</td>
<td>9.44</td>
<td>1.11</td>
<td>-</td>
<td>85.63</td>
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<tr>
<td>Global methane emission from manure management</td>
<td>0.15</td>
<td>0.50</td>
<td>0.00</td>
<td>0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.77</td>
</tr>
<tr>
<td>Grazing</td>
<td>2.93</td>
<td>3.89</td>
<td>0.34</td>
<td>0.23</td>
<td>4.58</td>
<td>0.31</td>
<td>12.27</td>
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<tr>
<td>Mixed</td>
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<td>0.02</td>
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<td>0.00</td>
<td>3.80</td>
<td>0.67</td>
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<tr>
<td>Industrial</td>
<td>3.08</td>
<td>4.41</td>
<td>0.34</td>
<td>0.35</td>
<td>8.38</td>
<td>0.97</td>
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<tr>
<td>Subtotal</td>
<td>18.77</td>
<td>54.57</td>
<td>9.57</td>
<td>9.79</td>
<td>9.49</td>
<td>0.97</td>
<td>103.15</td>
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</table>

Source: Steinfeld et al. (2006)
Table 6. Methane abatement strategies, mechanism of abatement, and considerations for use.

<table>
<thead>
<tr>
<th>Methane abatement strategy</th>
<th>Mechanism of abatement activity</th>
<th>Considerations when selecting abatement strategy</th>
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</thead>
<tbody>
<tr>
<td>Dietary composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase hemicellulose/starch</td>
<td>Increased passage rate; greater proportion propionate versus acetate; reduced ruminal pH</td>
<td>Shift methanogenesis to hind gut or manure, risk of subacute ruminal acidosis (SARA)</td>
</tr>
<tr>
<td>Decrease cell wall components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grinding</td>
<td></td>
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</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty acids</td>
<td>Inhibition of methanogens and protozoa; greater proportion propionate versus acetate; biohydrogenation</td>
<td>Effect on palatability, intake, performance, milk components; varies by diet, ruminant species; long-term studies needed</td>
</tr>
<tr>
<td>Oils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seeds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tallow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defaunation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical</td>
<td>Removes associated methanogens; less hydrogen for methanogenesis</td>
<td>Adaptation of microbiota may occur; varies with diet; maintenance of defaunated animals</td>
</tr>
<tr>
<td>Feed additives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanogens Vaccine</td>
<td>Host immune response to methanogens</td>
<td>Vaccine targets; diet and host geographical location differences</td>
</tr>
<tr>
<td>Monensin</td>
<td>Inhibits protozoa and gram-positive bacteria; lack of substrate for methanogenesis</td>
<td>Adaptation of microbiota may occur; varies with diet and animal; banned in the EU</td>
</tr>
<tr>
<td>Plant Compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>Antimicrobial activity; reduced hydrogen availability</td>
<td>Optimum dosage unknown; more in vivo research needed; long-term studies needed; may affect digestibility; residues unknown</td>
</tr>
<tr>
<td>Saponins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Essential oils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumarate</td>
<td>Hydrogen sink, greater proportion propionate versus acetate</td>
<td>Varies with diet; more in vivo research needed; long-term studies needed; may affect digestibility</td>
</tr>
<tr>
<td>Malate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Hook et al. (2010)
Figure 1. World greenhouse gas (carbon dioxide, methane, nitrous oxide and etc.) emissions. Source: Modified from World Resource Institute (2000)

Figure 2. Rumen fermentation through Embden Myerhof Parnas pathway. Source: Wanapat (2012)
Prof. Maneewan Kamonpatana Lecture

Sperm Sexing in Buffalo Using Flow Cytometry

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ABSTRACT

This literature review provides information on study in sperm sexing in buffalo using flow cytometry, which has been conducted since 2002 in our laboratory. Results shown below are from a series of experiments.

(1) Identification of difference in DNA content between X and Y sperm in buffalo was investigated. Semen samples collected from river type buffalo bulls were stained with Hoechst 33342 and incubated at 35°C for 60 min and then analyzed through a flow cytometer. The result showed that the difference in DNA content between X and Y sperm in buffalo was 3.6% with a sex accuracy of about 90%.

(2) A system of in vitro fertilization (IVF) of buffalo oocyte with sexed sperm was developed. The cleavage and blastocyst rates of oocytes inseminated with sexed sperm was lower (42.2%, 20.0%) than that with unsexed sperm (52.3%, 28.6%). Similar pregnancy rate was obtained after transfer of embryos to recipients from either fresh sexed and unsexed embryos (26.5% vs 26.9%) or frozen sexed and unsexed embryos (11.6% vs 15.4%). Eleven sexed buffalo calves (10 females and 1 male) and 10 unsexed buffalo calves (6 females and 4 males) were born following embryo transfer.

(3) A trial of artificial insemination (AI) with sexed sperm to produce sex-preselected calves in buffalo was performed. A total of 3863 recipients were inseminated with X-sorted sperm for a two years period from April, 2009 to March 2011. A total of 2006 calves (51.9%, 2006/3863) were born, of which 1786 were females with 89% of sex accuracy.

Results of this study indicate the feasibility of application of sexing technology to accelerate the genetic improvement in swamp buffalo and speed up the development of buffalo dairy industry in China.

Keywords: AI, buffalo, flow cytometry, IVF, sperm sexing

INTRODUCTION

Sexing of X- and Y-chromosome bearing sperm based on the difference in DNA content is the most reliable and repeatable method to produce sex-preselected animals. Since the first report by Johnson et al. (1989) in rabbits, flow cytometric sexing technology has been shown to be efficacious in several species including...
buffalo (Johnson, 2000; Seidel Jr & Garner, 2002; Maxwell et al., 2004; Lu et al., 2007; Liang et al., 2008).

There are more than 20 million of buffaloes in southern China (FAO, 2010) and most of them are swamp type. As the genetic character of small body type, low production of either milk or meat is the main negative property of the Chinese swamp buffalo. The milk yield is about 800 kg per lactation period. Chinese swamp buffalo was normally treated as working animals in the times of traditional agriculture. With the development of agricultural mechanization and urbanization, the draft performance of Chinese swamp buffalo is not enough to satisfy the needs of people living in a commercialized era. By means of new genetic and reproductive technology, it becomes an urgent task for current Chinese government to convert Chinese swamp buffalo to milk animal. Establishment of buffalo sperm sorting technology (Lu et al., 2006, 2007, 2010; Liang et al., 2008) provides a good technical basis to accelerate this process of change. The application of sex control technology, combined with other reproductive biotechnologies as IVF, AI, embryo transfer, and so on, could produce about the double number of milking buffaloes in the same period of time as in traditional reproductive technology alone, which could hopefully resolve the shortage of milking buffaloes in China and promote the development of buffalo dairy industry in southern China.

IDENTIFICATION OF X- AND Y-CHROMOSOME BEARING BUFFALO SPERM

The sperm sexing technology using flow cytometry involves staining of the sperm with DNA-binding fluorochrome (Hoechst 33342). Flow cytometric analysis is subsequently used to quantify the difference in DNA content between the X- and Y-chromosome bearing sperm, based on their differences in fluorescence intensity (Johnson and Welch, 1999). The difference in DNA content between X- and Y-sperm of many animals has been quantified (Johnson, 1992, 2000; Parrilla et al., 2004; O’Brien et al., 2005). However, no data on difference of DNA content in buffalo sperm is available until 2006 reported by Lu et al. (2006) from our laboratory.

Sperm sorting and analysis

The procedures for sperm sorting and analysis were those reported by Lu, et al (2006). Briefly, buffalo semen collected from six fertile Murrah buffaloes and six fertile Nili-Ravi buffaloes at Livestock and Poultry Breeding Station of Guangxi, China was extended in modified TALP solution and then stained with Hoechst 33342 at a concentration of 5 mg/ml and incubated at 35°C for 60 min. The stained sperm were sonicated for 2–5 s to remove the tails for more desirable orientation when passing through the orifice of the flow cytometer nozzle.

When the sperm passed through the flow cytometer the fluorescence emitted by the sperm was detected both by the 0° (forward) and the 90° (sideward) detectors and stored as frequency distributions. Only those sperm with proper orientation were gated and subsequently analyzed for the difference in DNA content between X- and Y-sperm populations. The nuclei of goat sperm were used as control sample to evaluate the alignment of the cytometer before the buffalo sperm nuclei were analyzed. A minimum of 50,000 signals of sperm nuclei per sample were recorded.
Six replicates from each bull were performed. The methods used for calculation and analysis of the difference (%) of the DNA content between the X- and Y-sperm populations was those reported by Garner et al., 1983.

Results

The analysis of the mean values of the fluorescent intensity revealed that the difference in DNA content was 3.59±0.11% for Murrah buffalo and 3.55±0.14% for Nili-Ravi buffalo (Table 1). Differences were found among males (within breed) ($p < 0.05$) but not between breeds ($p > 0.05$).

IN VITRO FERTILIZATION (IVF) OF OOCYTE WITH SEXED BUFFALO SPERM

The practical application of sperm sexing, synergistically with other assisted reproductive technologies (ART), could improve the efficiency of buffalo production both in biological and economic terms. However, there was no data available on IVF with sexed sperm in buffalo to produce sex-preselected embryo and offspring (Lu et al., 2007).

Sperm preparation and oocyte maturation

The procedures for sperm preparation and oocyte maturation were described by Liang et al, (2008). Briefly, sperm were sorted into X- or Y-chromosome bearing sperm enriched populations by a flow cytometer followed by deep freezing and storing in liquid nitrogen for later use. Frozen sexed and unsexed semen was thawed followed by a centrifugation in Percoll gradient to remove dead sperm.

Oocytes collected either from live buffalo by ultrasound-guided transvaginal ovum pick up (OPU) or from abattoir-derived ovaries were matured in vitro in TCM199 supplemented with 5% estrous cow serum and 10 mg/mL FSH at 38.5°C and 5% CO₂ in air for 22–24 h.

In vitro fertilization and culture

Ten to fifteen oocytes were added to a drop of 40 mL modified Tyrode’s medium supplemented with 0.6% BSA, 2.0 mM caffeine, and 20 mg/mL heparin. The final concentration of sexed or unsexed sperm in the fertilization drop was 1–2 x 10⁶ sperm/mL. Eight to 10 h after insemination, presumptive zygotes were transferred to culture medium containing TCM199 supplemented with 10% FCS on granulosa cell monolayers at 38.5°C and 5% CO₂ in air for 6-7 d.

RESULTS FROM EXPERIMENTS

Exp. 1: Embryo development after IVF with sexed and unsexed buffalo sperm

Results from Table 2 showed that the rates of either cleavage or blastocyst development from oocytes fertilized with sexed sperm were about 10% lower ($P<0.01$) than that with unsexed sperm.

Exp. 2: Freezing of buffalo embryos derived from IVF with sexed and unsexed sperm

Table 3 showed that no difference was found in survival rate after thawing between embryos derived from oocytes fertilized with sexed and unsexed sperm.

Exp. 3: IVF of oocytes derived from OPU and abattoir ovaries with sexed or unsexed sperm

There was a tendency that the percentage of blastocyst development rate of oocytes derived from abattoir ovary was higher after IVF than that from OPU,
however, the difference was not significant between the two sources of oocytes (P>0.05, Table 4).

**Exp. 4: IVF of oocytes from OPU donors of different breeds with buffalo sorted sperm**

There was no significant difference in either cleavage or blastocyst rates between two donor breeds (Nili/Ravi and Nili/Rave x Swamp) of oocytes inseminated with sexed sperm.

**Exp. 5: Pregnancy and birth of calves after transfer of fresh or frozen embryos produced by IVF of OPU-derived oocytes with sexed or unsexed frozen sperm**

The data from Table 8 showed that a total of 142 embryos produced by sexed and unsexed sperm were transferred. The pregnancy rate was rather low following transfer of embryos derived from sexed (26.5%) or unsexed fresh sperm (26.9%) and from sexed (11.6%) or unsexed (15.4%) frozen sperm. However, the female sex accuracy of calves (including abortions) was 85.7% (12/14) for the embryos from sexed X-sperm while only 46.2% (6/13) for the embryos from unsexed sperm. The proportion of abortions from pregnancies found in sexed embryos (3/14, 21.4%) was similar to that of unsexed embryo (3/13, 23.1%).

**ARTIFICIAL INSEMINATION (AI) WITH SEXED BUFFALO SPERM**

Artificial Insemination (AI) has been shown to be one of the most successful breeding strategies to effectively spread genes from the excellent bull for improvement of production traits.

AI with sexed sperm for production of sex-preselected offspring has been successful in cattle (Seidel et al., 1999); Sheep (Cran et al., 1997; Hollinshead et al., 2002); Horse (Buchanan et al., 2000) and Pig (Johnson, 1991; Grossfeld et al., 2005). There were two reports on the application of AI with sexed sperm in buffalo. One was from Presicce et al. (2005) in which a conception rate of 42.8% was observed in Mediterranean Italian buffaloes following Ovsynch protocol and AI with sexed sperm, and another was from our laboratory (Lu et al., 2010).

In our study, semen was collected from river type buffalo (Nili-Ravi and Murrah) and sorted for X-sperm enriched sample and finally frozen and stored in liquid nitrogen. One 0.25 mL-straw containing $2 \times 10^6$ sexed sperm, while the conventional semen 0.25 mL-straw containing $20 \times 10^6$ non-sexed sperm was used for AI once per recipient. Before the AI, dominant follicle on the ovary was detected by carefully rectal palpation and the sexed semen was artificially inseminated into the ipsilateral deep uterine horn with the dominant follicle of spontaneous estrous buffalo. The buffaloes used in the present study were swamp type and F1 crossbreed of Nili-Ravi/Murrah X swamp.

**Exp. 1: Effect of different bulls on pregnancy rate following AI with sorted sperm**

In this study, the conception rate of sexed sperm derived from NL388 was 55.0% (11/22), significantly lower than that of 83.6% (19/23) from ML445 (P < 0.05). This would suggest that in terms of economic efficiency, the selection of bulls for sperm sexing would be of great importance.

**Exp. 2: Difference in conception rate following AI with sexed sperm between heifer and parous buffalo cows**

The conception rate following AI with sexed sperm in heifers was 77.8%
(7/9), slightly higher than that of 67.6% (23/34) in parous buffalo cows, but not statistically different. Similar conception rate was observed in the primiparous cows (76.5%, 13/17) and the heifers (77.8%, 7/9). This would indicate a similar uterus condition and fertility in these animals.

Exp. 3: A trial for application of AI with buffalo sexed sperm

A trial for application of AI with buffalo sexed sperm was conducted in six counties of Guangxi province in China from April 2009 to March 2011. Most of the buffalo recipients used for the trial were those in household, which were raised in very small herds (2–5 buffaloes per family). AI with sexed X-sperm was done following spontaneous estrous in this trial. A total of 3,863 buffalo recipients were artificially inseminated and 2006 calves were born, of which 1,786 were female with a sex accuracy of 89.0%.

CONCLUSIONS

1. The difference of DNA content between X and Y buffalo sperm is identified as 3.6%.
2. In vitro production of sex-preselected embryos and offspring in buffalo is feasible.
3. AI with sexed sperm is successful in practice in term of rate of calve born and the sex accuracy.

REFERENCES


**FUNDING SOURCES**

This research was jointly supported by the National Natural Science Foundation of China (No. 31160458, No. 30360073); Guangxi Key Technology R&D Program (No. 1123005-1) and Guangxi Science Foundation Key Program (No. 2010GXNSFD013023).
Table 1. The mean±S.D. (n = 6) percent difference in DNA content between the X- and Y-chromosome bearing spermatozoa of buffalo (Lu et al., 2006).

<table>
<thead>
<tr>
<th>Buffalo Breed (%)</th>
<th>Murrah</th>
<th>Nili-Ravi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.65 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.55 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>3.52 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.69 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>3.72 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.38 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>3.52 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.56 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>3.59 ± 0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.60 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>3.59 ± 0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.56 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breed mean*</td>
<td>3.59 ± 0.11</td>
<td>3.55 ± 0.14</td>
</tr>
</tbody>
</table>

Mean values in the same column with different letters are significantly different (p < 0.05).
* The breed means are not significantly different from each other (p > 0.05).

Table 2. Cleavage and blastocyst development rates after IVF of oocytes with sorted and unsorted buffalo sperm.

<table>
<thead>
<tr>
<th>Sperm type</th>
<th>Replicate</th>
<th>No. oocytes</th>
<th>% cleavage</th>
<th>% blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexed sperm</td>
<td>12</td>
<td>926</td>
<td>42.2±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.0±1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Unsexed sperm</td>
<td>12</td>
<td>780</td>
<td>52.3±2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.6±2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Values in the same column with different superscripts differ significantly (P<0.01).

Table 3. Survival rate after thawing between embryos derived from oocytes fertilized with sexed and unsexed sperm.

<table>
<thead>
<tr>
<th>Embryo type</th>
<th>No. of embryos frozen</th>
<th>Survival after thawed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>From sexed sperm</td>
<td>172</td>
<td>122(70.93)</td>
</tr>
<tr>
<td>From Unsorted Sperm</td>
<td>153</td>
<td>112(73.20)</td>
</tr>
</tbody>
</table>

Table 4. Embryo development following IVF of oocytes from OPU and abattoir ovaries with sexed buffalo sperm.

<table>
<thead>
<tr>
<th>Oocyte source</th>
<th>Replicate</th>
<th>No. oocytes</th>
<th>Cleavage (%)</th>
<th>Blastocyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPU</td>
<td>5</td>
<td>155</td>
<td>56.7±12.9</td>
<td>16.0±7.8</td>
</tr>
<tr>
<td>Abattoir ovary</td>
<td>5</td>
<td>114</td>
<td>50.4±6.4</td>
<td>23.9±11.4</td>
</tr>
</tbody>
</table>
Table 5. Embryo development of oocytes from OPU donors of different breeds after IVF with sorted buffalo sperm.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Cleavage (%)</th>
<th>Blastocyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nili/Ravi</td>
<td>113/224 (50.4)</td>
<td>37/224 (16.5)</td>
</tr>
<tr>
<td>Nili x swamp</td>
<td>48/91 (52.7)</td>
<td>19/91 (20.9)</td>
</tr>
<tr>
<td>Total</td>
<td>161/315 (51.1)</td>
<td>56/315 (17.8)</td>
</tr>
</tbody>
</table>

Table 6. Pregnancies in buffalo following transfer of fresh or frozen-thawed embryos produced by IVF of OPU-derived oocytes with sexed or unsexed frozen-thawed sperm (Liang et al., 2008).

<table>
<thead>
<tr>
<th>Sperm/embryo</th>
<th>No. embryos transferred</th>
<th>No. pregnant (%)</th>
<th>No. live births</th>
<th>No. abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male Female</td>
<td>Male Female</td>
</tr>
<tr>
<td>Sexed/fresh</td>
<td>34</td>
<td>9 (26.5)</td>
<td>1 6</td>
<td>1 1</td>
</tr>
<tr>
<td>Sexed/frozen</td>
<td>43</td>
<td>5 (11.6)</td>
<td>0 4</td>
<td>0 1</td>
</tr>
<tr>
<td>Unsexed/fresh</td>
<td>26</td>
<td>7 (26.9)</td>
<td>2 3</td>
<td>2 0</td>
</tr>
<tr>
<td>Unsexed/frozen</td>
<td>39</td>
<td>6 (15.4)</td>
<td>2 3</td>
<td>1 0</td>
</tr>
</tbody>
</table>

Table 7. Pregnancy rate following AI with sorted sperm from different buffalo bulls.

<table>
<thead>
<tr>
<th>Bulls</th>
<th>No. AI</th>
<th>No. pregnant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL388</td>
<td>20</td>
<td>11 (55.0)</td>
</tr>
<tr>
<td>ML445</td>
<td>23</td>
<td>19 (82.6)</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>30 (69.8%)</td>
</tr>
</tbody>
</table>

a,bValues in the same column with different superscripts differ significantly (P<0.05).

Table 8. Effect of recipient cows with different times of birth on conception rate following AI with sorted sperm.

<table>
<thead>
<tr>
<th>Times of birth</th>
<th>No. recipients inseminated</th>
<th>No. pregnant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
<td>7 (77.8)</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>13 (76.5)</td>
</tr>
<tr>
<td>≥2</td>
<td>17</td>
<td>10 (58.8)</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>30 (69.8)</td>
</tr>
</tbody>
</table>
Table 9. Data on a trial of AI with buffalo sexed X-sperm.

<table>
<thead>
<tr>
<th>Name of county</th>
<th>No. recipients</th>
<th>No. calves born (%)</th>
<th>No. female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wuming</td>
<td>1119</td>
<td>440 (39.3)</td>
<td>386 (87.7)</td>
</tr>
<tr>
<td>Xingbin</td>
<td>910</td>
<td>534 (58.7)</td>
<td>478 (89.5)</td>
</tr>
<tr>
<td>Lingshan</td>
<td>1144</td>
<td>681 (59.5)</td>
<td>609 (89.4)</td>
</tr>
<tr>
<td>Hepu</td>
<td>517</td>
<td>277 (53.6)</td>
<td>241 (87.0)</td>
</tr>
<tr>
<td>Liucheng</td>
<td>173</td>
<td>74 (42.8)</td>
<td>72 (97.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3863</strong></td>
<td><strong>2006 (51.9)</strong></td>
<td><strong>1786 (89.0)</strong></td>
</tr>
</tbody>
</table>
Plenary Session 1: Buffalo for Food Security and Economy
Changing Faces of Swamp Buffaloes in an Industrializing Asia

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ABSTRACT
Swamp buffaloes are mainly found in East and South Asia and represents 20.51% of the world’s buffalo population. For centuries, swamp buffalo has played a major role in smallholder mixed farming system, however, the intensification of crop farming coupled with the increased farm mechanization in the past decade diminished the swamp buffaloes’ role as source of draft in small farms. Despite this development, their value as source of livelihood and food among rural farming families remains high, and thus, undoubtedly, the direction for its development can be shifted towards improving for meat and milk production. Genetic transformation is pursued thru cross breeding and backcrossing with riverine buffalo breeds. For a wide-scale crossbreeding program to be sustained there are necessary elements that need to be institutionalized, one important item is the establishment of Gene Pool of elite riverine buffaloes coupled with organized selection and testing from where genetic materials for wide-scale crossbreeding will be derived. This has to be complimented with a responsive AI and bull loan systems to ensure proper utilization of superior genetic materials and a scheme to stimulate sustained growth of village buffalo-based entrepreneurship.

Keywords: buffalo-based enterprise development, buffalo meat and milk production, Philippine carabao, swamp and riverine buffalo crossbreeding

INTRODUCTION
Economic growth in East and Southeast Asia in recent years in characterized by increasing industrialization and declining share of agriculture in the total economy. This is accompanied by rise in human population, increasing urbanization, increasing income and changes in food preferences. Demand for animal-derived products, such as meat and milk, has been noted to grow in this region at a rate faster than observed in more developed economies (Delgado et al., 1999). These livestock sector major change drivers are observed in countries where the world’s swamp buffalo population are mainly found.

To date, there are 32.75M swamp buffaloes and represent 20.51% of the world's buffalo population (FAO, 2012). The history of swamp buffalo is basically a history of smallhold land-based agriculture, since for centuries, the swamp buffaloes have played a major role in draft animal-dependent farming systems, mainly in the production of major agricultural crops, such as rice, corn, sugarcane and coconut. In recent years, however, developments in land-based agriculture in East and Southeast Asia, such as the expansion of irrigation facilities and farm mechanization, have significant impact on the use of draft buffaloes. Intensified rice production became more pronounced in irrigated areas and this has led to increased utilization of small
farm machineries, significantly displacing the draft buffaloes. The introduction of tractors for land preparation and transport of produce in corn, sugarcane and other crop production areas has had similar effects.

The interest in developing the already existing huge population of swamp buffaloes beyond being a draft animal, to improving the genetic potentials for meat and milk through crossing with riverine-type buffaloes is becoming profound. This lends more meaning in the light of the need to address the growing issue of low income among smallholder farming families amidst the rising cost of farm inputs, thereby creating significant impact on the net income derived from the traditional crop-dominant farming system. The technical aspect of such crossbreeding has been a subject of research interest for several years in view of the known differences in their chromosome numbers, swamp buffalo has 2n=48 while the riverine type has 2n=50. Today, there are enough evidences that this effort is feasible.

ASIAN SOCIO-ECONOMIC SETTING

Human population, landholdings and income

China and Southeast Asian countries with substantial swamp buffalo population has about 1.96 billion people as of 2012, representing 27.76% of the world's population (Table 1). Of interest is the fact that large percent of these people depend on agriculture, and among the people in land-based agriculture, the size of landholding has become smaller over the years. This trend happens in countries with slow industrial growth amidst the fast increase in human population. In this case, the generation of job opportunities outside of traditional agriculture cannot cope with increases in available labor and thus the relatively large number of people in the farming communities have to source livelihood from static, albeit decreasing cultivable land area.

Additionally, the rise in cost of farm inputs, such as fuel and fertilizer led to considerable increase in the cost of production. These simply imply that smallholders’ income from traditional crop remains low, as in the case of rice farmers. In the Philippines for example, while net income from rice production increase by an average of 12.11% per year from 2004 – 2010 mainly due to 8.16% annual increase in buying price of paddy rice (PhilRice, 2012), their net income at $363.97 per ha per cropping is considerably low. At twocroppings per year, this is translated to only $722.95 a year and given an average farm size of 1.3 ha per household, it would be difficult to support the basic needs of a family with five members. Additionally, due to inflationary effects, rural farming families are caught in a tight situation of declining purchasing power, and thus, measures to generate sources of additional income for the millions of farming families are for priority consideration.

Changing swamp buffalo population

Impact of industrialization: The changes in swamp buffalo population in East Asia and South East Asia over the last 50 years are shown in Table 1. There are five countries with sustained growth in buffalo population, namely, China, Cambodia, Lao PDR, Myanmar, and Vietnam, while other countries have registered
negative growth, namely, Indonesia, Malaysia, Taiwan, Thailand, and the Philippines.

In early 40’s, the phenomenon of declining swamp buffalo population, though relatively small, in an industrializing environment was noted in the southernmost island of Japan, as a result of the massive farm mechanization. The same trend was noted in Taiwan in the early 60’s. From the population of about 324,516 hd in 1960, the number declined steadily with power tillers increased at the rate of 3,500 units per year (Lin, 1975). To date, swamp buffalo in Taiwan is a zoo animal.

Substitution of buffalo with tractors also occurred as early as 1957 in the central plain of Thailand where swamp buffaloes were most dense (Na Puket, 1975), and this trend continued in the subsequent years due to aggressive government mechanization program. During the period 1960-2010, the reduction in swamp buffalo population is estimated to be 67.3%. Similar trend in swamp buffalo population was noted in Malaysia (Table 2). In these countries, the phase of industrialization has overtaken agriculture development as clearly manifested in the significant decline in the contribution of agriculture in the entire economy, ranging from only 12.0% in Malaysia to as low as 1.6% in Taiwan. In Indonesia and the Philippines, while there are recorded reductions in buffalo population, the rate of decline was not as drastic. In fact, there were recorded recovery peaks between 1990-2000, and increase in buffalo population is sustained in the Philippines until 2010 (Table 2). The recovery in population in the Philippines between 1990 and 2000 can be related with the entry of buffalo meat from India that started in 1994. Translated in live animal equivalent, the volume of annual import is equal to about 400,000 hd per year.

What is interesting is the case of China, a country where agriculture contributes only 9.7% to the total economy and yet, registered the highest growth rate in swamp buffalo population in the last 20 years. Elsewhere in South East Asia, countries with positive growth in buffalo population are those with greater contributions of agriculture to their total economy, and ranged from 20% in Vietnam to as high as 43% in Myanmar.

Other contributory factors: Some factors why farmers shy away from raising buffaloes is also interesting to be understood. The more apparent reasons are as follows:

Aging farmers: Majority of farmers directly engaged in small-holder mixed farming system in Asia are above 50 years old. They are ageing. In 10-15 years they will retire from working in the farm and they expect younger generation as replacement. However, the younger generation, if given opportunity, are moving out of their farming environment and seek out off-farm employment. This is particularly true for those who have gone through college education. Other than the fact that farm work is less attractive to them, the low level of income generated from small landholding is a major reason.

Farming convenience: Older farmers, just like the younger generation who may opt to stay in the farming setting due to lack of education and other off-farm employment option also embrace convenience. Tending their small land holdings, they find means how to carry out farming tasks more conveniently.
Mechanization is very attractive to them, and given access, such as government support for mechanization program, they readily adopt it.

**Market price of buffalo-derived product:** When swamp buffalo is raised purely for meat, one consideration in countries with slaughter ban, such as the Philippines, is the depressed price of buffalo meat vs cattle. This is related with the policy of promoting availability and usage of draft buffaloes during the early 60’s, the period when farming was largely dependent on draft animals. The ban allowed slaughter of male only after age of 7 years old, and of female only after 11 years old, the objective was to allow females to reproduce and be used for farm work, and males to be used for draft during their productive life.

Such policy created the impression that buffalo meat is very tough, essentially because what were legally available in the market come from retired and old work buffaloes. Up until this time, the consuming public has considered buffalo meat second class to beef, and thus is priced much less. For this reason, in the Philippines, raising buffalo purposely for meat is less attractive than cattle. It may take some time to change consumer’s negative perception of buffalo meat, even long after the removal of the slaughter ban. Some farmers sell their buffaloes and buy beef cattle for raising purposely for meat.

**GROWING DEMAND FOR ANIMAL-DERIVED PRODUCTS**

Many Asian countries have registered sustained economic growth in recent years and this has also resulted in increased establishment of urbanized areas. The rise in income among urban population has also brought about a corresponding shift in food preferences as demonstrated in greater demand for beef and milk. With the reduced land area for grazing and forage production, the only immediate option to meet the growing requirements is increase in imports of milk and beef in recent years.

It is easy to understand that sudden rise in meat demand in the fast-growing population of Asia can be met by intensive production of chicken and pork. This has taken place in China and Southeast Asian countries in significant magnitude, of course, with corresponding increases in imports of feed grains. Requirements for beef in these countries are met by massive imports of buffalo meat from India, with Malaysia and the Philippines leading. Indonesia imports about 16.0% of its beef requirements whereas China is nearly self-sufficient with only about 2.5% of its requirements coming from imports.

As a long-term development strategy, however, efforts in fast-growing economies in Asia have also included programs to enhance growth in their respective local dairy industry with massive infusion of stocks of "tropicalized" dairy cattle from Australia and New Zealand. This development approach is becoming more meaningful in most of the Asian countries that remain net importers of milk and dairy products as prices of milk in the international market have surged in view of the policy and regulatory measures in some exporting countries, and also due to unfavorable climatic factors that resulted in reduced production and thus in traded milk in the international market. With the rising demand for same dairy animals for restocking farms in post-BSE Europe and Latin America, however, prices of dairy breeder stocks have also significantly increased lately.
Utilization of the existing population of swamp buffaloes in hot and humid tropics and harnessing the age-tested abilities of the smallhold farmers to rear these animals to provide opportunities to earn additional income, and also to meet the growing domestic demand for milk and meat, against the backdrop of increasing farm mechanization, are good reasons to transform the huge number of draft animals into producers of milk and meat.

Given the abundance of low-cost labor among farming families, the production cost for milk and meat from crossbred buffaloes becomes competitive. With the net income derived from crop-dominant farming system as reference, it has been demonstrated that net income from milk of 1 to 2 crossbreds/backcross is sufficient to double the income of the smallholder family tending a hectare of rice. The added advantage in dairying is the derivation of cash income on a daily basis from the sales of milk while on the seasonal long wait for harvests from crops.

CROSSING SWAMP X RIVER BUFFALOES
Implementation of crossbreeding and backcrossing

The introduction of riverine buffalo genetic materials into distinctly swamp buffalo populated countries of China and South East Asia started as early as 1917 in the form of both live animals and frozen semen (Tables 3 and 4) as cited by Balaine (1988). Most of the breeds infused were Murrah Buffalo from India and Nili-Ravi from Pakistan.

The early introduction of riverine breeds resulted in crossbreds, either by way of natural mating between the introduced breed and the indigenous swamp buffaloes or by AI in the late 60’s or early 70’s. In China wide-scale crossbreeding between Murrah and the local breed of swamp buffalo started as early as 1965 through AI. By 1977, the Murrah x swamp crossbreds were mated with Nili Ravi to create a triple cross (Liu et al., 1986). In the Philippines the wide-scale use of AI for crossbreeding of Murrah and swamp buffalo was initiated only in 1984 after the setting up of buffalo semen processing laboratory. Since then, the F1 crossbreds were continuously inseminated with Murrah semen to produce backcrosses with increasing blood of riverine breed (Cruz, 2007, 2010). This wide-scale AI system is complimented with organized bull loan program. To date, the estimated number of crossbreds and backcrosses is almost 400,000hd with riverine blood line varying from 50% to 93.75%. In other SEA countries, the extent of crossbreeding has been at experimental scale as reported in Thailand (Konanta, 1986; 1994), Vietnam (Thac, 1979; Ly, 1985), and Taiwan (Lin, 1975).

Another interesting account of deliberate crossing between swamp and riverine type buffaloes was in Cuba. In a span of three years, in 1983-1986 Cuban government infused a total of 1,438 swamp buffaloes and 279 riverine types. These animals were crossed to produce F1 and backcrosses. In Australia, crossing were also reported between riverine breed and swamp buffaloes (Lemke, 2004).

To date, China and the Philippines are the only two countries in Asia that are pursuing large-scale crossbreeding and backcrossing of swamp buffaloes with the intent to produce critical population of animals with higher genetic potentials for milk and meat production.
Chromosomal analysis of water buffaloes and their crosses

The interest among scientists in the past has been anchored on the known fact that swamp and riverine buffaloes have different chromosome numbers: the diploid chromosome number of the swamp buffalo is 48 while that of the river buffalo is 50. The reduction in chromosome number in swamp buffalo is seen as the tandem fusion (telomere - centromere) of chromosome pair 4 and 9 of riverine karyotype. The general apprehension was based on other animal species of different chromosomes crossbreeding data indicating fertility problems among resulting offspring.

When crossbreeding between the 2 buffalo types occur, males and females of the F1 generation are heterozygous for the fusion with chromosome 2n=49. Of these chromosomes, 3 chromosomes included one metacentric, one submetacentric and one telocentric chromosome were not in pair. Through the G-band analysis, it was demonstrated that the metacentric chromosome in the three unpaired chromosomes belonged to the chromosome 1 of swamp buffalo, and the other two chromosomes corresponds to chromosomes I and 9, respectively, from river buffaloes, which may be homologous as they had G-band type (Huang, 2006).

Inter-se mating of F1 produces F2 hybrids of three different karyotype categories (2n=48, 2n= 49 and 2n=50). Chi-square tests on pooled data indicated that the distribution 1:2:1 ratio is expected if only balanced gametes with 24 and 25 chromosomes are produced by the F1 hybrids. Backcrosses (75:25) produced out of mating F1 (50:50) with swamp buffalo karyotype categories are 2n=48 and 2n=49. On the other hand, if F1 (50:50) is backcrossed with riverine buffalo the resulting F2 (75:25) has karyotypes of 2n=49 and 2n=50. In the three-quarters swamp and three-quarters river types, the respective karyotypic categories are in ratios approximating 1:1. The distribution of chromosome categories among the F2 hybrids and backcrosses suggests that only genetically balanced gametes of the F1 hybrids are capable of producing viable F2 and backcross generations (Bongso et al. 1983).

In China, three-way crossbred hybrids were obtained by crossing swamp buffalo x Murrah x Nili Ravi or swamp buffalo x Nili Ravi x Murrah. They had two chromosome categories viz. 2n=49 and n=50, respectively. The two types of karyotype exist not only in the progenies of three-way crosses, but also in the F2 hybrids and F3 hybrids of grading crosses. It could be observed that during the meiotic division, the F1 hybrid with 2n=49 chromosomes produced 24 synaptonernal complexes (SC), which consisted of 22 divalents, autosome trivalent and a XX divalents. During the synopsis, the meme 1 from swamp buffalo undergoes partial alignment with submetacentric chromosome 1 and telecentric chromosome 9 from river buffaloes. The synopsis is kept up until metaphase 1. The disjunction occurred during anaphase 1 when it was observed that the metacentric chromosome 1 from swamp buffalo was pulled on one pole to another pole, which resulted in production of two types of sperms viz. n=24 and n=25, respectively. The male river buffalo (2n=50) produced only one type of sperm (n=25). Therefore, the hybrids of three-way crossbred and F1 and F2 grading crossbreed hybrids had two types of karyotypes viz. 2n=49 and 2n=50. The ratio of the types of karyotypes was near 1:1 in the hybrids of three-way crossbred and the F1 grading crossbred hybrids (Huang, 2006).
Performance of crosses and backcrosses

*Milk Yield:* Chinese swamp buffalo has generally low milk production between 500-800 kg/lactation (Yang et al., 2007). However, selected Chinese swamp buffaloes have higher milk yield, as in the case of animals at government institutions where recorded average milk production is 1,092.8 kg/lactation. Under similar conditions, purebred Murrah and Nili Ravi milk yield/lactation were reported to be 2,132.9 kg and 2,262.1 kg, respectively (Yang et al., 2004).

The F1 crosses of swamp buffalo with Murrah breed had an average of 1,233.3 kg milk/lactation, equivalent to 12.3% increase in milk production. In the Philippines, the recorded yield of swamp buffalo x Murrah crosses is 4.14 kg/day and represents 176.0% increase, mainly because the milk yield of Philippine swamp buffalo is only 1.5 kg/day on the average. On the other hand, Chinese swamp buffalo crossed with Nili Ravi registered milk yield of 2,041.2 kg/lactation, an increase of 86.9% over the swamp buffalo parents.

Backcrossing the (MxNL) F1 with Murrah or NL F1 with Nili Ravi resulted in milk yield of 1,585.5 kg and 2,267.6 kg/lactation, respectively. Clearly, the backcrosses with 75% riverine bloodline have higher milk yield than FI crosses. Among the MUIT’ah backcrosses, the increase over the swamp parents is 45% and 28.5% over the F1 cross. Similar trend is demonstrated among Nili Ravi backcrosses, with reported increases of 107.5% compared to swamp parents and 1.0% compared to LF I crossbreds.

*Growth and Meat Production:* There is no difference in birth weight between the two breed groups, but the growth rate of crossbreds started to move ahead than that of the swamp buffaloes starting at age of 6 months up until 36 months, with growth advantage that ranged from 10-31.1%. At four years of age, FI crossbred (50:50) and backcrosses with 75% Murrah blood registered weight advantage of 9.8% to 2 1.4% over the swamp parents (Faylon, 1992).

In Australia, Lempke (2004) reported that the introduction of Riverine blood from the USA in 1994-1997 radically altered the productivity of TenderBuff. Growth rates in the crossbred from 3/8 and above are outstandingly greater than the purebred swamp available in the NT. Some 40% improvement in growth rates has been recorded in comparisons. Results from the NT Government Beatrice Hill Farm regularly confirm this trend. The more crossbred carcasses that are processed, the better the production data becomes.

International collaborations, the Philippines experience

The crossbreeding program in the Philippines is a result of various collaborations that involved many international institutions and entities that started as early as 1917 up until to date. These collaborations may be classified into three major areas, namely, genetics, system development and genetic utilization, and promoting enterprises as summarized in Table 5.

Phase I of germplasm infusion occurred between 1917 to 1956 and involved live animals from India on a government to government arrangement. Many bulls were assigned in the government breeding centers throughout the country and were used in natural mating. There were also Murrah bulls loaned out to private commercial buffalo farms for crossbreeding purposes. While there were good
number of crossbreds produced out of these efforts, the monitoring and mating of these animals were not properly pursued, and thus with time, the female crossbreds were mated by native bulls. Also, due to the absence of organized breeding program, the purebred dairy breeds which were distributed to several government institutions failed to increase in number and in quality as desired.

Phase II of genetic infusion started in 1981, this time through frozen semen of Murrah and Nili-Ravi from India. Organized AI to produce crossbreds for research under the 10-Year United Nations Development Programme-Food and Agriculture Organization (UNDP-FAO 1982 to 1992) project were initiated. The resulting F1 crossbreds were found to grow significantly faster than the swamp buffalo and to produce milk three to four times more than the native parents. It was also demonstrated that males and females F1 crossbreds (2N= 49) were fertile. However, based on the analyses of breeding and performance records, it is not recommended to pursue an F1 x F1 mating, thus the route pursued and expanded towards the end of the project and onward is the continued backcrossing with the riverine type. Backcrosses with increasing blood composition of the riverine breed registered linear increment in milk yield without detriment to the reproductive and adaptive performances. With these production potentials, the social and economic benefits accruing to rural farmers from raising crossbred and backcross carabaos instead of the local breed became pronounced.

On the basis of these encouraging results, the government established in 1984 the country's first frozen buffalo semen laboratory and implemented a national artificial insemination program for swamp buffaloes utilizing frozen semen of selected Murrah sires. Estrus synchronization procedures were also developed to permit synchronized breeding and allowed the coverage of many breedable females, which are scattered quite thinly in the villages all over the country, utilizing the relatively few available AI technicians.

The phase III of genetic infusion started in 1995 with the infusion of Bulgarian Murrah buffaloes. About 3,142 animals (216 males and 2,926 heifers), together with 3,000 doses of frozen semen from elite progeny tested bulls were imported between 1995 to 1998. As the number of Bulgarian Murrah available for importation became so limited on later years, importation of 2,038 Murrah from Brazil was made in 2010. This followed by planned importation of 1,025 Italian Mediterranean buffaloes and some 5,000 straws semen in 2013.

Development of organized genetic improvement program that involved selection and progeny testing among purebred dairy buffaloes started in 1995. Purebred dairy buffaloes were reared at institutional facilities and accurate animal ID, pedigree and performance data were taken. In 2000, purebred animals at the farmer coops were enrolled at the genetic improvement program. Collaboration with the Australian government thru the ACIAR towards refining the data analysis system was initiated in 1999 thru 2004.

Technical assistance from the Japanese government thru JICA followed in 2000 to 2005, and such cooperation focused on improving system for utilization of selected sires, improvement processing of buffalo semen, and refinement of AI system. These initiatives were very important elements for progeny testing system. Towards the middle of 2008, collaboration with the US government, thru its PL480
A program was initiated aimed at enhancing institutional capacities for buffalo R&D. This program focused on improving laboratory facilities and equipment and on human resource development.

Genetic conservation was put in place through the establishment of cryobank facilities with the assistance from KOICA and other partners such as the Taiwan Livestock Research Institute, Hankyong National University, and Institute of Animal Sciences of Korea.

As the crossbred and backcrosses animals have been produced and the numbers have continuously increased, farmers were organized and were trained to milk and assisted to set-up milk collection centers. The Korean (KOICA) and Japanese (KR2) governments assisted in this aspect through provision of post-production facilities and technical expertise to allow value adding of dairy products and facilitate viability of buffalo-based enterprises.

**INSTITUTIONALIZATION OF BUFFALO DEVELOPMENT PROGRAM**

The program of transforming the Philippine swamp buffaloes to producers of milk and meat has been institutionalized after the establishment of the Philippine Carabao Center and in China, after the establishment of the Guangzi Buffalo Research Institute. The components of the national program in the case of the Philippine experience are herein discussed.

**Establishing the ground for genetic improvement**

The fundamental initiative that is most consistent with the envisaged improvement in the productivity of the swamp buffalo is the establishment of germplasm pools from where superior materials can be obtained on a sustainable basis. Efforts along this line have yielded concrete results, as follows:

*Gene pools for selected native Philippine Carabao (PC)*: While exotic germplasm were introduced for the specific purpose of improving milk and meat, the government also ensured that the existing swamp buffalo germplasm are conserved for long-term genetic improvement program. The general premise is that through the years, domestic stocks of swamp buffaloes have adapted to the local conditions and therefore there are certain genes that can be very useful for future breeding and genetic improvement. Gene pools for the Philippine carabao were established in the three main islands of Luzon, Visayas and Mindanao. The animals are kept as Open Nucleus Herds (ONH), and selection of better stocks from the surrounding communities is done on a continuing basis. Selected animals outside of institution herd are taken in and shall form part of the ONH for the Philippine carabao. These animals have been chosen primarily for size, growth rate and reproduction ability.

There is also a swamp buffalo sanctuary in a separate island that is so well protected from the introduction of any exotic germplasm of buffalo of any form. Farmers are utilizing the indigenous buffaloes for their farming activities and this will certainly be carried through for many generations to come. Monitoring of the animals is regular.

*Gene pool for improvement for milk production*: Elite herds of "Bulgarian Murrah" are reared at the National Riverine Buffalo Gene Pool and at two separate institutions in the southern islands. Animals with outstanding performance at farmer-
cooperatives are also enrolled as part of the gene pool. With organized selection and testing system in place, the country is now assured of sustained sources of genetic materials for improvement of milk production. The system can produce about 400 bulls of good quality per year, with the top-ranking bulls subjected to organized progeny testing and then assigned as semen donors for use in the nationwide AI program, while the above-average bulls are used in the wide-scale bull loan for crossbreeding in the villages.

*Embryo Biotechnology Laboratory:* Attempts to hasten the envisaged genetic improvement have also led to the development of facilities and reproductive biotechniques that can be used as important tools in some specific areas not normally achieved through the traditional breeding techniques. To date, the facilities established at the PCC Central Research Station and in the satellite laboratory in Maharstra, India have developed technologies to produce high genetics embryos through the in-vitro system. These efforts are complemented with the newly refined ovum pick-up procedures, obtaining oocytes from superior donors for IVM/IYF as an alternative option to superovulation scheme that proved to be less predictable and more expensive. Likewise, the facility has just embarked on attempts to propagate superior genetics dairy buffaloes through the use of somatic cell nuclear transfer technique (SCNT).

*Use of DNA-based biotechniques as a tool for genetic improvement:* As can be gleamed from the latest studies in bovine, the use of recently developed biotechnologies, such as marker-assisted-selection (MAS), has provided adequate opportunities to enhance selection and thus genetic improvement. This area will therefore receive considerable attention in the future.

*Genetic evaluation system:* Breeding research that aims to improve the milk production potential of the riverine buffalo population in the country is carried out by putting in place a system of ranking and selecting the best animals. This is done by developing a BLUP animal model for determining the genetic merit of individual animals with milk production record. Initially, evaluation of cows was based solely on milk volume, but starting in 2005, milk fat and protein percentages were included as additional traits in the evaluation.

Junior bulls are also ranked accordingly and selected animals are subjected to growth performance, after which selected bulls are sent to semen laboratory for testing of semen quality. Top ranking selected bulls are then included for semen collection and processing and then into progeny testing. Progeny tested bulls become sires of future generation animals.

*Cryobanking of animal genetic resources:* Genetic materials in the form of frozen semen, embryos, DNA and tissues are also collected from distinctly different breed groups and lines as well as from outstanding animals in the gene pools and are cyropreserved and stored in the gene bank.

Included in the gene bank are samples collected from livestock species such as indigenous cattle, goat, sheep and the Tamaraw (2n=46), an animal within the buffalo family that is classified as an endangered species and is found only in the Philippines.
EXPANDING USAGE OF SUPERIOR GERMLASM

The utility of superior genetics obtained from the sustained selection and testing efforts is expanded by using females as dams of future sires while proven sires are used for AI. Outstanding sires tested and selected from the gene pool have been fully harnessed as semen donors in order to cover as many native swamp buffaloes as possible to effect the desired genetic improvement. Component activities/strategies on how to expand usage of superior genetics are as follows:

Semen Processing Laboratory: In support of genetic improvement efforts, the country established semen processing facilities as early as 1984, and to date, such facility houses 100 semen donors. Frozen semen from progeny-tested bulls are produced at a quantity more than sufficient to meet the national requirements, including the needs of the technicians of all local government units (LGUs) and nongovernment associations (NGOs) as well as private AI technicians.

Intensified Artificial Insemination and Bull Loan Program: In cooperation with the Local Government Units (LGUs), crossbreeding of swamp buffaloes with the dairy breed to improve the genetic potentials for milk is carried out nationwide. This system has current annual AI service coverage of about 100,000 head with planned expansion up to 250,000 in 2016. As a way of government subsidy to the genetic improvement program, frozen buffalo semen are provided free of charge up until now. However, as the scheme to privatize the AI services is gaining acceptance, frozen semen are provided to private AI technicians at cost. Provision of liquid nitrogen to preserve quality of frozen semen is at the shared account of national and local governments, but will likely be provided at a later stage at cost to private technicians who, in turn, charge for their services at a reasonable rate.

In the past two years, efforts were directed at privatizing the AI services by developing village-based private technicians in order to augment the limited technicians of the national government agencies and the local government units. Based on the data so far, these private AI technicians are more cost-efficient and more responsive in many respects compared to LGU technicians. Their main advantage is their constant presence in the village service area and their "pay-per-service" system that releases the government from costly subsidies in the form of salaries and allowances. Under a condition where animal ownership per farmer is only 1 to 3, and households are scattered widely in the rural communities, it appears that harnessing village-based AI technicians offers many advantages. In communities where advanced stage of privatization has been achieved, AI technicians are also trained as para-vets and they are also organized. As a group they source their AI supplies, including liquid nitrogen (LN2) and frozen semen at cost.

The AI is augmented with the bull loan program, which is undertaken in villages where AI services are not available. In fact, even in some areas where AI services are accessible, many farmers have high preference for natural mating, owing to very good success rate in this method compared to AI. A system of incentive is offered to farmers tending breeding bulls in the village wherein full ownership of the bull is awarded once the bull has sired at least 50 calves. Many farmers get their bull ownership in just a period of 2 years. In service areas of both AI and bull loan, the important consideration is to avoid inter-se matings of crossbreds. This is achieved by a programmed castrations of all crossbred males in
the field and ensuring that semen for AI and bulls for natural service are of purebred riverine buffalo genetics.

**SUPPORT TO ESTABLISHMENT OF BUFFALO-BASED ENTERPRISES**

Two approaches are being introduced in areas considered to be impact zones for the project. These areas are considered as such in view of the density of breeder stocks in the community likewise being a major consideration. In these communities, massive AI services are carried out with the intent of producing critical number of crossbreds all of the crossbreds and backcross males are for meat or for draft and females are retained as potential dairy animals. While this activity covers many animal raisers, the process is relatively slow owing to the long gestation of buffaloes and their late maturity. As a way of "shortcutting" the process, incubator modules composed of purebred buffaloes are introduced in the impact areas. These modules serve as show window for the farmers to see and appreciate the benefits of rearing the correct animal and adopting the proper management and breeding practices. In the impact areas, carabao raisers have been organized into cooperatives to collect, process and market milk and milk products in a systematic manner. Support to these cooperatives takes the form of organizational as well as technical trainings and the provision of post-production equipment, mostly to preserve the quality of the milk and assistance to market access.

In the National Impact Zone (Nueva Ecija), primary cooperatives involving thousands of families have formed into the Nueva Ecija Federation of Dairy Carabao Cooperatives (NEFEDCCO) to supply milk and dairy products to major urban markets. Throughout the country there are 13 regional impact zones.

**CONCLUSIONS**

There are compelling social and economic reasons for the decision to pursue wide-scale crossbreeding and continuous backcrossing of swamp buffaloes with the riverine buffaloes in countries such as China and the Philippines. While there were apprehensions about the technical feasibility of carrying out such wide-scale efforts, first because of the differences in the chromosome numbers of these two buffalo types, and second, by the initial data about chromosomal behavior suggesting some potential reproductive abnormalities, performance of both male and female crossbreds and their backcrosses obtained in the field have shown otherwise. What has been avoided so far is the inter-se mating of F1 (2n = 49) as there are resulting F2 offspring with undesirable phenotypic performance, more practically noted on F2s with 2n = 48. As a measure in the Philippines, crossbreds and backcrosses males are readily castrated and are destined for draft or for meat purposes.

Artificial insemination takes a major role in the genetic transformation of swamp buffaloes. The assignment of purebred riverine bulls in impact areas without AI services with the corresponding program to castrate the non-purebred males have guaranteed sustained backcrossing, generation after generation.

For wide-scale crossbreeding and backcrossing program to succeed, the mechanism needed for its implementation has to be institutionalized primarily because of the length of the required period, at least 15 to 20 years to achieve results of 3 to 4 generations of backcrossing. The establishment of Guangzi Buffalo
Research Institute in China and the Philippine Carabao Center in the Philippines are examples of institutional instrument needed to ensure sustained efforts throughout.

In the final analysis, the results of this genetic transformation of swamp buffaloes will find more meaning if the "new animals" designed to produce more milk and meat are fully utilized to benefit millions of farming families. The system should also recognize the requisites for "businessizing" the smallholders, rising them from subsistence way of husbandry to the level of entrepreneurship.

REFERENCES


Table 1. China & SEA GDP/Capita, agriculture as % of economy, % of labor in agriculture, buffalo population, and % change in buffalo population (1960 vs 2010).

<table>
<thead>
<tr>
<th>Country</th>
<th>Human Population</th>
<th>Agri as % of Economy (%)</th>
<th>GDP/Capita ($)</th>
<th>% of Labor in Agri (%)</th>
<th>Buffalo Population 2011 (hd)</th>
<th>% Change in Buffalo Population (1960 vs 2010)</th>
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<td>Myanmar</td>
<td>48,724,000</td>
<td>43.0</td>
<td>1,300</td>
<td>70.0</td>
<td>2.97</td>
<td>+174.2</td>
</tr>
<tr>
<td>Philippines</td>
<td>92,337,852</td>
<td>12.3</td>
<td>4,263</td>
<td>52.0</td>
<td>3.07</td>
<td>-5.2</td>
</tr>
<tr>
<td>Taiwan</td>
<td>23,315,822</td>
<td>1.6</td>
<td>21,592</td>
<td>5.2</td>
<td>0</td>
<td>-99.9</td>
</tr>
<tr>
<td>Thailand</td>
<td>65,926,261</td>
<td>8.6</td>
<td>5,382</td>
<td>49.0</td>
<td>1.69</td>
<td>-67.3</td>
</tr>
<tr>
<td>Vietnam</td>
<td>88,780,000</td>
<td>20.0</td>
<td>3,545</td>
<td>53.9</td>
<td>2.71</td>
<td>+27.7</td>
</tr>
</tbody>
</table>

Table 2. Declining swamp buffalo population in selected SEA countries, 1960-2010.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Indonesia</td>
<td>2,893,280</td>
<td>2,885,000</td>
<td>2,457,000</td>
<td>3,335,080</td>
<td>2,405,280</td>
<td>2,005,000</td>
</tr>
<tr>
<td>Malaysia</td>
<td>345,151</td>
<td>310,402</td>
<td>285,339</td>
<td>205,163</td>
<td>142,042</td>
<td>130,090</td>
</tr>
<tr>
<td>Philippines</td>
<td>3,452,000</td>
<td>4,431,500</td>
<td>2,870,270</td>
<td>2,764,950</td>
<td>3,024,400</td>
<td>3,270,400</td>
</tr>
<tr>
<td>Thailand</td>
<td>4,963,580</td>
<td>5,734,500</td>
<td>5,650,790</td>
<td>5,094,270</td>
<td>1,711,570</td>
<td>1,622,650</td>
</tr>
</tbody>
</table>

Table 3. Recorded transport of riverine frozen semen to South East Asia.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Origin</th>
<th>Breed</th>
<th># Straw/dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Philippines</td>
<td>1981</td>
<td>Pakistan</td>
<td>Nili-Rav</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>1983</td>
<td>Pakistan</td>
<td>Nili-Rav</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>1987</td>
<td>Pakistan</td>
<td>Nili-Rav</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>1982</td>
<td>India</td>
<td>Murrah</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>1984</td>
<td>India</td>
<td>Murrah</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>India</td>
<td>Murrah</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Italy</td>
<td>Italian Mediterranean</td>
<td>5000</td>
</tr>
<tr>
<td>Thailand</td>
<td>1979</td>
<td>India</td>
<td>Murrah</td>
<td>1000</td>
</tr>
<tr>
<td>Country</td>
<td>Year</td>
<td>Source of Genetic Material</td>
<td>Breed of Buffalo</td>
<td>No. (hd)</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
<td>----------------------------</td>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>male</td>
</tr>
<tr>
<td>Live Animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Philippines</td>
<td>1917</td>
<td>India</td>
<td>Murrah</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>1918</td>
<td>India</td>
<td>Nili-Rav</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>1947</td>
<td>India</td>
<td>Murrah</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>1947</td>
<td>India</td>
<td>Murrah</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1950</td>
<td>India</td>
<td>Murrah</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1953</td>
<td>India</td>
<td>Murrah</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1955</td>
<td>India</td>
<td>Murrah</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1956</td>
<td>India</td>
<td>Murrah</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>USA</td>
<td>Am-Murrah</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>1995</td>
<td>Bulgaria</td>
<td>Bul-Murrah</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>Bulgaria</td>
<td>Bul-Murrah</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>1998</td>
<td>Bulgaria</td>
<td>Bul-Murrah</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>Bulgaria</td>
<td>Bul-Murrah</td>
<td>670</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>Brazil</td>
<td>Murrah</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Italy</td>
<td>Italian Mediterranean</td>
<td>1025</td>
</tr>
<tr>
<td>China</td>
<td>1957</td>
<td>India</td>
<td>Murrah</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>1974</td>
<td>Pakistan</td>
<td>Nili-Rav</td>
<td>50</td>
</tr>
<tr>
<td>Thailand</td>
<td>1962</td>
<td>India</td>
<td>Murrah</td>
<td>Various</td>
</tr>
<tr>
<td></td>
<td>1979</td>
<td>India</td>
<td>Murrah</td>
<td>100</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Late 1970</td>
<td>India</td>
<td>Murrah</td>
<td></td>
</tr>
<tr>
<td>Taiwan</td>
<td>1957</td>
<td>Philippines</td>
<td>Murrah</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 5. Summary of international collaborations related to transformation of swamp buffaloes from draft to milk and meat in the Philippines.

<table>
<thead>
<tr>
<th>Theme</th>
<th>Institution/Entity</th>
<th>Date</th>
<th>Area of Collaboration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetics</td>
<td>Govt of India</td>
<td>1917 to 1956</td>
<td>Live Animals, Murrah (854 hd at various years)</td>
</tr>
<tr>
<td></td>
<td>Govt of Pakistan</td>
<td>1981 to 1987</td>
<td>Frozen Semen of Nili Ravi (3000 doses)</td>
</tr>
<tr>
<td></td>
<td>FAO-UNDP</td>
<td>1982 to 1992</td>
<td>Research on Crossbreeding between swamp x riverine</td>
</tr>
<tr>
<td></td>
<td>FAO</td>
<td>1994</td>
<td>Risk assessment on importation of live animals from India</td>
</tr>
<tr>
<td></td>
<td>Govt of Bulgaria</td>
<td>1995 to 1999</td>
<td>Live Animal Importation, Bulgarian Murrah (3142 hd various years)</td>
</tr>
<tr>
<td></td>
<td>Govt of Brazil</td>
<td>2010</td>
<td>Live Animal Importation, Murrah Breed (2038 hd)</td>
</tr>
<tr>
<td></td>
<td>Govt of Italy</td>
<td>2013</td>
<td>Live Animal Imporation. (1200 hd, Mediterranean breed; 4000 straw of frozen semen)</td>
</tr>
<tr>
<td>System Development and Utilization of Genetics</td>
<td>Japanese Govt thru JICA</td>
<td>2000 to 2005</td>
<td>Genetic Improvement, AI improvement, Semen Processing</td>
</tr>
<tr>
<td></td>
<td>Australian Govt thru ACIAR</td>
<td>1999 to 2004</td>
<td>Genetic Improvement focused on Animal ID, recording system and data analysis</td>
</tr>
<tr>
<td></td>
<td>Korean Govt thru KOICA</td>
<td>2010 to 2012</td>
<td>Cryobanking of AnGR, DNA based biotechnology, Semen Processing</td>
</tr>
<tr>
<td></td>
<td>US Govt thru its PL480 Program</td>
<td>2010 to 2013</td>
<td>Research and Development, Biotechnology Laboratories, Human Resource Development</td>
</tr>
<tr>
<td></td>
<td>Taiwan Livestock Research Institute</td>
<td>2008 to date</td>
<td>DNA-based technologies, Screening of Genetic Defects, Cryobanking</td>
</tr>
<tr>
<td></td>
<td>USDA</td>
<td>2012</td>
<td>Human Resource Development focused on Cryobanking of AnGR</td>
</tr>
<tr>
<td></td>
<td>International Buffalo Genome Consortium</td>
<td>2011 to date</td>
<td>DNA-based MAS, Buffalo Genome</td>
</tr>
<tr>
<td>Enterprise Development</td>
<td>Japanese Govt thru it 2KR fund</td>
<td>2010 to 2013</td>
<td>Dairy Product Processing, Establishment of Milk Collection Scheme for smallholders</td>
</tr>
<tr>
<td></td>
<td>Korean Govt thru KOICA and KAPE</td>
<td>2010 to date</td>
<td>Product Development, Product Standard, Product Traceability</td>
</tr>
</tbody>
</table>
Buffalo Livestock and Products in Europe

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General Secretary International Buffalo Federation, Coordinator FAO-ESCORENA Buffalo Network, Monterotondo, Rome, Italy
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ABSTRACT
Buffalo livestock and strategies are reported for all the countries in Europe, where buffalo specie is reared and used for food production, as Italy, Romania, Bulgaria, Germany, Macedonia, United Kingdom, Greece, Serbia, Albania, Ukraine, Hungary. Particularly Italy situation is discussed as in this country selection, milk recording and production, management, nutrition and reproduction techniques, quality food and marketing are really developed at top level. In Italy 50,000 buffaloes are recorded every month during the lactation, showing a milk yield of 2,220 kg in 270 days of lactation with 8.4% fat and 4.6% protein, as many champions produce more than 5,000 kg for lactation. Artificial insemination is largely applied. The National Association of Italian Buffalo breeders was instituted on 1979, the Buffalo Genealogical Book for the Mediterranean Italian Buffalo Breed on 1980, the famous mozzarella cheese obtained the Denomination Origin Protected on 1993, with Decree of Agricultural Ministry, approved by European Union, where milk and mozzarella characteristics are defined for consumer guarantee. In Italy 400,000 buffaloes are managed and fed in intensive system: the females are kept loose in paddock and mechanically milked twice a day; the males are managed in feed-lots or on slatted floor stables for fattening and are slaughtered at 15 months, achieving more than 400 kg live weight. Even if many products are appreciated coming from milk, as ricotta, provola, scamorza, treccia and other cheeses, or coming from meat, as steaks, roast, ham, bresaola, salami, the most important product in the Italian and international market is mozzarella, of which 36,000 tons are produced every year, with a value of 500 million euros. The consumption is 82% in Italy, 18% for export, particularly for Germany, France, USA, UK.

Keywords: buffalo livestock, management, Mediterranean Italian breed, products

INTRODUCTION
The buffalo population and economy in Europe shows two different trends: an increasing trend in Italy, where population, genetics, technologies, high quality products from both milk and meat lines and market are in progressing, and a decreasing trend in the most of other countries, where a draught animal disappeared as mechanization was introduced, without a selection of a dairy buffalo breed. Other countries more, where in the past buffalo species was not known, introduced dairy buffaloes, particularly from Italy, to create a new buffalo market, as U.K. and Germany.

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1. Italy

1.1. Selection, milk recording and production

Buffalo Genealogical Book was instituted by Italian Ministerial Decree on June, 23, 1980 and was held by A.I.A.(Italian Association of Breeders). The A.N.A.S.B. (National Association of Buffalo Species Breeders) had been instituted on 1979 and recognized on the 1994 by Agricultural Ministry. The Ministry, with Ministerial Decree n° 20154 has entrusted the management of the Book to A.N.A.S.B. on year 2000. In the same year other decree (D.M. 201992 on July 5, 2000) recognized buffalo enrolled in Genealogical Book pertaining to the only own race: “Mediterranean Italian” (Figure 1).

The animals rose in Campania and in Lazio Regions of Italy give the milk used for the production of the famous “Mozzarella di Bufala Campana D.O.P.” (Figure 2). The D.O.P. (Denomination Origin Protected) Mozzarella di Bufala Campana was recognized with the Ministerial Decree on May 10, 1993, published on the G.U. n.219 on 17/9/1993, and after from European Union; that means that this cheese mozzarella has to be produced in defined areas of the Provinces of Caserta, Salerno, Benevento, Napoli, Frosinone, Latina, Rome,(Foggia was added after), coming only from fresh milk of buffalo cows of Mediterranean Italian breed, registered in the Buffalo Genealogical Book. The Decree establishes the milk characteristics (fresh within 16 hours from milking, raw, minimum fat 7%), processing techniques (acidification, coagulation, stretching, moulding) and mozzarella characteristics. The logo is represented in Figure 3. The control and guardianship is effected by “Consorzio per la tutela del formaggio Mozzarella di Bufala Campana”, so the European consumer is guarantee that the logo means a quality product of the made in Italy, according the best standard of animal management, welfare and health, according also the best characteristics of mozzarella, as sanity, freshness, flavour and juiciness.

The main factors that have contributed to the development of buffalo in Italy in the past few years have been the following: no regimen of milk quotas; increase of the consumption of mozzarella in Italy and export in many countries in the world; the high price of buffalo milk (about 1.20 euro/ kg) in comparison with cattle milk (about 0.40 euro/kg), the high technology of farmers, high level of management and breeding, high genetic value of the herd, obtained by performance and progeny testing, animal recording and selection, artificial insemination applying, starting with an organization born more than 50 years ago.

Several cycles of progeny tests, with the publication on 1997 in the first time, of the genetic indexes of breeders of buffalo species, both males and females, are effected by ANASB. The execution of the milk recording in buffalo is applied according ICAR (International Committee for Animal Recording, Moioli, 2005), according to the Regulations of the Buffalo Species and to the norms emanated in the Central Technical Committee of the milk recording of the bovines and the buffaloes. For being able to be subordinate to the control, as a result of completely voluntary adhesion, the farms must fulfill to some prescriptions: they must be subordinate to the inspection from an expert of race of A.N.A.S.B.; they must possess the sanitary certificate from National Sanitary System that attests its indemnity and must have the bulls with genealogical certificate in order to admit
they to service.

For Mediterranean Italian Buffalo, the productive controls regard: the quantity of milk in kg, the determination of the percentage of fat and of proteins (kg and %) and the somatic cells. The beginning of the official lactation starts at calving, the first control cannot be carried before the five days from calving and not beyond 75 days. The duration of the reference lactation is 270 days, in any case the duration of the effective lactation must be indicated. Every milk control must be made on all the milking ordinarily practiced by the breeder in the 24 hours, annotating also the hour in which the same control is carried out, the quantity of milk found must be indicated in kilograms, the milk must be weighed with the balance or be determined with lactometers.

For every subject are reported the following data: number of current lactation, daily production expressed in kg milk, % fat, % proteins and number of somatic cells, the effective production from the calving for: kg milk, kg fat and proteins and the daily medium production, the milk production in comparison to the reference lactation of 270 days, “equivalente bufala matura” (E.B.M.) expressed in kg of milk, fat and proteins (Coletta and Caso, 2008). E.B.M is a hypothetical production for a buffalo that started his lactation in January at five years old; the productive ability is the ratio between E.B.M. of the single buffalo and the mean I.B.M. of the farm. All data are collected by ANASB, which decides on the selection goals which are presently to increase not only the milk quantities but specifically the mozzarella cheese production according to the mozzarella index:

\[
\text{Mozzarella (kg)} = \text{Milk (kg)} \times (3.5 \times \% \text{ proteins} + 1.23 \times \% \text{ fat} – 0.88) / 100
\]

In Italy there are 370,000 buffaloes and the mean milk production is over 2,200 kg for lactation, in confront of other Mediterranean countries where the maximum production is less than 1,900 kg. In Italy the milk production in 46,799 recorded buffaloes (ANASB, 2009) was 2,221 kg (8.24 % fat and 4.66 % protein) in 270 days of lactation (Table 1). Recorded buffaloes are raised in 290 herds with an average of 161.3 head per farm.

In the year 2010 milk production in 270 days of lactation was 2,180 kg with 8.47% fat, 4.59% protein. The recorded buffaloes were 50,240 with an increasing of 7.35% (ANASB, 2011). In other countries the buffalo productivity is lower, due to the fact that only Italy has undertaken a great deal of work on recording, on selection, on reproductive and genetic improvement, on health, on feeding and livestock systems, as is shown in the following analysis. The recorded buffalos on year 2006 were 40,425 with an increase of 1.2% regarding 2005. In Figure 4 is clearly evinced how the buffalo head number increased in the period 1981 – 2006 and this is the basis for the genetic development and the milk capacity improvement. On the basis of 26,462 considered lactations, we found on 2006 an average production of kg 2.178 with fat average of 8.09% and a proteins average of 4.67% (Coletta and Caso, 2008). In the reported period, the average production expressed in kg, has been increased slowly but constantly from 1990 with 1,893 kg for lactation until 2004 with 2,184 kg for lactation, until 2009 with 2,221 kg for lactation.

In the last few years, in Italy, milk composition has been improved: the average protein content has raised from 4.4% in 2002 to 4.6% in 2010 while the average fat
content raised from 7.3% in 2002, to 8.5% in 2010, without operating any selection for the character of protein and fat content. Moreover the possibilities for genetic improvement for milk quantity and quality will be higher, if the selection pressure will be increased reducing the number of bred females, discarding the low productive females. At the present time there are many females in Italy producing more than 5,000 kg milk/270 days of lactation ) until the maximum production of 5,600 kg with 8.32% fat and 4.63% protein.

There are in Italy two Bull Buffalo Centres for semen production: the COFA (Cooperativa Fecondazione Artificiale) in Cremona Province, Lombardia Region, in North of Italy, where there are many bulls with genetic potential to produce more than 4,000 kg milk/ lactation as Malandrino Bull and O-B-One Bull lines. The Chiacchierini Bull Centre in Perugia Province, Umbria Region, in Middle Italy: this one started a genetic selection programme with CIPAB consortium and actually produces semen from 16 tested bulls from different bloodlines, coming from mothers over 3,100 kg milk yield per lactation with more than 4.5% protein: there are Ciripicchio Bull with 4,494 kg milk/lactation and Jesce Sole Bull with 4,157 kg milk/lactation, as recorded in the best daughters, and Brillante, the best bull for pedigree index of 2010-2011 progeny test. This stud is one of the few European A.I. studs authorized as insect-proof quarantine barn, located away from the semen production zone. It is authorized for worldwide export for the excellent sanitary level. Chiacchierini Bull Centre produces sexed semen too, available from a lot of bulls.

Hereafter the selection will be directed at the improvement of the yield of mozzarella cheese, not simply for milk production, since the farm income is based firstly on mozzarella cheese, secondly on the sale of pregnant heifers, lastly on beef sales and finally on the sale of semen and embryos of high genetic value. The largest proportion of the buffalo population can be found in Provinces of Caserta and Salerno (Campania region), and the next localities for size of population are the Provinces of Frosinone and Latina (Lazio region), which are in the Denomination of Protected Origin (D.O.P.) area. The control and monitoring of pathologies is affected by the local veterinary services and by the “Istituto Zooprofilattico Sperimentale” (Animal Prophylaxis Research Institute), one for the Lazio region and another for the Campania region. The hygienic control of the milk production and of the milk products in the industry is of a particularly high standard. Research on the buffalo species is carried out by the Animal Production Research Institute, Monterotondo, Rome, where are the General Secretary of the International Buffalo Federation (IBF) and the FAO Inter-Regional Cooperative research Network on Buffalo that publish the Buffalo Newsletter, and by the Federico II University, Naples.

1.2. Management and nutrition

Italian buffalo management is today exclusively intensive, as in the past it was extensive too with calves milking from cows in the open air (Figure 5) separately or together with milking man by hands. This figure is disappeared, now existing only intensive system: dairy buffaloes are kept loose in paddocks close to the milking room, where the cows are submitted to udder control and mechanically milked twice
a day. The females are normally artificially inseminated in the paddock, using high
genealogy semen, preferably in February-March after oestrus induction, to obtain
calving before spring (about 50% fertility), as the milk is paid more in spring and
summer according the consumer demand. After one month from artificial
insemination the empty females are naturally mated (Figure 6), obtaining another
30% fertility with a total mean fertility rate of 80%.

The buffalo cows are selected too for udder and teat conformation and
adaptability to milking machine (Figure 7). Milk production is sustained by diets
with a high energy (from 0.85 to 0.95 MFU/kg dry matter-DM) and a high protein
concentration (14-16% crude protein on DM), based on maize and other silages,
cereal grains, soya, alfalfa or “graminaceae” hay and by-products. The feeding stuffs
movement and distribution is effected by mixing trucks along the feeding line in
paddock or in feed-lots (Figure 8); the movement and stocking of dung is also
mechanized; therefore there are no smallholders in Italy, but only farmers with an
average herd size of 161.3 head per herd. Heifers are also fed intensively in order to
achieve puberty before 20 months (Borghese et al., 1997, Borghese, 2005). The
heifers are housed loose in paddocks all year long, utilizing the same modern
systems used for dairy cows.

In intensive systems the buffalo cows normally receive unifeed composed of
maize silage, concentrates, hay, straw and sometimes by-products. For example, a
600 kg live weight buffalo cow producing 10 kg milk, would be fed 15.3 kg DM
(33% maize silage, 42% alfalfa hay, 8% maize grain and 17% concentrate with 38 %
proteins,) with 12.7 Milk Feed Units (FU), 2.1 kg crude proteins and 3.5 kg crude
fibre. Maize silage can be highly increased: some rations foresee until 60% maize
silage, 26% concentrates, 14% hay and straw, sometimes by products (tomato peel,
brewer grain residuals, sugar beet pulp) (Borghese, 2010).

Experimental diets (Barile et al., 2010) were carried out with maize or sorghum
silages (16% on DM): in the first diet maize silage was 71.2%, alfalfa hay 9.3,
concentrate 19%; in the second diet sorghum silage was 60.9%, alfalfa hay 10.1%,
concentrate 28.5%. In both groups milk FU/kg were 0.9, crude protein 15.6%, crude
fibre 21.2% on DM. Both diets produced more than 8 kg milk/head/day (8.03-8.78
respectively with maize or sorghum) with 8.74-8.47% fat and 4.98-4.78% protein
respectively during lactation 270 days long.

The calves are normally taken off the mothers, they receive colostrum in the
biberon (particular bottle) and after reconstituted milk, in single cage 1 or 2 months
after birth, to avoid infections and to control the consumption, after in multiple
boxes (Figure 9), where the calves receive milk replacers, starter concentrates and
good hay until the weaning (about 3 months for males, 3-5 months for females). The
males follow the meat line and they are managed in feed-lots or in slatted floor
stables for fattening (Figure 10), as the females are preferably managed in open air,
especially if pasture is available.

1.3. Buffalo food and market

The largest proportion of the buffalo population is localized in Provinces of
Caserta and Salerno (Campania Region), and the next localities for size of
population are the Provinces of Frosinone and Latina (Lazio region), which are in
the Denomination of Protected Origin (D.O.P.) area. The hygienic control of the milk and milk products in the industry is of a particularly high standard. The market is mainly based on mozzarella cheese, very famous one, not only for the local consumption according the traditional Italian cooking style, but also in many foreign countries. There are different types of mozzarella, the best one is produced in D.O.P. area (Figure 12) according the regulations: it is hand made by raw buffalo milk, soft, juicy and tasty, rich of live ferments, natural yeasts and microbes, it is coming from a difficult processing schedule, particularly for stretching phase (Figure 11), it changes taste during time, not preserving in fridge but in mozzarella water and the shelf life is about 5 days. The industrial mozzarella, even if produced in D.O.P. area according the regulations, is made by machines and microbes die during pasteurization, with the advantage of a longer shelf life, preserving in fridge (until more than 2 weeks) but the material is too compact and the taste is hard and anonymous; this product is distributed in supermarket and for export. After that there is a lot of false mozzarella, produced by mixing buffalo and cow milk or out of D.O.P. regulations. The basic price of mozzarella at cheese industry is 10 euro/kg, with a good profit, utilizing 4 litres of milk/kg mozzarella and starting from the milk price as 1.20 €/litre that is more than 3 times the price of cattle milk. The price in the shop increases as more as the quality of mozzarella and the distance from the site of production until 20-30 €/kg. The market is richer in Campania and Lazio Regions, where is easy to find shop with a lot of products coming from milk and meat industry. The mozzarella D.O.P. consumption is about 82% for the Italian market, 18% for the export, particularly for Germany (20% of the export), France (20%), USA (18%), U.K. (12%) (Borghese, 2005, 2010).

In the year 2010, 36000 tons of mozzarella was produced, with an increase of 12.5% respect to the 2009, with a sales volume of € 300 million at the production, € 500 million at the consumption (Borghese, 2011). Another very appreciated product is the ricotta (Figure 13), that is not really cheese because it is produced boiling the serum proteins remaining after the produced curd. After mozzarella market, now meat market is rapidly increasing: now there are some fattening centres for the production of excellent buffalo carcasses. Calve carcasses are appreciated for clear and tender meat (Figure 14) but normally the live weight at slaughter is 400-440 kg obtained at 15-16 months of age with 800-1000 g/d of daily gain, managed on slatted floor (Figure 10) to avoid bad smell of urine and faeces: young bulls without defect or pathologies, beautiful carcasses with conformation R (good), medium fattening (Figure 15) according Italian market requirements are obtained, 52% dressing percentage, 57% net dressing percentage, 62% meat on carcass, meat with low fat (less than 3%), very clear, tender and juicy, with good dietetic qualities: <50 mg cholesterol/100 g, unsaturated fatty acid/saturated fatty acid >1, iron >1.5 mg/100g.

The first quality cuts are well represented with good muscular growth and are sold at 14-25 €/kg for typical restaurants and many products, as bresaola, salami, cacciatorini are sold in typical shop together with buffalo cheese, as meat also obtained I.G.P. (Indication Geographic Protected) “Carne di Bufalo Campana”. Very appreciated and common products are: mozzarella, treccia, scamorza, crescenza, robiola, caciocavallo and other cheeses, ricotta, yogurt; meat and meat industry
products: bresaola, salami, sausages, caciorollo, cacciatorini (little salami) (Figure 16). Finally Italy is a reference point as buffalo importance in human food sustainability for high quality products.

2. Romania

Despite the fact that the origins of buffalo in the area are unclear, it is sure that they were introduced about 1000 years ago - by perhaps the Crusaders or the Islamic invaders. The buffalo found in the north in the Carpathians were, possibly, introduced 500 years earlier by the Avars (SAVE, 2011). But this theory is not real, because buffaloes coming from Mongolian countries have to be Swamp subspecies, on the contrary Romania buffaloes are typical Mediterranean, River subspecies and therefore coming from Near East (Borghese, 2012). Even though the origins are not yet clear, it is possible to see that the buffalo have adapted to their local environments: the Carpathian and Transylvanian types have hard hooves for moving over stones and have a thick winter coat. Wherever the buffalo live, this Riverine type loves to swim.

Various traditional products were made with their milk, meat and skins. Their muscle power was used on the farm as traction. Buffalo were valued for their frugality, longevity and triple-use. The negative side of the buffalo is, perhaps, part of the key to its downfall: the cows often only let down their milk for one person – usually the man of the family - they can also be aggressive and are extremely wilful and stubborn. These factors, along with the increased use in tractors and the promotion of high-yield cows led to the buffalo being replaced. Numbers in the last 20 years have decreased from tens of thousands down to, in some countries, too few to make breeding viable without import of new stock (SAVE, 2011).

The buffalo population in Romania was more than 200,000 head in 1996 (Borghese, 2005). Actually it is about 25,000 head, located particularly in Transylvania, classified as Mediterranean Carpathian breed. The SAVE Foundation (SAVE, 2011) organized an International Workshop on Conservation of Autochthonous Buffalo on 6-7 May 2011 in Sighisoara (Romania), as the real risk for Carpathian buffalo is the extinction. The conservation strategy is based only on subsidies, so the population is rapidly decreasing as family farmers don’t need more animals for draught and carry as in the past. The average milk production, but only in few recorded animals, in 2008 was 1,800 kg per lactation (274 days) with some champion producing until 3290 kg per lactation (Vidu, 2010) with fat percentage moving from 5.2 to 6.2% and protein from 3.5 to 3.9%. The animal recording is at good level (669 recorded animals in 2009) and artificial insemination too with 2041 effected A.I. in 2008 (Vidu, 2010). The mean age at the first calving is 36 months, calving interval is 485 days. Buffaloes are still used today on small private farms for draught and the goal of the selection process is to create a dual-purpose type of animal (milk and meat), realizing good daily gains (600-800 g), in order to slaughter the males at 22 months with 460 kg of live weight. At present the calves are also fattened to be slaughtered at four months (100 kg of live weight).

The animals are housed and tied during the winter due to the unfavourable weather conditions and fed with hay, bran, concentrates, silage, grazing on pasture in the warm season (Figure 17, 18, 19). As Romania buffaloes can have these
performances, but only if correctly managed, they need soon a project to increase rapidly the milk production capacity in all the population, even applying artificial insemination with Italian semen, to create a milk-cheese market and justify a buffalo economy in the country. Subsidies are not a long-term, sustainable solution for conservation of the species, one or two animals for family. Policy changes can lead to immediate decrease in numbers as subsidies are cut or the focus of them merely changes. Connecting with nature conservation in protected areas by grazing provides a cost-effective eco-management system whereby buffalo can obtain a monetary value without requiring a commercial activity. This, in turn, can be coupled with agri-tourism activities, use within extensive production systems and linked to local traditions and ethnic minorities.

In many of the countries concerned, availability of land and land ownership has been negatively affected by historical processes. Land has changed hands, been collectivised or, in some places, national borders have changed. Often it is impossible to find out who really owns a piece of land, this is compounded by the fact that many traditional farms are so small-scale that, to buy a land parcel large enough for a commercially viable buffalo herd would mean tracking down many previous owners and their relations in order to negotiate a purchase (SAVE, 2011).

Markets for buffalo products (dairy products and meat) exist but could be improved upon. Common dairy products are yogurt, butter, soft cheese and typical cheese as Vladeasa and Braila (Borghese, 2005).

3. Bulgaria

The mechanization entered in the agriculture and led to a decrease in the buffalo population. For this reason it was necessary to change the genetics of the native buffalo from draft type to milk type. 20 pregnant buffalo cows and 10 bulls from the Indian Murrah breed from India, 50 buffalo cows and 4 bulls from Nili Ravi from Pakistan were imported in Bulgaria in 1962 and 1974 respectively. This activity was affected systematically under the scientific management of the Buffalo Research Institute in Shumen and the National Animal Selection Centre (Alexiev, 1998). This was the beginning of creating a new milk breed, the Bulgarian Murrah (Figure 20, 21) with genetic potential 2,000 kg milk yield, 7.5% fat content and 550-600 kg body weight of adult buffalo cows.

For the purpose of optimizing genetic improvement of the buffalo population in respect of milk ability, a selection program based on genetic level of the population and on the artificial insemination was developed by Alexiev (1979) and improved by Peeva (2000). In the program an estimation of the most important traits was made, as well as of fixed and variable parameters. A model, including four pathways of genetic transmission and inbreeding depression was developed (Alexiev et al., 1991). According to the programs of the authors an annual genetic gain of 1.06% and 1.89% for milk yield was realized, respectively. The number of buffaloes in Bulgaria at 01.11.2008 was 8968, including 5,153 dairy buffaloes (Peeva, 2009). On the private farms, where the population is concentrated the reduction is 36.0%, whereas on the state and cooperative farms this reduction is 98.8% compare to 1990. The interest in buffaloes increased during the last years which led to building buffalo farms with capacity from 10 to 100 dairy buffalo cows. From the total number of the
buffaloes, 43.8% are rearing in farms with capacity up to 20 buffaloes and 56.3% with more than 20 (Peeva, 2009).

As a result of the crossing up to now the buffalo population is more than 80% of Bulgarian Murrah breed. As the results of the crossing of Bulgarian Local buffalo with Murrah breed during the last decades came substantial transformations on its type and body conformation. The cows from Bulgarian Murrah breed are characterize as animals with deep and wide thorax and body compact in comparison with Bulgarian buffalo cows. Another evidence of the presence of genetic capacity for high milk newly population is the fact that many buffalo cows have a milk yield above 2500-3000 kg and some of them – more than 4500 kg. The champion of the population is crossbred F2 from which is obtain 5349 kg with 6.64 % fat for 305 days (Peeva, 2009).

During 2009 from Italy was imported semen of Mediterranean buffaloes to cross with Bulgarian Murrah. The main purpose of the crossing is to increase genetic diversity in buffalo population, to decrease inbreeding and improve the body conformation of the animals. The mean values of the lifetime traits in the Bulgarian Murrah are follow: productive life is 1451.18, which is equal to 3 years and 4 months (Ilieva and Peeva, 2007), longevity is 2,646 days (7 year, 3 months) as for culled cows at first, second and third lactation is respectively 1254, 1708, 2435 days (Peeva and Ilieva, 2007); lifetime milk yield – 5,851 kg; milk yield per day of lifetime time – 1.97 kg; lifetime lactation period – 858 days; milk yield per day of lactation period – 6.64 kg; lifetime lactations number – 3.67; lifetime calving interval – 1288 days; milk yield per day of productive life – 4.43 kg (Peeva, 2009).

The percentage of culled cows for low productivity is 19%, including buffalo cows having milk yield below 200 kg per lactation of 120 days; the prolapses takes 11% of the total culled cows, including vaginal and uterine prolapses; the proportion of culled cows with short lactation is 7%; the culled cows for old age (over 8 lactations) are 9% of total: this shows that the longevity of buffaloes is longer than in cattle (Peeva and Ilieva, 2007).

Main source for meat are male calves. Investigations regarding the fattening abilities of buffalo calves show considerable differences by comparison with beef calves (Dimov and Peeva, 1994). The average daily gain of buffalo calves is between 650 and 1083 g. The most effective slaughter body weight is 400 kg (Dimov and Peeva, 1994). The buffalo has lower dressing percentage compare to cattle. For suckling calves it is about 56%; to 4 months of age it is 59.4%; from 6 to 12 months it is 45%; from 12 to 18 months it is 47% and over 24 months 45.3% (Peeva, 2009). The average age at first calving is within the range from 32 to 40 months on different farms in the country and different breeds (Peeva, 2000). The calving interval range from 436 to 505 days according breed, nutrition and management of the farms (Peeva, 2000). Buffaloes were raised on the State farms, kept tied in closed sheds, machine milked and fed maize silage, alfalfa or grass hay, straw and concentrates. The animals were manage in separate groups according to physiological conditions: suckling calves, females four to twelve months, heifers, pregnant heifers, dry cows and milking cows.

After the changes in the political and social-economic system in 1989, buffaloes were transferred to the new private farms, where scientific and genetic
activities were limited and the animal numbers have drastically declined. Actually, there are only 9,200 head, of which 5,880 are cows of Bulgarian Murrah in Bulgaria (Borghese, 2011). These animals are submitted for milk recording and to artificial insemination. Milk recording, selection, artificial insemination and progeny testing are coordinated by the Buffalo Research Institute in Shumen. Products: White brine cheese, typical yoghurt, salami, sausages, Pastarma. The most of the market is link to the typical buffalo yogurt, very appreciated and to meat byproducts.

4. Germany

In Germany there are now 2111 buffaloes, in 14 different Regions, but particularly in Sachsen (434), in Baden-Wurttemberg (389) and in Brandenburg (287) (Thiele, 2009; Borghese, 2010). The Germany is an example of adaptation capacity of buffaloes to cold climates; they can stay on the snow without problem (Figure 22). Normally the animals are managed in the stables during winter and on the pasture during spring and summer (Figure 23). The population started with 625 head imported by Italy and Bulgaria in 2001, showing now typical characteristics of Mediterranean or European breed, much more similar to Balkan type (Figure 24, 25) than to Bulgarian Murrah. The population had a quick increasing linked to a rich market of high quality products, coming from milk and meat processing as mozzarella and other cheeses, cream, yogurt, sausages, meat boxes, and also beauty products (Figure 26).

Germany is another example of a new and rich market, invented by the buffalo farmers. All buffalos are recorded in the German livestock controlling system. According the report related to the 2 biggest buffalo herds of Sachsen (Saxony), Dr. Golze reported the level for traits of fertility, milk and growth performance relating to the years 2004/2005 (Guglielmetti, 2007): weight at birth was found to be 44.7 kg for male buffalo calves and 39.5 kg for female buffalo calves respectively. Weight at 3 months was 147.0 kg for male calves and 132.4 kg for female calves. Weight at 9 months was 351.2 kg for male calves and 305.7 kg for female calves. Males were used for breeding from the age of two years (Guglielmetti, 2007).

Buffalo heifers were first mated at the age of 18 to 24 months. Age at first calving was 35 months on average. Calving interval was on average 633.5 days. The buffalo bulls were commonly slaughtered at a weight between 540 and 760 kg (Guglielmetti, 2007). Regarding milk performance Dr. Golze reported (Guglielmetti, 2007) that in the Chursdorf herd over a 305-day lactation period, milk yield was on average 2,232 kg in the first lactation and 2,577 kg in the second lactation. Fat yield was 193.7 kg in the first lactation and 237.7 kg in the second lactation. Protein yield was on average 101.0 kg and 123.7 kg for the first and second lactation respectively. There was found a big variation for these traits.

A recent study (Guglielmetti and Golze, 2009), conducted by the Saxon Regional Office for Environment, Agriculture and Geology, required male and female young buffalos to be slaughtered at an age of 647 days (561 to 757 days, n = 12). Weight at slaughter was 549 kg on average. The dressing percentage was 56.7%, whereas the weight of the two halves was 307.1 kg. The percentage of valuable parts was found to be 62.5% on average. The meat taken off the M.
longissimus dorsi contained 21.4% raw protein, 2.5% raw fat, 75.0% water and 1.1% ash. At 48 hours after slaughter pH was at 5.5. The drip loss was 3.5%, loss after grilling 32.6% and loss after cooking 47.3%. Shrinkage on chilling was 3.6% after 14 days and 4.2% after 21 days. According to Minolta CR300 the meat colour was 33.3. Tenderness was measured: 5.2 kg at 48 hours post mortem, 3.4 kg after 14 days and 2.8 kg after 21 days.

5. Macedonia

The buffalo farms in Macedonia are very few, 4 or 5 at all perhaps, and the total population is very reduced, probably 175 animals (SAVE, 2011), but nobody from many people knows exactly the reality; there is a farm in Debreshte village (near Ropotovo), with some local dairy cows and 12 buffaloes of Mediterranean breed, but small and compact, that were bred on natural pasture (Figure 27, 28). The first problem of the farmer is that they have no male and very high consanguinity. The proposed solution is the introduction of artificial insemination by Italian semen to increase the milk production, actually very low, and to introduce different and better genetic basis (Borghese, 2010).

In Mojanci village (near Kocani), there is a family farm with 8 buffaloes in the farmyard close their house, of the same Mediterranean breed (Figure 29), but the animals were bigger than in the previous farm. The farmer produces simple cheese that is sold in local market (Figure 30). The reality is that a programme to save and develop buffaloes in Macedonia is a priority to maintain biodiversity, to conserve buffalo genetic, that was introduced 5 centuries ago with Turkish invasion, to develop animal farms and typical products for local market and as a basis for tourist economy. The project presented by the Animal Science Institute to the Agricultural Ministry, will be carried out with the cooperation of Italy (Borghese, 2011).

6. United Kingdom

There are a maximum of 2500 breeding females in the UK and probably a maximum of 1,200 milking animals and maybe less (Borghese, 2010).

As for milk yields, the average is 1,500 kg per year. This was due to poor quality buffalo imported from Romania and also some mis-information through ignorance of the nutrition of buffalo. An imported way of bulls too have been from the North of Romania. The best animals are without doubt those that show signs of Bulgarian Murrah ancestry, and indeed the sires of over half of foundation animals were Bulgarian (from AI). Lactation length is about 300 days in the best (with perseverance) but considerably less in some, particularly in the first lactation. Calving interval is usually a bit over 365 days (Wood, 2009).

7. Greece

In Greece, due to the rapidly changing socio-economic conditions, including the mechanisation of the agricultural sector and the substitution of buffalo milk by milk produced by imported dairy cattle, the number of buffaloes has declined dramatically over the last decades. As a result, from the 75,000 animals counted at the end of the 50s, today only few head remain. There are 2,503 buffaloes in Greece (Ligda, 2009). Currently the population has increased, reaching 3,137 animals by the
end of 2010 (SAVE, 2011) with 28 herds distributed in 21 localities in western, central and northern Greece; most animals are found in the area of Lake Kerkini National Park where they are considered an integral part of the protected area. All herds are monitored by the Centre for Livestock Genetic Improvement (CLGI) in the framework for the conservation of genetic resources in the livestock breeding sector run by the Ministry of Rural Development and Food. Herd books and records are kept by CLGI in collaboration with the Association of Buffalo Breeders of Greece (Kazoglou et al., 2011).

Lactation length varies from 210 to 280 days with an average lactation milk of 700-1,000 kg while the age at first calving is 36-48 months (Borghese, 2010). The age at slaughter for young stock is 15-17 months and the weight at slaughter is 350-400 kg. The cows are milked twice a day at the farm by hand. Buffaloes are not used for draught, but only for milk and meat production. The dairy products obtained from buffaloes are yogurt, white cheese in brain, butter, kaimaki and cream. Each farmer has his own bulls available for natural service in fields and used in proportion 1 to 8-15 cows. Artificial insemination is not applied. Efforts for buffalo production are made by researchers of Greek Focal Point for the Preservation and Conservation of the Animal Genetic Resources, at the Aristotle University of Thessaloniki, with the support of the Ministry of Agriculture to rehabilitate buffalo production and to let buffalo farming, at present under protection, to become an economic viable activity. Really the buffalo market is much reduced.

8. Serbia

There are 1200 buffaloes in Serbia (SAVE, 2011) of Mediterranean breed, Balkan type (Figure 31, 32). They are kept in extensive low input production system (Figure 31, 32). The number of buffaloes and trend of population is stable, in the last ten years. Activity on conservation is supported by the Ministry of Agriculture, through the subsidies and program for crossbreeding in pure breed (Stojanovic, 2011). Buffaloes could have an important role for high quality food (production of health and safety food, organic production, particularly appreciated are local fresh cheeses, Figure 33), ecological production and integral development of rural areas, anticipating combination of agriculture and rural tourism (Stojanovic, 2011).

9. Albania

Albanian buffalo is an autochthonous breed, classified in the group of Mediterranean breed. The mantel is black or dark grey and rarely with white spots. Horns arched back and side inward bent. Buffalo population is 321 Albania head (SAVE, 2011). They have been used mainly as draught power; however Albanian farmers and consumers have been interested for their milk and meat products: milk yield is around 450-600 kg in the first lactation going up to 850-980 kg in the third one; its fat and protein content are respectively 3-10.2% and 5.3-6.8% (Papa and Kume, 2011). Fertility rate is round 80-85% and days open 120-150 days. During 2010 more than 21 farmers, in four regions of Albania, have received subsidies for buffaloes: as result the size of buffalo population is increasing; the breeding nucleus in four farms have been established. Until now 13 male lines are selected. Nevertheless there is the need to control inbreeding level and provide breeders for an
exchange of the breeding stock (Papa and Kume).

10. Ukraine
There are 115 buffaloes in Ukraine (SAVE, 2011). The percent of crossbreds with Murrah is very high. The Mediterranean Carpathian breed is present too. Some animals were imported by Armenia on 1984. The Ukrainian buffalo population has the real risk of extinction. In 2004 private initiatives collected rare and unique animals for tourist attraction: one part stays spread over Western Ukraine and the other is located in Kiev region (Jacobi, 2011). The “Saving of Agro-biodiversity of Carpathian mountains” is working to stop decline of buffalo population, to create nucleus herd farms, to support contact among the owners, to regulate problem of inbreeding, to search private people who are ready to create small buffalo farms and village communities working in cooperation production (Jacobi, 2011). As buffalo cannot survive as zoo animal only for tourist attraction, but need to become milk and meat producer.

11. Hungary
A small but tenacious population is living in Hungary for many years (Cockrill, 1974). They were introduced by the Turks in 16th century and the population is named Carpathian or Mediterranean Hungarian Breed (Figure 34, 35). There has occurred a decline of population due to loss in economic value of working buffaloes (Karpati, 1997). According SAVE (2011) there are 200 buffalo head in Hungary. According direct information by the Ministry of Agriculture and Rural Development, there are 2000 buffaloes, most of them living in National Parks, as gene reserves. Now, according a new project, some new modern farm are going to be created, introducing Mediterranean Italian head of high genetic value from Italy, with the purpose to originate a market of milk and meat quality products.

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Table 1. Italian buffaloes (ANASB 2009).

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<td>N° Recorded buffaloes</td>
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<td>% Protein</td>
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Figure 1. Mediterranean Italian breed, Tor Mancina farm, Rome (Borghese photo, 2006).

Figure 2. Mozzarella and the colours of Italian flag: green, white, red.
Figure 3. Logo of Mozzarella di Bufala Campana DOP.

![Figure 3](image3.png)

Figure 4. Recorded buffaloes in the years 1981-2006 (Coletta and Caso, 2008).

![Figure 4](image4.png)

Figure 5. Extensive system: calf with cows. Tor Mancina farm, Rome (Borghese photo, 2006).

![Figure 5](image5.png)
Figure 6. Intensive system with mating. Tor Mancina farm, Rome (Moioli photo, 1994).

Figure 7. Beautiful Mediterranean Italian buffalo cow in intensive system. Tor Mancina farm, Rome (Borghese photo, 2006).

Figure 8. Intensive system: feed-lot. Tor Mancina farm, Rome (Borghese photo, 2006).
Figure 9. Calves managed in box.

Figure 10. Intensive system for fattening. Tor Mancina farm, Rome (Borghese photo, 2006).

Figure 11. Milk processing and mozzarella production.
Figure 12. Typical Italian mozzarella di Bufala Campana DOP “Aversana type”.

Figure 13. Typical Italian ricotta.

Figure 14. Calves buffalo carcasses.

Figure 15. Rump of young bull with conformation R and fatness 2+.
Figure 16. Scamorze, bresaola and salami from buffalo.

Figure 17. Buffaloes on Transylvania pastures in Meschendorf (Borghese photo, 2011).

Figure 18, 19. Buffaloes on Transylvania pastures in Meschendorf (Borghese photo, 2011).

Figure 20. Bulgarian Murrah bull (Alexiev photo, 1998).
Figure 21. Bulgarian Murrah herd (Alexiev photo, 1998).

Figure 22. Mediterranean buffalo on the snow in Sachsen (Guglielmetti photo, 2007).

Figure 23. Buffalo on the pasture in Sachsen (Guglielmetti photo, 2007).

Figure 24. Mediterranean buffalo young bull, Chursdorf, Germany (Manfred Thiele photo, 2008).
Figure 25. Mediterranean buffalo cow, Chursdorf, Germany (Manfred Thiele photo, 2008).

Figure 26. Typical buffalo products from Germany.

Figure 27. Mediterranean Macedonia breed, Ropotovo, Macedonia (Borghese photo, 2008).
Figure 28. Buffaloes on pasture, Ropotovo, Macedonia (Borghese photo, 2008).

Figure 29. Family management in Mojanci village, Kocani, Macedonia (Borghese photo, 2008).

Figure 30. Simple cheese in Mojanci village, Kocani, Macedonia (Borghese photo, 2008).
Figure 31. Mediterranean Serbian cow (Srdjan Stojanovic photo, 2011).

Figure 32. Mediterranean Serbian cow (Srdjan Stojanovic photo, 2011).

Figure 33. Fresh cheese (Srdjan Stojanovic photo, 2011).
Figure 34. Mediterranean Hungarian buffalo.

Figure 35. Mediterranean Hungarian buffalo.
Developments of Buffalo Industry in America

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ABSTRACT

The buffalo population in America nowadays arrives to 4.2 million heads, approximately (nearly all in South America, and 90,000 in Central and North America). This means only the 2% of the world population, but America actually has the higher annual average increase of buffalo population comparing to all the other continents (12%). This species has been introduced into the continent in the end of the XIX century, being utilized first for draught, later on for meat production and finally for dairy, in a slow process at the beginning, that has speed up in the last 50 years. Still nowadays in some American countries the buffalo is entering as a draught animal (Panamá, Guatemala, Ecuador, etc.) occupying afterwards its space as meat and milk producer. From Argentina in the south to Canada in the north, the buffaloes are mainly riverine type. There are limited swamp buffalo populations in the northern Brazil (Pará State), Venezuela, Cuba, Guyana, and United States. In nearly all the countries buffalo meat production systems have been developed, more intensive in some cases and less in others, all of them with a great efficiency. Reproduction biotechnologies are used since years ago, the same as intensive pastures management. Selection programs are being followed to improve meat and dairy productions. There are large areas with natural pastures waiting to be utilized for buffalo meat production. As a draught animal, the buffalo is essential mainly for the African palm plantations, and also for the transport of sugarcane, for pulling carriages (carts), boats, etc. The buffalo dairy industry has been increasing in a significant way during the last 30 years, with spectacular results, mainly in Brazil, Venezuela and Colombia; and in a smaller scale, in Cuba, Costa Rica, Guatemala, Bolivia, México, United States, etc. Meat and dairy products are supplying internal markets, but also are exported. Argentina and Brazil are working the buffalo hide, and they produce, through an excellent manufacturing industry, products as first quality leather goods, polo stirrup leathers, engine joints, soles, belting, saddles, luggage, handbags, chamois (suede), upholstery, boots, etc. In most of the cases, in America, the buffalo production is located in middle size farms; and in some cases, in large scale farms or ranches. Actually the American buffalo population could be enough to accelerate its diffusion in the small exploitations in order to change radically the life quality and the income of millions of people that are working and living in little family farms developing an economy of subsistence. Official information referred to buffaloes is generally limited in America. This information has been used for this paper, plus the information supplied by several breeders associations trough the continent, by universities, and by personal communications with breeders, researchers, etc.

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**Keywords:** by-products, dairy, draught, markets, meat, production systems, social effects

**INTRODUCTION**

Buffaloes in America have only 220 years of presence, approximately. At the end of 1800 buffaloes were brought into South America, for draught power. Swamp buffaloes were imported from Asia by French to Guiana and by Dutch to Surinam. English imported Riverine breeds to Guyana and Trinidad. Brazilians brought Mediterranean breed buffaloes from Italy to Marajó Island, in the delta of the Amazon River. After the 60’s of last century buffalo population increase turned to be explosive, especially in South America. More recently, Riverine buffaloes from Trinidad, and Swamp buffaloes from Guam and Australia, were introduced in Central and North America. Actually the buffalo population in America reached the number of 4,225,000 heads: 4,130,000 in South America, and 95,000 in Central and North America (Zava, 2011). Most of them are located in the equatorial and tropical regions. The buffalo is the only livestock that produces efficiently in all the Brazilian ecosystems. The buffalo has expanded as producer of meat, milk, and draught power all through Brazil, Venezuela, Colombia, Argentina, Central America and Caribbean, United States and Mexico. And it is opening its way in the rest of America.

**MATERIALS AND METHODS**

The different situations and developments of buffalo industry in countries of North, Central and, mainly, South America sub-continents are described. In Central America, among the Caribbean Countries, Cuba has 63,000 buffaloes (Zava, 2011). Swamp buffaloes are distributed in all the provinces, in marginal farms, managed in extensive breeding systems for meat and draught power production. The Trinitarian breed buffaloes and its crosses (60% of the national population) are milked, reaching daily productions of 10-12 litres. In these herds, males are fattened, and slaughtered with 420 kg of life weight (AWBA, 2001). In 55 dairies of all the country it was reached an average of 1,400 litres per lactation (Valdés et al., 2006). In Continental Central America there are around 15,000 buffaloes, distributed mainly in Panama, Guatemala and Costa Rica. In Belize there is only a herd of 350 heads, 200 in Honduras and 350 in Nicaragua. In Panama, there are 5,000 buffaloes, owned by few breeders (Roldán, 2007). In Guatemala there are 8,000 buffaloes, distributed in different regions. The 80% are owned by 20 breeders, and the rest by small farmers. After the first population of Trinitarian breed, buffaloes Mediterranean and Murrah were introduced, and then Mediterranean semen was imported. Buffalo meat is sold in some cities at the same price of cattle meat. They export the leather to Mexico for shoes manufacturing. In Panama and Guatemala, buffaloes are used to transport through humid lands coconuts, pineapples, bananas, mangos and the African palm fruits. The buffalo feeds itself with the local vegetation and stands perfectly the high humidity and temperatures of tropic. A palm’s by-product is a main ingredient of the buffalo’s diet: the palm pith meal (Roldán, 2007). In Costa Rica there are 2,100 buffaloes, owned by 40 breeders, used mainly for draught in the palm plantations. In the last...
years new buffalo dairy farms were organized. The Agricultural School of Humid Tropical Region has buffaloes for draught (to carry the milk) and for dairy, producing yoghurt and creams (Rosales, 2007; Zava, 2011). Buffaloes were introduced into Trinidad and Tobago to carry the sugar cane. These Caribbean islands have an equatorial climate and high temperatures the whole year. After observing their excellent adaptation to food and management conditions, the coconuts plantation companies began to use buffaloes to clear weeds among palm trees, to pull carriages specially designed for the collection of coconuts; and also for meat production (Taboada, 2011). The bubaline population now is reduced to 5,700 heads (Zava, 2011), due to the exportations into more than 14 countries of America, and to the tourist developments realized in fields close to the coasts. The 50% of the population is of Buffalypso or Trinitarian breeds (Bennett et al., 2007). Buffaloes for meat production are managed in different production systems, varying from extensive (rational grazing on Pangola Grass pasture with loads ranking from 1 to 1.75 heads per acre), to semi-intensive systems (diurnal access to pastures and nocturnal housing in sheds), and finally to feed-lot. In the first case they reach their slaughtering weight (350-400 kg) with 24 months of age; in the second, with 15-18 months; and in the third they enter with 12 months of age and in 6 months they are ready for slaughtering with 400 kg. The animals fed with ration gain 850 grams of life weight per day. The ration includes mainly sugarcane, molasses, corn, soy meal, minerals, etc. (Taboada, 2011). Since 1990 there is a Research Dairy Project with Trinitarian buffalo cows in the Aripo Experimental Institute, where they obtained the following average values in the milk composition: fat = 7.15%; protein = 4.03%; no fat solids = 8.84%; total solids = 16.07%; Calcium = 0.23%; ashes = 8.5%; and lactose = 5.6% (Rastogi and Rastogi, 2004). Regarding North America, 10,000 buffaloes are located in Mexico, all of them bounded for meat production. One breeder has more than 5,000 heads in Chiapas, Veracruz and other southern tropical states (Zava, 2011). In a ranch near Puebla city, with 1,000 heads, they fatten buffalo males in natural fields and sell meat cuts to supermarkets in very good prices: common cuts in USD 20.00/kg and premium cuts in USD 45.00/kg (Coronel, 2008). Canada joined to buffalo production since 1999, and already has 1,000 heads. There are three dairy buffalo herds, one in British Columbia, another one in Ontario and the largest in Toronto (Zava, 2011). Actually there are 6,000 buffaloes in the United States. The buffalo began to develop there since 1975, due to the action of breeders of the States of Florida, Louisiana, Arkansas, California, etc., supported by the University of Florida, and years later, by the University of Davis in California. They first developed meat production in marshy lowlands of Florida, and in fields of Louisiana, Texas and Arkansas. Then dairy productions began in California and then in Vermont and other states. There are also buffaloes in New York, Montana, Oregon and Washington. They began with buffaloes of Carabao or Swamp breed, then with Trinitarian breed; and then they inseminated and transferred embryos with Mediterranean, Murrah and Jafarabadi breeds. Today in the US each breeder sells individually the buffalo meat produced by him. For lacking of scale they were not able to implement a supermarket commercial chain. Mainly, the meat selling go into restaurants that are close to the buffalo farms, focusing to its low cholesterol and in the good ratio of poli-insaturated fat acids,
those groups as CLA (Conjugated Linoleic Acids), especially those Omega 3 and Omega 6. The buffalo meat in US also reached excellent results in sausages manufacturing (AWBA, 2007) (Zava, 2011). **South America** has more than 4 million buffalo heads, mainly located in Brazil. Venezuela and Colombia began with riverine buffaloes coming from Trinidad and Swamp buffaloes from Australia. Brazil, together with Venezuela and Colombia, had during the last 30 years a great expansion in meat and milk production with buffaloes. In the last years in Argentina the expansion of agriculture is displacing animal production towards the tropics, producing a significant increase of buffalo meat production, and more recently, dairy production. With 300 heads introduced in 100 years, **Brazil** has 3.5 million buffaloes, divided in 25,000 herds. Four breeds were spread: Mediterranean, Murrah, Jafarabadi and Carabao. The 62% of buffaloes are located in the North Region (Amazon), the 9% in the North East, 6% in the Centre West and 22% in the South East and South Regions. More than the 50% of the population are Murrah breed and its crosses, and 20% Mediterranean breed. The dairy productions have different levels, depending on management. The most are in extensive systems, with one milking, and have an average production of 1460 litres. With two milking the average is 2,500 litres, and adding genetics, 3,000 litres. One of the greatest individual productions reached 5,142 litres. Nearly 92 million litres per year are produced, coming from 82,000 dairy buffalo cows that belong to 2,500 herds. And 150 dairy factories are processing 45 million of buffalo milk litres per year (Bernardes, 2007). Four pastoral ecosystems are utilized for rearing buffaloes in brazilian Amazon Region in grazing production systems: 1) Natural pastures of floodable lowlands in the Marajo Island, Delta of the Amazon River; 2) The natural pastures of floodable lowlands areas of the coasts of the Low and Middle Amazon River; 3) High fields natural pastures; 4) High fields implanted pastures. In Brazil prevails the grazing system in buffalo breeding for meat and in dairy. Frequently, they use for dairy production the supplementation with volume (sugar cane, cutting green grass, silages, etc.) during the periods of worst forage supply (autumn and winter), that, due to the reproductive cycle, in buffalo cows coincides with the period of higher milk production. Is unusual the supplying with concentrates. In the tropic and sub-tropic, in the rearing herds, is usual to observe calving levels higher than 80 and 90%. The males reach the slaughter weight (near 430–480 kg.) with 18–24 months of age, and with 30–36 months in the dairy herds. In Brazil the buffalo meat annual production reaches at least 155,000 ton, resulting of 743,000 heads slaughtered. It’s leather, in spite of a real demand, especially for exportation, is still scarcely exploited, mainly due to the great dissemination of the slaughterhouses, that turns the transport expensive and reduces the process scale (Bernardes, 2006). The buffalo in **Venezuela** was limited in the poor and floodable lowlands, but in the last 35 years it spread out through all the plains and also the mountains, producing a “red and white” revolution in the local livestock industry. Actually there are 350,000 buffaloes, producing 105 million litres of milk per year and 17 million kg of meat. And they occupy more than 700,000 hectares, improving them with their presence (Coirán, 2008). The 57% of the exploitations combine buffalo with cattle. Within them, the 60% focuses the double purpose of milk and meat. Natural pastures are utilized in the 60% of the cases. In floodable areas there
is an extensive system for meat production. In these areas of difficult access they work with not much domesticated animals, no more than twice a year (health, sale, etc.). In the double purpose systems, the use of land is more intensive (fenced fields, implanted pastures, drainage, etc.). A reduced group of ranches are focused to the buffalo dairy production, with semi-intensive managements based in diurnal grazing, and concentrate supplementation, hay and/or cutting green grass during night, with two milking per day. They have near 6 litres of average daily production (1,500–1,700 litres per lactation of 250 days). Each 5 litres of milk they obtain 1 kilo of mozzarella cheese. Most of the buffaloes are situated in the Venezuelan Plains Region (75% of humidity, 26°C of average temperature with maxims of 40 – 45°C, 1,500 to 2,000 mm of rain per year, concentrated in the 6 months). The buffaloes are prevailing cross-bred (66.7%). The Murrah is the predominant pure breed (26.7%), and second is the Mediterranean breed. The size of the average herd varies from 50 to 200 heads (Reggeti, 2007). Colombia had 380 buffaloes in 1977 and reached 150,000 in 2011. They began using them for draught power in African palm plantations, then for meat production and finally for dairy. They do works like ploughing, transporting, pulling great carriages, tasks that before were realized with machinery or with mules, and they colonize areas where cattle couldn’t be introduced. Actually machinery is improving and is used again, and buffaloes are being bounded for dairy and beef. Trinitarian breed buffaloes were first imported to Colombia. Afterwards, a great number of Murrah, Trinitarian and Mediterranean buffaloes came from Venezuela; and Murrah from Brazil. Also came from Brazil semen Murrah and from Italy semen Mediterranean (Sanint, 2006). The North Coast Region have the 60% of buffalo population, bounded for meat and milk, with a climate varying from arid to humid in the different territories, with temperatures surpassing the 40°C and average rainfall of 1,200 mm during the 6 months of the rainy season. Are plain lands with fertile soils and variable drainage, ranking from 0 to 50 mts. above sea level. The Middle Magdalena Valley Region, with a variable climate that changes from humid to dry, with temperatures of 29 to 40°C, with an altitude ranking 120 to 250 mts. above sea level, and with an annual average rainfall of 2,300 mm distributed in two rainy seasons per year, has arid woods and humid tropical woods, with a plain and a topography lightly undulated. This region groups the 35% of buffaloes in the country, bounded to breeding, weaned buffalo calves breeding and fattening. And fattening is done through intensive and extensive pastoral systems, and feed lot. There are a great number of farms and ranches with pastoral production systems working with ecological certified production, with identification and tradability. Buffaloes are fattened in intensive grazing systems, in rotational grazing on improved pastures, some of them (Climacuna Grass, Angleton Grass, Estrella Grass, Pangola Grass) with more than 12% of Brute Protein (where buffaloes have an average gain of 1 kilo of life weight per day), and some others (like the Brachiaria sp.) with 4 to 8% of BP. They reached milk production above 4000 litres in some individuals. And also there are exploitations for meat, where they achieve a production of weaned buffalo calves weighing 300 kg. We find also housing production systems for both industries. Already they are working successfully in the factory of meat, milk and hide, with first quality products (Roldán, 2005; Zava, 2011). In Argentina, buffaloes
increased from 1,300 heads in 1976 to 120,000 in 2012. This increase has been mostly vegetative, adding importations mainly from Brazil, and also from Paraguay and Italy. The buffaloes are located in 11 of the 23 argentine provinces, mostly in Formosa (40,000 heads) and Corrientes (35,000), in the North East Argentine Region. The buffalo breeding cows are 45,000 and are slaughtered 17,500 buffalo males per year. In the argentine humid sub-tropic buffaloes surpass cattle adding a 60–70% in weight gain, and a 15–20% in the calving rate. They reach easily 220 kilos with 8 months of age, 480 with 24 months, and 550 with 27-30 months, arriving in that way to the slaughtering weight one year earlier than cattle, improving the carcass quality. The slaughtering carcass yield ranks from 52 to 54%. The beef is lean, with excellent colour and tenderness. The suckling or baby buffalo of 11 months with 250–300 kilos of weight has good market niches in northern medium cities. The meat buffalo production systems evaluated are extensive (with loads ranking from 0.5 to 0.7 heads per hectare), are located in natural pastures fields of the Paraná River Basin, mostly in open low fields and also in high fields, with humid subtropical climate, north of 31° south latitude, with 1000–2500 mm. annual rainfall and temperatures varying from 7 to 43°C (average 25°C), where cattle produce not much. Calving rate, with good management varies from 80 to 98% (vs. 60–75% of cattle). The daily weight gain is remarkable: 700 gram/day pre-weaning and 500 gram/day post-weaning. The reposition of females is done, always in low quality natural grass, when they reach 2/3 of their adult size (350 kilos) and 2 years of age (vs. 2 to 3 years in cattle). Buffalo cows are discarded at 18–22 years of age, and buffalo bulls at 6–7 years. In Bolivia there are 10,000 buffalo heads, all of them in the east of the country, with high humidity, rivers and high temperatures, typical of the subtropical climate. A dairy farm in Santa Cruz de la Sierra produces a daily average of 6 litres and a milking average of 1.672 litres (maximum 2,600 litres). They have a modern milking parlour with 16 milking machines: milk and cheese are processed in modern plants that are sanitation and efficiency paradigms. They also fatten buffalo calves and sell the beef that they process, with promotion that emphasize the rate of cholesterol, 30% lower than that of cattle. They produce several types of cheese, among them processed cheese and ricotta, under a trademark, and sell through aggressive market promotion. Other ranches breed and fatten buffaloes, slaughtering males with 450 kg and 24 months, reaching excellent prices in supermarkets. They have Murrah and Jafarabadi buffaloes and its crosses, although most are Mediterranean. Buffaloes are also spreading into Beni Department, in the bolivian amazon region (Zava, 2011). In Paraguay there are about 10,000 buffaloes spread all over the country, mostly in small herds of 20 to 50 buffalo cows. Only few ranches, that own the 50% of the national herd, breed and fatten buffaloes rationally and successfully, in natural pastures fields, with 1,600 mm annual rainfall and high average temperatures, even in winter. Whole males are slaughtered at 24–28 months, without suffering hormonal changes in the carcass. Pregnancy in buffalo cows is higher than cattle: 70-87%. Demand of fatten buffaloes is excellent, with the same price of cattle. There is good demand for purchasing and processing hides. In Ecuador buffalo population is about 1,300 heads. One ranch located 90 km. from Guayaquil city have a dairy with Trinitarian breed buffalo cows, and they sell in good prices
buffalo males for draught power in African palm plantations. The females are kept for the dairy herd. They began to process mozzarella cheese. Perú actually has around 1,000 buffaloes, in Loreto Department, near the city of Iquitos, in the Peruvian Amazon. French Giana, Surinam and Guayana have together no more than 1,000 buffaloes. The first animals were brought for draught power more than 100 years ago. Afterwards were introduced Murrah breed buffaloes for dairy production.

RESULTS AND DISCUSSIONS
In America the buffalo became an exceptional animal for draught power in tropics and equator, mostly in the plantations of Central America, Caribbean, Colombia, Guyana, Surinam, French Guiana, and for transportation in the Amazon Region of Brazil. In North America its meat and milk products have great market niches to be exploited. In Central America and Caribbean, besides its increasing use for draught, buffalo has revealed as an excellent beef producer, and recently, also as milk producer. And its products are being demanded in the same area of production. In South America, buffalo is producing a revolution in livestock of Venezuela and Colombia, replacing the surviving cattle breeding with an efficient production with buffaloes. In Brazil, in its equatorial, tropical, subtropical areas, buffalo surpass cattle in efficiency for meat and milk productions, and spreads all through its enormous territory, with an excellent demand for dairy products and local markets for beef. The same occurs in the subtropical area of northern Argentina, where the buffalo produces meat with efficient calving rates and weight gains; with slaughtering at early age and very good carcass quality. Dairy is more recent but it has an excellent internal market for its products. The rest of the countries of South America, are doing the first steps with buffalo. America today is the continent with the highest annual growth in buffalo population. Buffalo consolidates its suitability for meat, milk and draught in different regions, being irreplaceable in areas with most rigorous ecosystems of tropic and equator. American buffalo population reached already the necessary scale to maintain a high growing rhythm. The productive efficiency turns the buffalo into a developing valuable tool for nearly all the regions, and also to improve the income of small owners with small familiar business.

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Thailand Buffalo Strategic Plan 2012-2016

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REASONS AND THE NEED FOR SETTING UP BUFFALO STRATEGIES OF THAILAND

Most of buffalo farmed in Thailand are swamp buffalo with a small number of dairy buffalo and crossbreds. Buffalo has been for centuries a major component of Thai farming. Buffalo was regarded as a mobile fertilizer plant and used for drafting purpose in the field before being replaced by the tractor over the last 30 years. The use of machinery instead of buffalo power has partly caused a decline in the number of buffalo farming. According to the statistic of Department of Livestock Development (DLD), the number of buffalo in 1978 was 6.5 million and decreased continuously during the past 10 years from 2001 to 2011 the number of buffalo decreased from 1.61 million heads from 0.451 million families raising to 1.23 million buffalo, with only 0.271 million families raising which is caused by a change in the way of rice farming with tractors replacing the buffalo labor. There have been a lot of sales of entire herds of buffalo to slaughter houses. The remaining buffalo raisers do not raise buffalo as a career. Therefore, they do not pay attention to the management of the buffalo herd, breeding improvement and disease prevention of the buffalo leading to low productivity of buffalo and not having enough buffalo for consumption. A strategic plan of buffalo is necessary to drive the development of buffalo to the right direction with participation of all sectors. It is essential and critical to maintain the existent of buffalo in Thailand. The DLD as a government agency responsible for livestock production in the country has established a strategic plan for the years 2012-2016 (number of buffalo and raisers shown in Table 1 and Figure 1).

PRESENT SITUATION

The numbers of buffalo and buffalo raisers by region in the year 2011 are shown in Table 2. There were 271,112 households engaged in buffalo raising with a total number of 1,234.17 with an average herd size of 4.50 buffalo per family. Most of buffalo were farmed in the Livestock region 3 or 45.27 percent, followed by the Livestock region 4 or 27.78 percent in the country; both regions are located in the Northeast of Thailand. The top 5 provinces with the most number of buffalo is Ubon Ratchathani (123,428), Surin (109,166), Sirsaket (91,519), Buriram (90,956) and Sakon Nakon (78,135).
SWOT ANALYSIS

The buffalo industry has strengths, weaknesses, opportunities and threats. This information is used as a basis for analysis and then the development of industries strategies for R&D. The analysis includes ‘using strengths to take advantage of opportunities; ‘using opportunities to overcome weaknesses’; and ‘minimising weaknesses to avoid threats’. The challenge is to improve productivity along the supply chain.

**Strengths**
- Easily farmed and suitable for small farming systems of self-sufficiency
- Manure can be used as fertilizer
- Labor is used to substitute machinery to reduce farming costs
- Buffalo utilize efficiently low quality roughage, with same temperament which is suitable for use in agriculture and the tourism sector
- Buffalo is important to the economy, society and local tradition and culture
- Meat and milk products are healthy and nutritious to satisfy specific market (niche market)
- Leather is of high quality

**Weaknesses**
- Are associated with very poorly educated and elderly farmers, hence, adoption of new technologies for career development in buffalo industry is slow
- Lack of buffalo farming associations or networks
- Lack of an improved genetic program
- Lack of an improved health management
- Breeding sires are not commonly farmed because it is considered as a burden
- Farmers do not realize the importance of forage crops
- Public pasture areas have been encroached leading to buffalo receive insufficient nutrients, slow growth with low fertility
- High level of mortality rate
- Buffalo has a limit on the use of labor, therefore, it is not suitable for a large scale agricultural area
- Lack of involvement of the research community and limited number of researchers in this field

**Opportunity**
- The Government campaign to encourage farmers to use buffalo labor to reduce production costs
- A national policy on organic agriculture is a national agenda
- Buffalo farming has helped to produce organic fertilizer
- Neighboring countries have a great demand for live buffalo both meat and breeding buffalo
- Buffalo meat is good for the health of patients and the elderly
- Buffalo leather is in great demand both within the country and abroad

**Threats**
- As the impact of the free trade agreement (FTA) there will be an increase in importation of meat and visceral organs for consumption from overseas and this could cause a decline of buffalo prices
The establishment of ASEAN Economic Community in 2015 may result in a flow out of the buffalo to other countries. This could lead to a reduction of breeding dams for production within the country. The promotion of some public sector projects causes the farmers to shift from buffalo farming because of the area problems, such as rubber and oil palm planting. There are no definite legal measures to control or prevent the killing of buffalo cows and pregnant female buffalo. Insufficient R&D funding.

THAILAND 5-YEAR BUFFALO STRATEGIC PLAN (2012-2016)
The Strategic Plan’s vision is: “Sustainable production development, strengthen lifestyle of buffalo raisers.”

**Mission**
1) development of a buffalo production system for commercial purpose
2) motivating the farmers for buffalo conservation and sustainable development
3) increase the productivity of buffalo
4) public relation and promotion of the benefit of buffalo raising to the public continuously and widely
5) improve the regulation, law and order in accordance with current situation
6) raise awareness of the buffalo value to farmers and youth in the development of baffalo raising
7) integration with other agencies and organizations involved in the development of bufflaoes in a multilateral cooperation manner

**Purpose of the Strategic Plan**
The buffalo strategic plan has four main purposes:
1) to develop buffalo farming as a secure and sustainable career
2) to create incentives for the conservation and development of the buffalo
3) to strengthen the network of buffalo farming
4) to increase the buffalo production capacity of farmers.

**Target of the Strategic Plan**
The buffalo strategic plan has three main targets:
1) to promote the export of buffalo meat and drafting buffalo abroad each year, not less than 2,000 heads
2) create a network of conservation and development of the buffalo raiser groups to 100 groups per year
3) improve productivity of buffalo in the promoted groups to increase by 15 percent per year.
BUFFALO DEVELOPMENT STRATEGY IN FOUR AREAS:

1. Strategies for Thailand buffalo production development system as a marketing and processing business
   1.1 support farmers to establish a community enterprise to be able to control the production volume, serve as marketing agency to create bargaining power in the market by creating a network linking production with marketing
   1.2 registration of buffalo farmers and establish Buffalo Information Center for collection and dissemination of information on the production and marketing of buffalo
   1.3 support the establishment of a Thailand Central Export Market for buffalo to control the quantity and quality of output and promote buffalo farming
   1.4 conducting projects to promote the production of buffalo meat and breeding buffalo for export in cooperation with financial institutions, the project has duration of repayment of low interest and capital loan in accordance with the production of buffalo breeding
   1.5 supports the processing of buffalo to increase in value added
   1.6 promotes quality of buffalo meat consumption in order to achieve acceptable and reasonable price.
   1.7 updates procedures to control the movement of animals and carcasses to facilitate the marketing system

2. Strategies to conserve Thai buffalo development and cultural heritage on buffalo
   2.1 promote and encourage existing farmers to sustainably develop buffalo raising by supports the integration, strengthen the learning process for farmers, allocate of budget subsidies to farmers to manage the development of buffalo breeding programs / projects of from the community groups and creates learning and prototype networking group
   2.2 create Thailand Buffalo Conservation Networking and Development at all levels by establishment of conservation groups in every village or district that has a potential
   2.3 support cultural, traditional and intellectual heritage of the community associated with buffalo
   2.4 transfer of local wisdom and intellectual of Thailand buffalo to public and younger generations, introduce buffalo farming in the educational curriculum system and a media support the learning of young people, establishment of school and learning center for buffalo in the community as non-formal education system in the appropriate sites
   2.5 campaign through various media for people to realize the value of a buffalo
   2.6 promote conservation tools, invention and development of tools used for buffalo
   2.7 conduct an annual national buffalo event by setting up the buffalo competition widely in different regions and in the national competition to encourage farmers and the general public aware of the importance of buffalo breeding selection and conservation of the species in Thailand which is known as the largest swamp buffalo the in the world
2.8 conserve buffalo breeding dams by:
   strengthen law enforcement and punishment seriously especially
ekilling of pregnant and mature female buffalo of reproductive age for
breeding purpose to preserve the species
   campaign and public relations to educate the conservation of genetic
of native buffaloes species and not to consume buffalo fetuses.

3. Strategies to increase efficiency of Thailand buffalo production by
   support the production for commercial and conservation
   3.1 for breeding improvement, support the breeding of buffalo by the
network and provide better buffalo breeding sire attracting more farmers
involvement
   3.2 expand the buffalo breeding using biotechnology in order to supply the
breeding needs and multiplication with rising demand
   3.3 for feeding, support the use of good quality forage and agricultural by-
products to increase efficiency and reduce production costs by farmers or groups of
farmers as growers/ producers/distributors
   3.4 improve animal health by designation of proactive animal health action
plans to prevent infection and parasitic diseases as well as zoonosis and to enhance
monitoring and laboratory diagnostic of animal diseases

4. Strategies for research and studies to support commercial buffalo
   production and conservation
   4.1 Urgent research and studies
      The study of buffalo labor in cropping and grazing and other
      benefits of buffalo
      Research and studies of market mechanisms
      Research and studies of the slaughter and processing of buffalo meat
      and leather for various products
      Research and studies of the value of Thailand buffalo
      Research and studies to strengthen of farmers who raise buffalo
      Research and studies of meat and buffalo leather for various
      products
   4.2 Research for knowledge
      Research and development of animal breeding, animal disease,
bio technology and management to increase productivity
      Research, studies and development of buffalo information center
      Study and use of herbs for the buffalo medication
      Communicate research findings to farmers

BENEFITS EXPECTED FROM THE IMPLEMENTATION OF THE
STRATEGY
   Economic aspects
   1) During the year 2012-2016, it is expected to have 120,300 buffalo calves
born according to the strategic plan, representing the value of US$59.61 million.
   2) Expected buffalo manure produced by 120,000 buffalo with 2 tons each
to be 240,000 tons, worth US$33 each or a total US$7.92 million.
Social aspects

1) Make the community happy and having a good quality of life from reducing expenditure (using buffalo labor and manure instead of chemical fertilizer) and income from the sale of the buffalo.

2) Resulting in the continuity of cultural heritage of indigenous knowledge related to buffalo as a sustainable community identity.

Environment aspect

1) The buffalo manure can be used instead of chemical fertilizer to help protect the environment and reduce global warming.

REFERENCES


Table 1. Number of buffalo and their raisers in Thailand during 2002 – 2011.

<table>
<thead>
<tr>
<th>Year</th>
<th>Buffalo (heads)</th>
<th>Farmers (families)</th>
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</thead>
<tbody>
<tr>
<td>2002</td>
<td>1,617,358</td>
<td>451,283</td>
</tr>
<tr>
<td>2003</td>
<td>1,632,706</td>
<td>461,152</td>
</tr>
<tr>
<td>2004</td>
<td>1,494,238</td>
<td>371,086</td>
</tr>
<tr>
<td>2005</td>
<td>1,624,919</td>
<td>393,352</td>
</tr>
<tr>
<td>2006</td>
<td>1,351,851</td>
<td>300,929</td>
</tr>
<tr>
<td>2007</td>
<td>1,577,798</td>
<td>377,816</td>
</tr>
<tr>
<td>2008</td>
<td>1,359,807</td>
<td>218,905</td>
</tr>
<tr>
<td>2009</td>
<td>1,388,685</td>
<td>300,852</td>
</tr>
<tr>
<td>2010</td>
<td>1,190,886</td>
<td>258,955</td>
</tr>
<tr>
<td>2011</td>
<td>1,234,179</td>
<td>271,112</td>
</tr>
</tbody>
</table>

Source: Information center DLD: www.dld.go.th/ict/th/
### Table 2. Number of buffalo and buffalo raisers by region in the year 2011.

<table>
<thead>
<tr>
<th>Main regions</th>
<th>Livestock regions</th>
<th>Number of buffalo (heads)</th>
<th>Farmers (families)</th>
<th>Average number per family</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
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Source: The DLD Information Center (www.dld.go.th/ict/th/)

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**Figure 1.** Trends of numbers of buffalo and their raisers during 2001–2011.
Plenary Session 2: Sustainable Buffalo Production
Prospect of Nutrition and Feeding for Sustainable Buffalo Production

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ABSTRACT

Buffalo (Bubalus bubalis) is known as efficient convertor of poor quality forages into high quality milk and meat. Buffalo is mainly categorized as Asian and Mediterranean with two main sub Species (River and Swamp type) present in Asia. At present, around 95% of the world buffalo population is contributed by Asia; where animals are mostly fed on low quality roughages and crop residues with poor nutritive value resulting in poor production, reproduction with delayed onset of puberty in heifers and high mortality in young stock. Recent investigations showed that use of bST in buffalo fed high energy density diets and increasing the level of rumen un-degradable proteins in the diets of lactating dairy buffalo, improved the production and reproduction performance. Furthermore, use of stair step feeding system in growing heifer reduced the age at puberty with significant reduction in the cost of feeding. Further, milk fed calves can be successfully and economically weaned as early as 8 weeks of age without any adverse effects on growth when given free access to good quality starter diet. Despite the recent developments in buffalo nutrition, a number of other areas; like establishment of nutrient requirements for lactating buffalo according to stage of lactation and production status, requirements for growing heifers aimed to reduce age at puberty, development of milk replacer for young buffalo calves, use of various feed additives and performance enhancers, changes in rumen microbial ecology and reduction in methane production, still need considerable attention.

Keywords: buffalo, early weaning, heifer nutrition, lactation, nutrition, production, reproduction

INTRODUCTION

The role of nutrition in achieving high and sustainable livestock productivity is fundamental and imperative. Proper nutritional management is the key to a successful animal reproduction and health program. Over the last four decades a number of animal-nutrition-based technologies and practices have been developed and applied with varying degrees of success. Some technologies have produced profound beneficial effects and have been employed widely; whereas, others have shown potential on research stations but have not been taken up by farmers. The world population is growing at a tremendous rate and is expected to reach 8000 million by the end of 2020; therefore, adaptation of recent developments in various scientific and technological fields is important to sustain the growing food demands (Mehra, 2001).

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Buffalo (Bubalus bubalis) is classified into two sub species swamp and water buffalo (chromosome n= 48 and 50, respectively) contributing as a major source of food (milk and meat), power, fuel and leather especially in developing countries. Buffalo is distributed worldwide; however, around 95% of the total world buffalo population is present in Asia with India, Pakistan and China as major buffalo holding countries. In these countries, animals are fed on low-quality roughages; agricultural crop-residues/and industrial by-products containing high fibrous materials resulting in poor growth, production and reproduction. Contrary to cattle, buffaloes are unique in their capability to efficiently utilize poor quality feed resources, through better rumen fermentation (Wanapat et al., 2000) as well as better nitrogen utilization (Devendra, 1985), indicating natural potential of animal to survive and produce in tough environment with limited feed resources. However, improper and inadequate nutrients availability has resulted in low milk production, poor growth, high mortality rates and poor reproduction performance (Qureshi et al., 2002; Tiwari et al., 2007; Sarwar et al., 2009; Pasha and Khan, 2010).

Several authors have reviewed the world buffalo distribution and regional feeding practices in buffalo (Borghese, 2009; Pasha and Khan, 2010). Recently, Sarwar et al. (2009) comprehensively documented the research on locally available feed resources such as crop residues, industrial by-products, ruminally protected fats and proteins, micronutrients, performance modifiers and their impact on growth, milk yield and reproductive performance of buffaloes. However, over the past few years new developments have been published in different areas of buffalo nutrition; energy density, RUP to RDP ratio in lactating dairy buffalo, bST in lactating buffalo, compensatory growth in heifers and starter based early weaning programs in young calves are some examples.

Therefore, the objective of this review is to summarize previous findings with recent developments in the area of buffalo nutrition, and to discuss future prospects of buffalo nutrition in different segments of buffalo production.

WORLD BUFFALO POPULATION AND MILK PRODUCTION

Water buffalo is classified into the genus Bubalus, species bubalis. River (water buffalo) and swamp are the two sub species of buffalo with different morphology (body weight, shape & frame), genetics (50 and 48 chromosomes, respectively) as well as purpose of rearing. Swamp type buffalo is mainly kept for draught purpose and meat with relatively less milk production of around 600 kg per year whereas, river buffalo is kept as a dairy animal with higher milk production up to 2,000 kg per lactation. It is also used for meat production due to its larger body size. Buffalo is disturbed worldwide due to its inherent ability to produce high fat milk and better conversion of poor quality feed resources. In some regions of the world buffalo is native whereas, in other parts it was imported due to its distinct qualities. Buffalo is present in every continent and region of world varying in ecology, climate, topography and socio-economic conditions. According to FAO (2008), the world buffalo population is about 177.25 million heads: out of this Asia is contributing 95% of the total world buffalo population with 172 million heads; 4 million are in Africa (mainly in Egypt) contributing 2.3%; South America with 4.1 million heads and contributing 2.4%; in Australia 0.040 million contributing 0.02%
and Europe with 0.500 million heads contributing 0.3% to the world buffalo population.

Each subspecies of buffaloes further contains different breeds that are domesticated and used for different purposes. Given below is the list of domesticated buffalo breeds with their distribution and milk production (Table 1).

In developing courtiers, having considerable populations, buffalo is an integral part of human food as well as economy especially for smallholders. Whereas, in developed countries, the buffalo population is increasing due to better quality of milk, higher demands for buffalo milk related products and good quality beef. Current world milk production is shown in Figure 1; whereas, change in annual milk production of each country status is given in Table 2.

BUFFALO PRODUCTION AND FEEDING SYSTEMS

India, Pakistan and China are among the largest buffalo population holding countries in Asia. Majority of the buffalo in these counties is raised on mixed, either cut and carry or grazing system where animals are fed on low-quality roughages; agricultural crop-residues/and industrial by-products containing high fibrous materials, low level of fermentable carbohydrate low and quality protein. Low milk yield, poor reproductive performance (seasonal breeding behavior, anestrous, and longer calving interval) and low growth rate have been reported in buffaloes (Qureshi et al., 2002). Further, seasonal changes in the supply of feed stuffs due to traditional forage sowing and harvesting practices results in scarcity of good quality fodder during several months of year. This inadequate supply of quality fodder has been identified as one of reasons for poor performances (Sarwar et al., 2009). Therefore, over the past few decades researchers have been working to improve fodder quality and crop residues to ensure consistent nutrients supply with varying degree of success (Sarwar et al., 2006). Equal emphasis has been given to improve feed utilization and to enhance production through genetic improvement.

China is the third largest buffalo holding country of the world. Despite the huge variety of genetic resources, the majority of buffalo in China is Swamp type. China imported the Murrah buffalo from India in late 1950s and the Nili Ravi form Pakistan in late 1970s. Research on these exotic breeds in the areas of breeding, feeding behavior, frozen semen, artificial insemination and cross breeding has shown encouraging results. Comparison of milk production potential of different buffalo breeds is given in Table 3.

Pakistan is the second largest with buffalo population which is main dairy animal in the country (Table 4). More than 85% of the total buffaloes are raised in herds of two to five animals indicating the dependence of huge number of families linked with buffalo farming. At present, buffalo production and feeding systems are characterized by their location, herd size, feeding practices and marketing opportunities (Khan, 2009). Based on feeding and production, four systems are prevalent: 1) Rural subsistence smallholdings 2) Rural market-oriented smallholdings 3) Rural commercial farms and 4) Peri-urban commercial dairy farms with the respective herd size of 2 to 3, 3 to 6, 10 to 30 and 15 to 100 animals (Pasha and Khan, 2010).
India is the first country in the world for the number of buffaloes and milk production (about 84.4 million tons, of which 54 are buffalo milk). In India, crop-livestock mixed farming prevails where majority of rural families own livestock. Ruminants dominate livestock production and are fed based on ‘Low External-Input System’ utilizing crop residues and by-products (FAO, 2011). However, production improvements through better nutrition, production, reproduction and biotechnologies have improved the buffalo management. India possesses a variety of dairy buffalo breeds that are among the top milk producing breeds of Asia including Murrah, Nili-Ravi, Surti and Jaffarabadi breeds. Among the other distinct countries of Asia with considerable buffalo population includes: Philippines, Thailand and Vietnam. In these countries, majority of population is kept by the poor farmers having limited resources and access to other economic opportunities.

MANAGEMENT OF MILK FED BUFFALO CALVES

Raising the calves is a labor intensive and costly segment of livestock production. Therefore, commercial farmers especially in peri-urban areas give little importance to rearing of male calves. Increasing milk prices has developed a wrong concept among the farmers that rearing calves is not profitable; therefore, farmers prefer selling milk instead of feeding to young calves. Buffalo male calves, in such commercial production systems have been reported to be slaughtered within first ten days of their life. Those surviving, are mainly kept with dam for milk let down and allowed to suckle a very limited amount of milk, and weaned around one year of age (Khan et al., 2007). Whereas, in modern calf rearing practices newly born dairy calves are separated from dam and fed milk/MR along with good quality calf starter concentrate. Further, buffalo calves are fed available green fodder, and only few farmers provide them with concentrate. In peri-urban commercial areas male calves have been reported to be slaughtered within first 6 to 8 days of their life. Younas and Yaqoob (2002) reported that calves reared conventionally weigh around 60-80 kg after one year. The inadequate feed resources and imbalanced conventional feeding practices have been identified as major cause of low growth rate. Male calves obtained from conventional rearing system later become the part of commercial fattening system. To promote calf rearing, government has started a program for the fattening of calves. Incentives are given to farmer to increase the meat production in Pakistan. With rapidly increasing demands of meat and meat products, limited feed resources and rapidly changing environment the need of time is to focus on efficient growth and production systems.

Management of buffalo calves varies with system of production; in semi intensive system improper management and feeding causes high mortality up to 51.8% for calves under one month of age with 5% higher mortality in male calves (Ramakrishna, 2007). Whereas, in commercial dairy farms feeding and health care practices are neglected resulting to even 81.09% mortality (Tiwari et al., 2007). The main cause of high mortality in commercial dairy is the inadequate feeding and health facilities like colostrum feeding, improper milk feeding, naval cord disinfection and timely treatment (Tiwari et al., 2007). In another study authors reported an average mortality rate of 79.50% in peri-urban buffalo dairy farms (Ahmad et al., 2009). Among the feeding practices only 8% of the farmers were
feeding calves within first three hours after birth, whereas 83.3% farmers fed colostrum after placenta expulsion due to the myth prevailing that colostrum feeding can delay time of placenta expulsion. The amount of milk fed to young calves has a positive influence of the health status and growth performance (Appleby et al., 2001). Therefore according to NRC (2001) calves should be fed @ 10% of their birth body weight. In another study Khan et al. (2007) investigated the management conditions provided to calves reared in commercial dairy farms and reported that mortality rate was relatively low (17.98%) but due to high risk of mortality the calves were sold with ten days after birth. Keeping in view the above mentioned limitation, efficient and economical method of weaning can help us bridging the gap associated with conventional rearing practices.

EFFECT OF NUTRITION IN EARLY WEANED BUFFALO CALVES

Weaning of calves from milk at an early age is practiced to reduce cost of milk feeding (Owen and Larson, 1982; Quigley et al., 1991). From birth to weaning, calves undergo a transition from monogastric to ruminant that is greatly influenced by the type of feeding. The feeding of calf starter rations has been believed to enhance the ruminal VFA production necessary for early development of a functional rumen (Beharka et al., 1998; Zitnan et al., 2005). To date limited information is available about the performance of buffalo calves in response to weaning.

Azim et al. (2011) investigated the effect of milk replacer (MR) feeding rate and early weaning diets on growth performance and economics of feeding in Nili-Ravi buffalo calves. Twelve buffalo male calves, average age 15-21 days and BW 41 ± 2 kg were assigned to three treatment groups: Group A fed MR at 8% of BW for the entire period of 90 days, Group B calves fed MR @ 4% of BW and early weaning diet (EWD) ad libitum, and in Group C calves were fed MR @ 4% of BW and EWD for first 45 days and later on EWD only. Average daily gain was higher in Group C compared to other treatments (0.52 vs. 0.42, 0.46 Kg/d); similarly, DMI was highest in Group C compared to those in Group A and B (1.00 vs. 0.65, 0.72 kg/d). Feed efficiency was greater for group A (1.55) compared to Group B and C (1.62 and 1.94, respectively). Cost of feeding for each kg of live weight gain in Group C was 52 and 25% less than calves in group A and B, respectively. At the end of experiments digestibility was determined. In trial I, at week 8, digestibility of both DM and CP was higher in group A (96.28 and 94.32%) than B (87.53 and 85.92%) and C (88.32 and 84.46%). Similar trend of DM and CP digestibility was found in trial II (at 12th week). Dressing percentage of group C was the highest; whereas, carcass composition and weights of non-carcass organs were not affected by dietary treatments. Authors concluded that feeding buffalo calves the MR at 4% of their BW, weaning off milk at 45 days and ad libitum access to starter during post-weaning period was the most economical system.

Contrary to cattle calves, nutrient requirements and management of milk fed buffalo calves has not been studied extensively. According to NRC (2001) young calves should be fed starter rations containing 18% crude protein. However, studies in cattle have shown that calves can be fed starter diets with CP levels up to 22% of diet (Bartley, 1973). Zicarelli et al. (2007) compared different protein levels in
starter diets on harmonic growth in terms of biological efficiency of growth (BEG), live weight, body weight gain and morphological measurements of buffalo calves. Sixteen female buffalo calves of age 49.0±1.3 days were divided in two feeding groups. The T and C groups were fed starter diets containing crude protein 28.5 % and 21% on dry matter basis, respectively. Investigators reported no differences on biological efficiency of growth, live weight, body weight gain and morphological measurements. Blood urea nitrogen concentration was higher in T group compared to the C group (8.53 mmol/L vs. 4.66 mmol/L, respectively). In another study, Gude et al. (2007) elucidated the effect of chromium picolinate supplementation for 3 consecutive days before and 5 days abruptly in calves weaned off milk at day 90 of age. Calves were separated from dam immediately after birth. Plasma cholesterol and indol levels decreased after weaning in both groups. Authors concluded that abrupt weaning of buffalo from liquid to solid feed at 90 d of age cause no stress. Further, supplementation of chromium reduced weaning stress indicated by lower plasma cortisol levels.

More recently, Afzal (2012) studied the influence of weaning regimen on intake, growth characteristics, and plasma blood metabolites in male buffalo calves. Twenty-four male buffalo calves were each assigned to one of three treatments groups: continuous milk feeding (CMF), limited milk feeding (LMF), and early weaning (EW). Within each treatment, calves were weaned from milk at three different levels of development: 12 weeks (CMF), 10 weeks (LMF) and 8 weeks (EW) of age. For the first 3 days after birth, calves in all three treatments were fed colostrum, and were then moved to individual milk feeding at 10% of BW for the next 6 weeks. Thereafter, the provision of milk to the CMF group was reduced by 16.5% each week (through week 12), using week six intakes as a base. The LMF calves were fed milk at 7.5%, 5%, 3.5%, and 1.5% of BW during week 7 through 10, respectively. Lastly, calves in the EW group were fed milk at 5% and 2.5% of BW during weeks seven and eight, respectively. Calf starter feed was also provided ad libitum from week 2 through week 12 and individual intakes were recorded daily. Blood samples were taken form week 6 through 12, on weekly basis and plasma was harvested after centrifugation. Whereas, the BW, heart girth, withers height, and hip width were measured at the start of experiment and later on weekly basis. Study results revealed that total weight gain, average daily gain (ADG), and body measurements were the same across all three groups. Milk intake was lowest, whereas calf starter intake was highest in the EW calves compared to the other treatment groups. Dry matter intake was higher in the EW and LMF calves compared to the CMF calves. The feed efficiency was greater in the CMF calves compared to the LMF and EW treatment groups. Blood glucose concentration was similar among the treatments. However, blood urea nitrogen was greater in the EW calves compared to the CMF and LMF groups. Plasma concentration of non-esterified fatty acids (NEFA) was higher in the EW calves compared with the CMF calves. In the light of these results, authors concluded that buffalo calves can be successfully weaned as early as 8 weeks of age without negatively affecting growth performance.
NUTRITION OF GROWING BUFFALO HEIFERS

Heifer rearing is the most expensive part of the buffalo production with feed cost contributing 63% to 84% of the total cost (Moore et al., 2009; Razaquee et al., 2010) representing a large expense to the overall farm operation. The objective of heifer rearing program is to provide quality heifer that can reach its maximum lactation productivity potential. Heifers attaining 55 to 60% of their mature body weight are considered to be ready for breeding (Freetly et al., 2001). One of the major limiting factors in delayed puberty of buffalo heifers has been the low cost feed resources with poor nutritional quality.

Conventionally, buffalo heifers are reared on seasonal green forages and crop residues resulting in poor growth rates and delayed onset of puberty (Jabbar, 2004; Bhatti et al., 2007). Average age of puberty in buffalo heifers is around 37 months (Rehman, 2006). Investigators have documented age of puberty between 18 to 24 months of age in Nili-Ravi buffalo heifers (Jabbar, 2004). Researchers from India determined energy and protein requirements for maintenance and growth for Nili-Ravi buffalo heifers (Paul and Patil, 2007). Thirty growing buffalo heifers (weighing 119 kg) were randomly divided into six equal groups and fed six diets consisting of three levels of energy (55%, 60% and 65% TDN) and two levels of protein (10% and 12% CP) in ration in a factorial arrangement. Animals were given ad libitum access to feed until they reached 400 kg live BW. Energy and protein requirements at different stages of growth were estimated by partitioning of the metabolizable energy (ME), CP and metabolizable protein (MP) as intake for maintenance and body weight gain by regression analysis. The ME (kJ), CP (g) and MP (g) requirements for maintenance at different body weights were 443-542, 5.89-9.38 and 4.03-6.30 kg\(^{0.75}\) d\(^{-1}\). The values for ADG were 26-53, 0.24–0.48 and 0.18-0.31 g\(^{-1}\) ADG d\(^{-1}\). The predicted requirements matched well with their corresponding actual intake values across the data range.

Studies in cattle have shown that heifers when fed restricted amount for a certain period of time and fed on high energy diets later resulted with relatively higher gain. Similar observations were documented by Anjum et al. (2012b) who evaluated the compensatory growth potential and age of puberty in Nili-Ravi heifers. In this study, buffalo heifers were fed on three energy restriction periods followed by high energy feeding in each period. Twenty two heifers, age between 6-8 months old, and average BW 99 kg, were assigned to dietary treatments: control group fed continuously according to NRC recommendations and stair-step nutritional regimen (SSNR). In control group, TMR were formulated according to large dairy breeds heifer’s nutrient requirements for growth rate of 0.6 kg/day at 100, 200 and 300 kg BW during phase I (from 8 to 13 months), phase II (from 14 to 19 months) and phase III (from 20 to 25 months), respectively. In the SSNR treatments heifers were fed on low energy diet (80% ME of control) for first 4 months and for next two months with high energy ration (120% ME of control). Results revealed no difference in daily DMI, weight gain and FCR in heifers fed control and SSNR diets. Age of puberty was also similar between treatments (18-23 months of age). Days of puberty (649 and 639 days) and average live body weight at the time of puberty BW (382±14.00 vs. 364±12.48 kg) were not affected by the method of feeding. However, cost of feed was reduced by Rs. 1669/ in heifers fed in...
SSNR group compared to the control group. Study concluded that buffalo heifers can be reared successfully on SSNR feeding regimen without negative impact on reproductive traits and reduced cost of feeding.

In another investigation Anjum et al. (2012a) compared the effect of low energy TMR followed by higher energy ration on growth potential of heifers during a 120 day study. Nili-Ravi buffalo heifers of age 6-8 months, weighing 99 kg were assigned to two dietary treatments: 1) control with ME 2.55 Mcal/kg of DM and 2) stair-step diet fed on diet containing ME 2.03 (SLE) Mcal/kg for 120 days and followed by high energy diet with ME 3.10 (SHE) Mcal/kg of DM for 60 days. Average daily gain of heifers on SHE diet was higher (0.82 kg/day) compared to those fed in control group with gain of 0.58 kg/d. Whereas, ADG was lower in SLE group compared to control group 0.51 vs. 0.61 kg/day, respectively. Similarly, feed conversion ratio was better in SHE and control treatment compared to SLE fed heifers. Study concluded that similar growth rates can be attained in buffalo heifer through compensatory growth feeding method using higher energy ration (120% of NRC recommendations) with reduction in cost of feeding by 17% of the total cost. Recent work by Javaid et al. (2011), compared different levels of RUP and RDP in bull calves and reported a linear decrease in DMI with increasing level of RDP in diet. Dry matter digestibility increased linearly with increasing levels of RDP in diets. Authors concluded that growing calves can effectively utilize straw based TMR (16% CP) with RDP contributing 82% of dietary CP contents without any negative impact on rumen and blood metabolites.

NUTRITIONAL MANAGEMENT OF LACTATING BUFFALO

Buffalo is major contributor of the milk produced in Asia especially Pakistan and India. Nili-Ravi and Murrah have been established as top milk producing buffalo breeds. Buffalo like other ruminants are capable of fermenting feed stuffs to fulfill their energy needs and to synthesize proteins from fermentation end-products. Recent research in ruminants has been focused on rumen ecology and manipulation of rumen microbes (Ørskov and Flint, 1989; Martin, 1998; Weimer, 1998). Most of these studies have been carried out in temperate areas where animals were fed on high quality roughages with high levels of concentrates. However, in Asia high environmental temperature, hot humid summer and dry seasons, has led to low and inconsistent availability of feed and nutrients throughout the year; therefore, producing adverse effect on rumen metabolism as well as production performance of buffalo (Wanapat et al., 2000; Wanapat, 2004). Despite the lack of good quality feed supplies in tropics, buffaloes have been reported to use local feed resources efficiently resulting in better rumen fermentation and enhanced productivity (Wanapat et al., 2000). In a comparative study by Devendra (1985), a better nitrogen utilization was documented in swamp buffalo compared to cattle. This superiority is particularly noticeable in situations where the feed supplies are low in quantity as well as quality. Furthermore, in an earlier work of Franzolin (1994), documented that digestibility of each nutrient in feed is 2 to 5% higher in buffalo compared to cattle. Better utilization of poor quality feed stuffs may be attributed to variation in rumen microbiota (bacteria, protozoa and fungi). Wora-anu et al. (2000) documented a higher population of rumen cellulolytic, proteolytic and amylolytic bacteria in
swamp buffaloes compared to cattle fed on similar diets indicating the variation in rumen micro flora enabling them to utilize poor quality high fiber feed stuffs.

Nutrient requirements of buffalo are different from dairy cattle due to difference in climatic adaptability, nutrient utilization and quality of feed offered. Paul et al. (2002) compiled data from 33 studies carried on lactating dairy buffalo to predict requirements for DM, total digestible nutrients (TDN), crude protein and digestible crude protein. Animals were given ad libitum feed access; feed intakes and milk production were recorded daily and in majority of experiments feeding consisted of roughages with roughage and green forage contributing 66% and 44% of the total DMI. Results revealed that maintenance requirements for DM, TDN, CP, and digestible crude protein (DCP) were 59.9, 35.3, 5.43 and 3.14 g/kg W^{0.75}, respectively. In a recent work on lactating Nili-Ravi buffalo, Afzal et al. (2007), documented factors affecting milk yield and lactation length for 426 records of animals kept at National Agriculture Research Institute, Islamabad, Pakistan. Animals were fed seasonal green forage ad libitum along with a daily allowance of 2 kg concentrate for each animal. Authors reported an average milk yield of 1,831.6 ± 530 liters per lactation during an average lactation length of 273± 52.8 days. Milk yield was found to increase with lactation number as well as lactation length. Study concluded that milk production has a positive correlation with parity and lactation length. Ahmad and Bilal (1995) summarized data on 437 lactation records of Nili-Ravi buffaloes from year 1978 to 1993 maintained at Livestock Experiment Station, University of Agriculture, Faisalabad. Results revealed that persistency of lactation and peak milk yield averaged 91.31 ± 0.55 kg and 239.15 ± 7.74 kg, respectively. Calving during months of hot and humid summer was more persistent (92.80 ± 0.52) compared to other seasons. Importantly peak milk yield of 250 kg was obtained during hot dry months of year. The lactation persistency was found to be highly correlated with lactation length. Whereas, milk yield per lactation and peak production were not affected by the lactation number. Researchers from Italy documented the nutrient requirements for lactating Mediterranean buffalo (Bartocci et al., 2002). Twenty buffalo farms were selected, and on the basis of daily milk production were divided into three groups; low (<8 liter/day), intermediate (8-9 kg/day) and high milk producers (>9 kg/day). Lactating animals were fed silage based diets. Average daily DMI was 16.75 with highest DMI (17.06 kg/day) in herds with animals producing milk over 9 kg/day (high milk producer). A similar trend was reported in high producing herds for daily milk production, average milk protein value and highest milk fat value. Authors suggested that rations should be formulated according to production potential of the lactating animals.

Protein levels in the diet of lactating dairy buffalo varies around 12% of DM basis. Sivaiah and Mudgal (1978) suggested the administration of 126.03 g to 166.34 g (Kurar and Mudgal, 1980) of digestible crude protein /100 g of milk produced protein, while according to Rai and Aggarwal (1991), the concentration of crude protein on dry matter should be between 11% and 14%. Changes in protein levels of diet can affect the concentration of protein in milk as well as blood urea concentration. In a series of two experiments Campanile et al. (1998), verified the effect of changing protein levels in diets on milk production, milk quality and changes in plasma metabolites. Two experiments were conducted on eight buffaloes.
Each experiment was further divided into three periods P1 (40 days), P2 (21 days), P3 (21 days) and fed rations containing CP% of 9, 12 and 9, respectively. Animals were fed a silage based total mixed ration (TMR). Milk production (kg/d), milk protein and quantity and MU increased with increase of CP% in diet. Blood urea increased with increasing protein levels showing a strong link. Javaid (2007) conducted the study to evaluate the effect of varying ruminally degradable protein (RDP) to undegradable protein (RUP) ratio on milk production and reproductive performance of early lactating Nili-Ravi buffaloes. In this trial, twenty four early lactating Nili-Ravi buffaloes (n=8/treatment), were used in a Randomized Complete Block Design to evaluate the effect of varying RDP: RUP on DMI, nutrient digestibility, N balance, milk yield and its composition, and reproductive performance. Three iso-nitrogenous and iso-caloric diets were formulated. The C diet was balanced to contain RDP: RUP of 50:50. The other two diets, medium (MRUP) and low (LRUP) ruminally undegradable protein were formulated to have RDP: RUP of 66:34 and 82:18, respectively. Increasing RDP to RUP ratio resulted in linear decrease in DMI in early lactating buffaloes. Linear increase in DM digestibility was observed with increasing RDP to RUP ratio. Neutral detergent fiber digestibility decreased linearly with increasing RDP to RUP ratio. Crude protein digestibility and blood pH remained unaltered across all diets with increasing RDP to RUP ratio. A positive N balance in early lactating buffaloes was observed across all diets and decreased linearly with increasing RDP to RUP ratio. Linear decrease in milk yield, milk protein and fat yields was observed with increasing RDP to RUP ratio. Percentage of fat, total solids, solid not fat and lactose remained unaltered across all diets. Study concluded that feeding diets containing 50:50 (RDP: RUP) increased DMI, milk yield in lactating dairy buffalo.

Buffaloes are capable of efficiently utilizing non protein nitrogen sources. Therefore, use of urea in the diets of buffalo has been studied extensively. Ahmad et al. (1983) observed the impact of complete dairy ration based on molasses and urea (containing 13.4% CP, 17.9% CF and 65.5% TDN) on 75 Nili-Ravi buffaloes and cows of three different genetic groups. Controlled group was offered conventional ration based on wheat straw and concentrate mixture (containing 50% wheat bran and 50% cotton seed cake). The average milk yield for control and experimental groups was found to be 5.5 and 6.9 kg, respectively. In a 90 days experiment, Shah and Muller (1982) studied the economic impact of feeding poultry litter to lactating cows and buffaloes and observed a non-significant difference in milk yield between control and experimental group of buffaloes (6.761 vs. 7.281). Furthermore, Tahir and Ahmad (1983) conducted an experiment on twenty buffaloes randomly dividing animals into four groups (A B, C and D). Group B, C and D were fed with three levels of urea i.e. 0.5%, 1% and 5% respectively. Group A was fed on ration containing cotton seed cake as a source of energy and found that average milk yield was 9.54, 9.52, 7.93 and 7.46 liters in the group A, B, C and D respectively. Authors concluded that vegetable protein source could safely be replaced urea in the ruminant ration but its increased level produced deteriorating effect on milk production. In another experiment Iqbal (1983) fed buffaloes on four rations containing 0, 0.5, 1.0 and 1.5 % urea, respectively. The average values for milk yield in group A, B, C and D were 9.60, 9.68, 7.96 and 7.53 liters per day. A slight
increase in milk production was reported in animals fed on ration containing 0.5% urea; however, sudden decrease in production was observed with increasing level of urea in the diet.

Effect of bovine somatotrophic (bST) hormone on milk production in lactating dairy cattle has been documented by several investigators. Ludri et al. (1989) carried out a study to determine the effect of bST on milk production in lactating dairy buffalo. Thirty Murrah buffalo, at mid-lactation stage, producing milk from 5 to 15 kg daily, were assigned to three treatments: 0, 25, 50 mg injection of bST for 14 days. Animals were fed on a mixture of green sorghum and corn forage ad libitum and concentrate ration 1 kg for every 2.5 kg of milk produced on daily basis. Results of this study revealed that buffaloes injected with the 50 mg bST daily produced 19% and 29.5% more milk compared to control group during the post treatment week 1 and 2, respectively. Authors concluded that administration of bST improved the production significantly. Ahmad (2009) evaluated the effect of long term bST administration on production performance of Nili-Ravi buffalo. Animals were divided into two groups: group A with no bST and group B injected bST 250 mg after every 14 days. Animals were fed according to NRC (2001). Maintenance requirements of animals were met through seasonal green forages while milk production requirements were fulfilled with a concentrate ration of CP 18% and ME 2.45 MCal/kg. Administration of bST enhanced milk production by 18%, 5.9%, 35% compared to those in group during the year 1, 2 and 3, respectively. However, lactation length was unaffected by bST administration in control vs. bST (253 vs. 260 days). Animals treated with bST were profitable earning a net profit of Pakistani Rs. 4227/animal during the lactation period of 305 days. Authors concluded that long administration of bST at 250 mg/animals repeated after every 14 days improved production, reproduction and profitability in Nili-Ravi buffalo.

In another investigation, Jabbar et al. (2009) evaluated the effect of increasing energy density of diets on production performance of bST administered lactating Nili-Ravi buffalo. Animals were fed on low energy density (LED) 85%; medium energy density (MED), 100%; and high energy density (HED) 115% of the NRC, (1989) standards. All the animals were injected with bST (every 14 days), and were fed on a straw based TMR during a period of 90 days. Daily milk production was highest 8.8 kg/day in animals fed high energy ration compared to 8.2 and 7.9 kg of milk produced by MED and LED fed animals, respectively. Dry matter intake was lower in high energy ration; whereas, feed efficiency improved linearly with increasing energy density of diet. Study concluded that bST administered lactating buffalo should be fed on TMR with 15% higher energy than NRC recommendations for improving milk production and feed efficiency. In a 2x2 factorial experiment, Italian buffalo were fed four diets; control, control diet plus 0.3 kg of calcium salts of long chain fatty acids, control diet plus 320 gm of bST and control diet plus 0.3 kg calcium salts of long chain fatty acids plus 320 gm of bST (Secchieri et al., 2005). Animals were fed corn silage based TMR with CP around 17% of DM. Bovine somatotrophic hormone was administered every 21 day. Milk production was higher in animals administered bST and Ca salts of long chain fatty acids. Body condition was reduced by bST administration; whereas, addition of fats reduced the weight loss. Short chain fatty acid decreased by addition of fats with calcium salt.
However, long chain, medium chain and unsaturated fatty acid concentration in milk increased by bST administration. Investigators reported a decrease in milk protein percentage in buffalo fed control diet plus calcium salts of long chain fatty acids. Authors concluded that addition of calcium salts of long chain fatty acid and injection of bST enhanced milk production in Italian buffalo.

**EFFECT OF NUTRITION ON BUFFALO REPRODUCTION**

To date, information about the effect of nutrition on reproduction of dairy buffalo is limited. Contrary to cattle, buffalo has been reported to have low fertility (Drost, 2007) leading to delayed age at first calving in heifers, and longer calving interval as well as seasonal and poor estrous behavior (Qureshi et al., 2002; Patro et al., 2003; Rakshe, 2003). Age at first calving can be reduced through proper nutritional management of growing heifers (Zicarelli, 2006). Qureshi et al. (2002) reported that age at first calving can be reduced significantly if heifers are fed properly to achieve higher daily gains. Borghese et al. (1994) documented an ADG of 562 vs. 465 g/day in heifers fed rations higher vs. low energy diets (5.56 MFU/d vs. 4.42 MFU/d, respectively) indicating that high energy rations improved daily gain and consequently reduced age at puberty. Qureshi et al. (2002) investigated the role of nutrition on reproduction and blood metabolites. Results of the study revealed that feeding higher protein rations to dairy buffalo resulted in higher plasma urea levels and reduced conception rate. Further, body condition score and post-partum ovulation were correlated with ME intake. Javaid (2007) reported a decrease in BUN and MUN with increasing levels of RDP in the diet. No differences were found for days open with change in RDP to RUP ratio. However, a higher conception rate was reported for buffaloes fed on rations low RDP to RUP ratio. Study concluded that feeding diet containing 50:50 RDP: RUP improved reproductive performance through increased feed intake and reduced BUN concentration.

Silent heat during summer months has been identified as a limiting factor in buffalo reproduction. In an earlier work of Zicarelli (1997) it was revealed that fall in temperature, and rains induced cyclicity in buffaloes. Therefore, it was suggested that proper nutrition along with proper housing management (showering or wallowing pond) can improve estrus behavior during summer through reduction in oxidative stress. Kahlon and Singh (2003) documented that supplementation of α-tocopherol (3,000 mg/week) reduced the oxidative stress and improved cyclicity in buffalo heifers. Other research found niacin and vitamin E + selenium supplementation beneficial in improving the reproductive efficiency of buffalo (Ezzo, 1995; El-Barody et al., 2001; Nayyar et al., 2002). In a recent work Sarwar et al. (2007) investigated effect of feeding sodium bicarbonate (SB) during summer months in early lactating Nili-Ravi buffalo. Supplementation of 1.5% of sodium bicarbonate (SB) during summer month’s improved reproductive efficiency through increased DMI and reduced services per conception. Authors explained that SB supplementation helped in reducing effect of cellular acidity (CO₂) due to higher metabolic rate in early lactation. Most recently work by Ahmad (2009), reported that administration of bST with a dose level of 250 mg after every 14 days, improved reproductive performance traits, post-partum estrous (98 vs. 160), service period
(115 vs. 207) compared to control groups (no bST) in Nili-Ravi buffalo. Although several authors have previously documented that delayed first calving and longer inter-calving interval is present in conventional buffalo farming practices. However, work from Zicarelli (2010) demonstrated that, if the nutritional requirements of growing heifers are met properly and the culling rate is reasonable, the first calving age and the inter-calving period in buffalo are similar to those reported in dairy cattle.

REFERENCES


Table 1. Domesticated buffalo breeds of the world.

<table>
<thead>
<tr>
<th>Name</th>
<th>Distribution</th>
<th>Lactation Duration (days)</th>
<th>Milk Production (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatolian</td>
<td>Turkey</td>
<td>224</td>
<td>1009</td>
</tr>
<tr>
<td>Azeri</td>
<td>Iran, Azerbaijan</td>
<td>200-220</td>
<td>1200-1300</td>
</tr>
<tr>
<td>Azi-Khel</td>
<td>Pakistan</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bangladeshi</td>
<td>Bangladesh</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bhadawari</td>
<td>India</td>
<td>274</td>
<td>780</td>
</tr>
<tr>
<td>Bulgarian</td>
<td>Bulgaria</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Murrah</td>
<td>Bulgaria</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Carabao</td>
<td>Philippens</td>
<td>NA</td>
<td>400 (Local)</td>
</tr>
<tr>
<td>Egyptian</td>
<td>Egypt</td>
<td>180</td>
<td>1850</td>
</tr>
<tr>
<td>Jafarabadi</td>
<td>India</td>
<td>350</td>
<td>1800-2700</td>
</tr>
<tr>
<td>Jerangi</td>
<td>India</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Kundi</td>
<td>Pakistan</td>
<td>320</td>
<td>2000</td>
</tr>
<tr>
<td>Khuzestani</td>
<td>Iran &amp; Iraq</td>
<td>210</td>
<td>1800</td>
</tr>
<tr>
<td>Lime</td>
<td>Nepal</td>
<td>351</td>
<td>875</td>
</tr>
<tr>
<td>Manda</td>
<td>India</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Meshana</td>
<td>India</td>
<td>305</td>
<td>1800-2700</td>
</tr>
<tr>
<td>Murrah</td>
<td>India</td>
<td>305</td>
<td>1800</td>
</tr>
<tr>
<td>Nagpuri</td>
<td>India</td>
<td>243</td>
<td>825</td>
</tr>
<tr>
<td>Nili Ravi</td>
<td>Pakistan, India</td>
<td>305</td>
<td>2000</td>
</tr>
<tr>
<td>Parkote</td>
<td>Nepal</td>
<td>351</td>
<td>875</td>
</tr>
<tr>
<td>Romanian</td>
<td>Romania</td>
<td>274</td>
<td>1800</td>
</tr>
<tr>
<td>Sambalpuri</td>
<td>India</td>
<td>350</td>
<td>2400</td>
</tr>
<tr>
<td>Surti</td>
<td>India</td>
<td>350</td>
<td>2090</td>
</tr>
<tr>
<td>Tarai</td>
<td>India</td>
<td>250</td>
<td>450</td>
</tr>
<tr>
<td>Toda</td>
<td>India</td>
<td>200</td>
<td>500</td>
</tr>
</tbody>
</table>
Table 2. Worldwide buffalo milk (whole, fresh) production from year 2006 to 2009.

<table>
<thead>
<tr>
<th>Country</th>
<th>Milk production (tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albania</td>
<td>10</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>35,088</td>
</tr>
<tr>
<td>Bhutan</td>
<td>389</td>
</tr>
<tr>
<td>Brunei</td>
<td>65</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>7,022</td>
</tr>
<tr>
<td>China</td>
<td>3,000,000</td>
</tr>
<tr>
<td>Egypt</td>
<td>2,702,960</td>
</tr>
<tr>
<td>Georgia</td>
<td>5,400</td>
</tr>
<tr>
<td>Greece</td>
<td>158</td>
</tr>
<tr>
<td>India</td>
<td>62,860,000</td>
</tr>
<tr>
<td>Iran</td>
<td>279,054</td>
</tr>
<tr>
<td>Iraq</td>
<td>23,000</td>
</tr>
<tr>
<td>Italy</td>
<td>210,000</td>
</tr>
<tr>
<td>Malaysia</td>
<td>10,659</td>
</tr>
<tr>
<td>Nepal</td>
<td>1,031,500</td>
</tr>
<tr>
<td>Pakistan</td>
<td>21,622,000</td>
</tr>
<tr>
<td>Philippines</td>
<td>240,000</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>41,600</td>
</tr>
<tr>
<td>Syrian</td>
<td>3,600</td>
</tr>
<tr>
<td>Turkey</td>
<td>32,443</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>33,198</td>
</tr>
</tbody>
</table>

Table 3. Comparison of milk performance in different buffalo breeds (kg/day).

<table>
<thead>
<tr>
<th>Breed</th>
<th>Lactation length (days)</th>
<th>Milk yield Average (Kg)</th>
<th>Milk yield per day (Kg)</th>
<th>Highest daily milk yield (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>280.4±20.2</td>
<td>1092.8±207.4</td>
<td>3.79</td>
<td>6.60</td>
</tr>
<tr>
<td>M</td>
<td>324.7±73.9</td>
<td>2132.9±78.3</td>
<td>6.57</td>
<td>17.40</td>
</tr>
<tr>
<td>N</td>
<td>316.8±83.6</td>
<td>2262±663.9</td>
<td>7.14</td>
<td>18.40</td>
</tr>
<tr>
<td>MLF₁</td>
<td>313.7±96.7</td>
<td>1240.5±479.8</td>
<td>3.95</td>
<td>7.57</td>
</tr>
<tr>
<td>MLF₂</td>
<td>313.9±90.1</td>
<td>1423.3±534.5</td>
<td>4.53</td>
<td>8.30</td>
</tr>
<tr>
<td>NLF₁</td>
<td>326.7±96.4</td>
<td>2041.2±540.9</td>
<td>6.25</td>
<td>16.65</td>
</tr>
<tr>
<td>NLF₂</td>
<td>321.4±118</td>
<td>2325.6±994.4</td>
<td>7.22</td>
<td>19.35</td>
</tr>
<tr>
<td>NMLF₂</td>
<td>317.6±78.4</td>
<td>2294.6±772.1</td>
<td>7.22</td>
<td>18.80</td>
</tr>
</tbody>
</table>

L = Local; M = Murrah; N = Nili-Ravi (Bingzhuang et al., 2003)
Table 4. Production performance of Nili-Ravi buffaloes in Pakistan.

<table>
<thead>
<tr>
<th>Performance trait</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>First lactation milk yield (litres)</td>
<td>1800</td>
</tr>
<tr>
<td>Overall lactation milk yield (litres)</td>
<td>2100</td>
</tr>
<tr>
<td>Average lactation length (days)</td>
<td>290</td>
</tr>
<tr>
<td>Age at first calving (months)</td>
<td>48</td>
</tr>
<tr>
<td>Service period (days)</td>
<td>230</td>
</tr>
<tr>
<td>Dry period (days)</td>
<td>250</td>
</tr>
<tr>
<td>Calving interval (days)</td>
<td>540</td>
</tr>
<tr>
<td>Gestation period (days)</td>
<td>310</td>
</tr>
<tr>
<td>Fat % in milk (%)</td>
<td>6.5</td>
</tr>
<tr>
<td>Total solids in milk (%)</td>
<td>17</td>
</tr>
</tbody>
</table>

Figure 1. Current worldwide buffalo milk (whole, fresh) production.
Brief Introduction to the Development of Chinese Dairy Buffalo Industry

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ABSTRACT
This review briefly introduces the current situation of development of dairy buffalo industry in China with regard to implementation and national service network construction of buffalo improvement, research progress and application of reproductive biotechnology and nutrition regulation on production performance of dairy buffalo, buffalo milk production and product processing, distribution and optimization for the industrial layout and development, as well as several exploration models worked effectively in counties.

Keywords: China, dairy buffalo, development, improvement, industry

INTRODUCTION
Water buffalo have been domesticated and utilized for approximately 7,000 years and, used mainly for draught with a long history, have played an important role to promote the development of agriculture in south of China. According to the latest statistical data on FAO website updated on 16 Jan. 2013 (FAOSTAT, Production, Live Animals, 2013), in 2011, China has the third largest buffalo population of 23,382 million, just ranking after 112,916 million in India and 31,726 million in Pakistan.

Water buffalo, accounted for 1/5 of the total number of cattle in China, mainly distribute in 18 provinces, and more than 80% in 9 provinces, i.e. Guangxi, Yunnan, Guangdong, Guizhou, Hubei, Sichuan, Hunan, Jiangxi and Anhui, each of which has the buffalo populations exceeding 1 million. Although the buffalo population In China is quite massive, representing 11.97% of the world’s total, however, the total milk production is still very low, representing only 3.33% of that (Table 1).

In China, more than 99.9% indigenous buffalo are swamp type, with lower milk and meat production when compared with river type buffaloes, i.e. Murrah, Nili-Ravi and Mediterranean buffaloes, and were selected for draft purpose in the vast rural area during their long historical cultivation. With the improvement of modern agricultural mechanization, the role of Chinese indigenous buffalo changed gradually from draught to milk-meat has become inevitable.

Actually, water buffalo milk processing in China can be traced back to as early as the late 19th century, when milk were collected from indigenous buffaloes to make local flavor dairy products in a few of coastal regions, such as Guangdong, Accepted April 10, 2013; Online November 11, 2013.
Zhejiang, etc., but the scale didn’t distribute industrially because of the limitation of milk supply. The milk performance of indigenous buffalo was increased greatly via crossbreeding with and improvement by several imported foreign river buffalo breeds since the 1950s, thus, the buffalo milk supply is lifted up dramatically.

In the recent 2 decades, with the rising of people's living standards, buffalo milk, as a nutrient-rich and safety-higher natural food, becomes gradually for public cognition, which continuously pushed up the market demand of buffalo milk and its products, meanwhile, pointed out the direction for China's buffalo cultivation and development of the industry.

DAIRY BUFFALO BREEDING AND MILK PRODUCTION
Crossbreeding
(1) Crossbreeding by Murrah and Nili-Ravi buffalo

Chinese indigenous buffalo are swamp type with smaller body-size and lower milk and meat production when compared with river type counterpart. An adult indigenous buffalo cow weighs about 250-400 kg and yields an average of milk for about 500-600 kg per lactation, or up to 800-1,000 kg after selection, with about 7.5% milk fat, 5% protein and 20% dry matter (Yang et al., 2005). To improve the production performance of Chinese indigenous buffalo and to increase the economic benefits for buffalo rearing, Murrah and Nili-Ravi breeds were imported from India and Pakistan in 1957 and 1974, respectively to crossbreed with the indigenous buffaloes, After exploration and research for decades, production performance of Chinese indigenous buffalo hybrid Buffalo greatly is improved greatly, esp. in milk production. It is reported that after crossbreeding of indigenous buffaloes with dairy buffaloes (Murrah and Nili-Ravi), the milk yield of the first and second generation Murrah crossbreds respectively reached 1,240.5 kg and 1,423.3 kg, which were 13.5% and 30.2% higher than that of selected indigenous buffaloes (P<0.01); the milk yield of the first and second generation Nili-Ravi crossbred in a lactation respectively reached 2,041.2 kg and 2,351.3 kg, which were 86.8% and 115.2% higher than that of local buffaloes (P<0.01); the milk yield of the triple crossbreds and offspring of triple crossbreds respectively reached 2,294.6 kg and 1,994.9 kg, which were 109.98% and 82.55% higher than that of indigenous buffaloes (P<0.01). The contents of milk fat, lactoprotein and dry matter in crossbred milk are 7.9%, 4.5% and 18.4%, respectively (Yang et al., 2007).

(2) A preliminary result of crossbreeding by Italian Mediterranean buffalo

The remarkable progresses indicated that the optimal way for improving the milk and meat performance of Chinese buffalo is multiple breeds (triple or more) crossbreeding or upgrading. For this purpose, several thousand Mediterranean buffalo semen straws were imported from Italy in 2007 so that more exotic bloodlines can be involved in the crossbreeding and improvement in buffaloes in China.

Currently, more than 300 hundred animals were born with varieties of pure Mediterranean buffalo, F1 of Mediterranean buffalo × Murrah buffalo, F1 of Mediterranean buffalo × Nili-Ravi buffalo and F1 of Mediterranean buffalo× Guangxi native swamp buffalo in the national buffalo breeding farm in Guangxi Buffalo Research Institute, China.
Studies were carried out to determine the climate adaptation in Guangxi and growth performance (body weight, length and height, the chest and abdominal circumferences, as well as the physiological and biochemical substance) of the F1 produced from frozen semen straws of Mediterranean buffalo introduced into China. The results of F1 of Mediterranean buffalo were similar with ones of the Murrah and Nili buffalo and there was no difference between the hybrid offsprings crossbred Mediterranean, Murrah and Nili-Ravi with Chinese native buffalo, which demonstrated that the Mediterranean buffalo was not only able to fully adapt to the environmental and climatic conditions in south of China, especially in Guangxi, but also showed good growth advantage.

**Reproductive biotechnologies accelerate the speed of elite breeding and expand the scale of improvement**

Reproductive biotechnologies in buffaloes were initiated in the early 1980s and remarkable progress has been made in the recent few years in China. The first IVF buffalo calf was born in Guangxi University (GXU) in 1993, and *in vitro* embryo production was improved remarkably since the first IVF buffalo twins and the first OPU-IVF buffalo were born in GXBRI in 2001 and 2004, respectively. Currently, several hundred calves have been produced via OPU-IVF-ET technique in the latest decade.

In addition, the successful cryopreservation of buffalo embryos guarantees the safe transportation and plays an important role in the nation-wide commercialization of embryo transfer (ET). Zhang et al. (2004) reported that the survival rate of vitrificated-thawed IVP hybrid embryo is 64.4% and the three calves were born after transfer the embryos into the surrogate Chinese swamp buffalo cows. From 151 riverine frozen embryos transferred in indigenous or hybrid buffalo, 18 calves were produced in GXBRI during 2002-2006, in which pregnancy rates of single- and double-embryo transfer were 23.5% and 28.6%, respectively.

Taking into account the huge number of indigenous buffaloes, embryo biotechnology allows Chinese breeders to use low-yielding (but reproductively efficient) indigenous females as surrogate mothers for the production of elite riverine calves through OPU-IVF-ET. The calving efficiency per year of the individual could be improved 7-10 times while the donors received OPU twice a week for 3 months continuously.

Also, Reproductive biotechnologies in buffaloes allow only one generation (4-year period) to produce 100% riverine-bloodline buffaloes via OPU-IVEP-ET system, when comparison that traditional upgrading has to take 3 three generations (12-year period) to cultivate only 82.75% riverine-bloodline buffalo crossbred exotic riverine buffalo bull with indigenous swamp buffalo cow, which compress dramatically the breeding years (reviewed by Yang et al., 2012).

**Sex pre-selection controls the gender of offspring**

To meet the demand from different breeders and farmers, the calf’s sex could be pre-selected through sexed semen: Sex predetermination of a calf is useful for both breeders and commercial farmers. Male calves could be selected preferentially by semen processing unit and beef farm whereas females by dairy farm.

Studies conducted in GXU demonstrated that Murrah and Nili-Ravi buffaloes were selected for flow cytometric sperm separation, with sorting
accuracies for X- and Y-bearing spermatozoa of 94% and 89%, respectively (Lu et al., 2006). The cleavage and blastocyst rates by using the sexed spermatozoa fertilized in vitro with abattoir-derived oocytes were 42.23% and 19.89%, respectively. The female twins were born in February 2006 after transfer of the presumed hybrid X-embryos (Lu et al., 2007). Liang et al. (2008) reported that embryos obtained from either OPU or abattoir-derived oocytes fertilized in vitro with the sexed sperm had similar developmental competence with regard to cleavage (57.6% v. 50.4%, respectively) and blastocyst development (16.0% v. 23.9%, respectively) rates. Furthermore, using frozen-thawed sexed versus unsexed semen did not affect rates of cleavage (50.5 vs. 50.9%, or blastocyst development (15.3 vs. 19.1%) after IVF using OPU-derived oocytes. The study provides a proof of concept for further research and a wider field application of these technologies in buffalo.

Up to the end of 2011, 9816 buffalo cows were inseminated with total of 19113 sexed semen, including 17715 X-semen and 1398 Y- semen, to deliver 4517 sex-controlled calves with an average 52% conception rate and a 89.03% accuracy rate of sex control. The success of pilot-scale experiment lays a solid foundation for large scale implementation of sexed semen, as well as plays a positive role on speeding up the elite breeding and promoting the development of buffalo dairy industry in China.

**Feed development and nutrition regulation makes maximum use of the production performance of dairy buffalo**

Development of feed resources for dairy buffalo mainly includes development of regional feed resources, evaluation of feed nutritional value, database construction of feed nutrient, efficient utilization via application and combination of pretreatment technologies, i.e. alakalization, ammoniation and silage, etc. Crude feed after ammoniation, alkaline treatment can greatly improve the nutritional value, increase the content of crude protein and crude fiber digestibility. For example, ammoniated rice straw can make crude protein content and rate of crude fiber digestibility increased from 3.5% to 10% and from 40~50% to 45~55%, respectively. The ultimate goal for development of feed resource is physiological characteristics in different key periods of feed resources and regional of milk cow of milk buffalo growth and lactation stage nutrition needs of ration formulation and application. The ultimate goal of feed resource development is, based on the features of regional feed resources and the physiological characteristics of dairy buffalo in key periods, to formulate diet formulation and application to meet their nutritional requirements in growth, lactation and other stages.

Buffalo nutritional needs are influenced by many factors, such as breeds, feed, feeding and management, etc., and are not static as well; it changes with the breeding selection and hybrid combination. Currently, researches on buffalo nutritional needs mainly focuses on milk protein, calcium, phosphorus, energy demand. For example, the application of gradient feeding experiment, digestion and metabolism experiment and comparative slaughter experiment helped us to obtain the requirement parameters and metabolic rules on energy, protein, calcium, phosphorus, etc. in different physiological stages.

At present, the researches on buffalo rumen microbial were mainly conducted to determine microbial species and its composition, mechanism for
ruminal fiber digestion, production of methane in the rumen, the relationship between diet composition and metabolic pathways (ruminal methane production and other), and rumen micro-ecology control technology, etc. The research results are important significantly to understand the unique buffalo rumen microbial resources for development and utilization of these resources.

Aiming at the issues in forage resource shortage and low efficiency in buffalo feeding, etc., studies on key technology and application of buffalo nutrition regulation and efficient utilization of feed are investigating to create and improve the key method and system of digestion, metabolism and optimal regulation on buffalo nutrition and feed optimization, and to reveal the related absorption and metabolism mechanism of energy and protein. Also, some studies are at the initial stage to explore the influence of optimization technology (e.g. dietary carbohydrate and protein structure) and additives applied technology (e.g. plant-derived antioxidants, enzymes, microbial agents and non-ionic surface active agent and others) on performance of growth and reproduction, quality of products and status of health in buffalo, and so on.

**Brief summary of milk production of water buffalo in China**

The FAO data of buffalo milk total output and average annual milk yield per animals for the last 4 decades (1971-2011) in China, India, Pakistan and the world were shown in Table 1 and Table 2, respectively (FAO, Production, Livestock Primary, 2013). Buffalo milk total output in China rose from 1.035 M tons in 1971 to 3.1M tons in 2011 with the average annual growth rate of 2.78%, which is lower than that in India, Pakistan and the world (4.12%, 3.74% and 3.83%, respectively) (Table 1). The average annual yield per buffalo in China increased from 356.9 kg in 1971 to 543.3 kg in 2011 (Figure 2) with the average annual growth rate of 1.06%, which is also lower than that in India and the world (1.78% and 1.41%, respectively), but higher than that in Pakistan (0.42%) because of the annual yield per buffalo was maintained at very high level (from 1,600+ kg to 1,900+ kg).

According to the "China Dairy Industry Yearbook 2009", in 2008, the populations of improved dairy buffalo and milking buffalo are about 400 thousand and 80 thousand, respectively. The average annual yields per milk buffalo are 1543 kg in Guangxi, 740 kg in Yunnan, 766 kg in Guangdong, 800 kg in Hubei, 800 kg in Henan, 825 kg in Fujian and 958 kg of the average in these provinces, respectively.

**The present situation of water milk processing in China**

After application of buffalo crossbreeding and improvement since 1970s, the total volume of buffalo milk used for processing are greatly increased to stimulate the development of buffalo milk processing. Some hybrid buffalo farms and then several small buffalo milk processing plants were established in main buffalo-production area, e.g. Guangxi, Guangdong and Hubei from the end of 1970s to the early 80's. Many demonstration bases of buffalo dairy development were sprouted, especially after the implementation of "China - EU buffalo development project" in 1997-2002, in cities and counties with good conditions on buffalo crossbreeding and improvement and and achieved good results to further promote the development of buffalo milk processing industry.

At present, China's buffalo crossbreeding and improvement has implemented
throughout all 18 buffalo-production provinces. However, the processing factories specialized in buffalo milk processing are rare, even enterprises involved in buffalo milk processing are only 18 up to 2005, processing capacity accounted for about 20% of China's buffalo milk production. At present the main buffalo milk products are: pasteurized milk, yogurt, milk beverages, condensed milk, butter and cheese, in addition, some special flavor products are produced by local traditional methods in some regions, such as milk bread, milk tofu, milk ginger juice, Urum etc. Also in some regions fresh milk are sold directly to consumers due to scattered milk buffalo and lack of milk storage equipment.

As shown in Table 3, data of production quantity of butter and cheese processed from buffalo milk from 2001 to 2011 in China from FAO statistics indicated that the average growth in cheese production is much higher than that of butter (FAO, Production, Livestock Processed, 2013).

Construction and distribution of china’s network for buffalo crossbreeding and improvement

Construction of national service network for buffalo crossbreeding and improvement

Firstly, a National Coordination Committee on buffalo crossbreeding and improvement under the leadership of the Ministry of agriculture is necessary to unify the deployment for the tasks throughout China and guide in the provincial level. Similarly, the provincial committee in each province can aim at the same purpose and guide in the district- and county- level. Then, technical service network forms at five different levels, i.e. the provincial poultry and livestock improvement station, the prefecture-level nitrogen transfer station for frozen semen, county livestock improvement station (or animal husbandry and veterinary station ), town and village bovine artificial insemination station (site).

Taking Guangxi as an example, at of the end of 2010, the region has established 13 liquid nitrogen transfer station for frozen semen. There are 96 counties involving in buffalo crossbreeding and improvement with a total of 1,997 AI stations (sites) and 2,611 AI technicians, of which 1610 were working for the village AI sites. In additional, multi-level, multi-channel trainings were organized for the AI technicians by animal husbandry departments at all levels in order to improve their service techniques and skill.

Optimizing the regional distribution of dairy buffalo industry

In order to make the distribution of milk production in China is more scientifically reasonable, China has issued “11th Five-Year Development Plan (2006-2010) and Long-Term Goals Until 2020 for the Dairy Industry” and has created five dairy production regions for specialization based on local conditions, i.e. Large City Surrounding Region, Northeast-Inner Mongolia Region, North China Region, West Region and South Region (USDA, 2010). The South Region covers 13 provinces and has the population of no more than 1 M cattle whereas more than 5 M buffaloes, thus the region is also called the South Buffalo Dairy Region and will vigorously develop dairy buffalo. During the construction and development of the South Region, Guangxi, Guangdong and Yunnan become the 3 priority provinces because of the advantages on buffalo herds and industrial foundation, etc.

In the South Buffalo Dairy Region, industrial sub-regions should be
optimized and divided into breed improvement area, crossbred buffalo feedlot area, milking buffalo rearing area, and dairy processing area according to combining the function characteristics of each link in the water buffalo dairy industry development with the developmental foundation in different region. Also, in the milking buffalo rearing area, licensed fresh milk purchasing stations and enterprise milk collection zones should be distributed reasonably to ensure an orderly development for dairy production.

The current exploration models of development of buffalo dairy industry

In order to better support the development of buffalo dairy industry in China, Guangxi has the maximum amount of buffaloes and is the earliest province implementing in improvement of native buffalo and development of buffalo dairy industry. With good technological reserve and extensive mass foundation, several exploration models, e.g. “company-cooperative-farmers”, “orchard-grass-buffalo-marsh gas” and “company-base-farmers”, have been formed to adapt to different regions and levels of economic development, furthermore, these models are gradually expanding to other regions to improve the degree of organization and create a sustainable path toward the buffalo dairy production. The followings are the typical models formed in counties and companies:

1) Company–cooperative-farmers in Lingshan County, Guangxi

Lingshan County, Guangxi is famous for milk buffalo rearing and development and is entitled in “Hometown of Milch Buffalo in China”. After 40 years of development, milk buffalo has become a local characteristic advantage resources. At present, Lingshan County has become the largest production base of milk buffalo and development base of buffalo dairy industry in Guangxi. The exploration model of “company- cooperative-farmers”, companies support the farmers to build standard cow sheds, extend buffalo improvement and high-quality pasture grass, and recover buffalo milk with insured price as well, to promote the milk buffalo feeding on large-scale, standardized, intensive development. In addition, Promotion of “adoption” or “foster” mode via providing buffalo cow to the poor families helps them become rich.

2) orchard-grass-buffalo-marsh gas in Beiliu City, Guangxi

Beiliu City launches the buffalo improvement program since 2002. A 3-dimensional milk buffalo ecological feeding mode (called orchard-grass-buffalo-marsh gas) was set up to make full use of the local advantage resource of huge litchi, longan planting area. This mode links the orchards, pasture grass, buffalo, faeces, marsh gas and fertilizer as an ecological chain to not only fully utilize the orchard and provide adequate and stable feed source for buffalo dairy industry development, but also further promote the development of related industries and the comprehensive recycling use of resources to create a high quality, safe, pollution-free green mode.

3) company-base-farmers in Dongyuan Company, Hepu County, Guangxi

Guangxi Dongyuan Ecological Agriculture Technology Co., Ltd. (hereinafter referred to as “company”) is founded in October 2005. Milk buffalo feeding in the company are guided by the market and are formed in large-scale, mechanization, harmless treatment, scientific management, localization of feed utilization. Now the
Company has more than 3000 hybrid buffaloes, of which 2576 are in 3 milk buffalo feeding demo bases and 576 are in 152 households in 4 demo villages. This “company-base-farmers” mode combines the leading company, demo base and village farmers together to achieved a sustainable path for dairy buffalo feeding with good economic, ecological and social benefits.

At present, many other different but similar models are developed in other provinces according to local resource advantage and environmental conditions, for example: “company + peasant household”, “company + demonstration area + farmers”, “dairy farmers association”, and so on. But no matter what kind of models are, they take the market as the guidance, vigorously support the leading enterprises, give full play to driven role and radiation effect of leading enterprises, keep the production, processing, sales channels smooth, extend the industrial chain, increase the added value, improve industrial economic benefits, and finally ensure the buffalo industry healthy, sustained and rapid development.

CONCLUSIONS
The milk buffalo industry in China is an emerging and sturdily growing industry, and has great development potential and vitality. In recent years, China’s central and local governments attached great importance to water buffalo industry and introduced of relevant policy support to support the development of the industry. Buffalo dairy industry in China ushered in a new hitherto unknown opportunity. At the same time, the development of dairy buffalo industry is a systematic engineering with heavy workload and large difficulty, involving wide disciplines and many departments, thus, it needs full support and continuous guidance from the government. But we have reasons to believe, in the near future, the milk buffalo industry is bound to become China's another pillar industry in agriculture and rural economy and important ways toward income increase for rural farmers.

ACKNOWLEDGEMENTS
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Table 1. Whole fresh buffalo milk production in the world, China, India and Pakistan (tons), 1971-2011.

<table>
<thead>
<tr>
<th>Year</th>
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<th>China</th>
<th>India</th>
<th>Pakistan</th>
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<tbody>
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% of world in 2011 % 3.33 67.03 24.69

Ave. Annual growth, % 3.83 2.78 4.13 3.74

Population in 2011 (hd) 195266180 23382130 112916000 31726000

% of world pop. in 2011 100 11.97 57.83 16.25

Source: FAO, 2013
Table 2. Average annual yield per buffalo in the world, China, India and Pakistan (kg), 1971-2011.

<table>
<thead>
<tr>
<th>Year</th>
<th>World</th>
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<th>India</th>
<th>Pakistan</th>
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<td>1327.5</td>
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<td>2006</td>
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<td>528</td>
<td>1603.9</td>
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<td>1578.3</td>
<td>543.3</td>
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<td>1934.8</td>
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</table>

Ave. Annual growth, %

1.41  1.06  1.78  0.42

Source: FAO, 2013

Table 3. Production quantity of butter and cheese produced from buffalo milk in China (tons), 2001-2011.

<table>
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<tr>
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<td>9300</td>
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</tr>
<tr>
<td>2011</td>
<td>9300</td>
<td>12400</td>
</tr>
</tbody>
</table>

Ave. Annual growth, %

0.05  1.47

Source: FAO, 2013
Buffalo under Threat in Amazon Valley, Brazil

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ABSTRACT

Since 1986 after a visit to Brazil, the eminent scientist and FAO expert Prof. Dr. William Ross Cockrill considered the Brazilian Amazon Valley as the “buffalo paradise” due the utilization by grazing buffalo of a profitable off-take from the land. In the regions of lower and middle Amazon River due tropical environmental condition of the grassland soils, the flood plains are closely related water level of the rivers, which show a five meters difference between the driest season (November and December) and fuller (May and June). Thus in western Pará region where the flood period coincides with the most intense rainy season and less rainy with the ebb of the rivers many small ponds and lagoons are seasonally formed which give a natural excellent conditions for livestock, that is highlighted by abundance of native forage with high nutritive value. At the time of full, pastures are flooded, making grazing possible only for buffalo species that moreover causes weight loss and even death of other animal species, especially cattle. The current development process in the floodplains areas in western Pará region is leading to progressive degradation of ecosystems. The large livestock animals, cattle and buffaloes represent one of the most important socio-economic activities of small and medium producers. However, many factors have threatened the buffalo breeding in this region and generated controversy about the viability of this important economic sector in the region, and case any social measures and advanced breeding techniques are not taken into the problem, buffaloes will tend to disappear from the regional scenario. The disorderly occupation of these areas and the lack of economic alternatives for the riparian "justify" the intense exploitation of natural resources because they depend for survival. From 1975 to 2000, the buffalo population in Brazil has increased by about 13% per year, making it one of the fastest growing in the world flocks, however at the regional level since 2000 when the population reached about 200 thousand heads began a decline of buffalo herd, which now came in 2012 to less than 110 thousand heads. Although buffaloes showed higher productive and reproductive performances when compared to cattle, on the other hand this fact is mitigated by the unclear role that buffalo seems to plays in a floodplain controversy conflict where it has being accused of altering the floodplain environment and interfering with other productive activities such as fishing and agriculture. In this paper it is discussed the main causes and actors involved in that conflict which were classified as social like the land question, farmers, smallholders and riverside communities, religious groups, Accepted April 10, 2013; Online November 11, 2013.
nongovernmental organizations – NOGs, media, local riparian unions and associations, financial institutions as well as factors affecting the buffalo management production system such as errors in the herd management, health inefficient herds sanitary control, problems of inbreeding, price of milk and meat, indiscriminate animals export and slaughter, neglect of government agencies and lack of alternatives for new opportunities for buffalo development seems to be directly related to the problem.

Keywords: Amazon valley, Brazil, buffalo, floodplain, management

INTRODUCTION

Buffalo is a precious animal genetic resource, especially for tropical areas where it plays an important role in the livelihood earning of the rural inhabitants of many regions. Despite of the all the characteristics and peculiarities, buffalo has been under threat in some areas of the world, as now occurred in Brazil, especially in the Amazon valley which was once considered by FAO as the “buffalo paradise”. In spite of that there are people working on regional basis against such aberration. A group of farmers, technicians and scientists are looking for a regional and international cooperation on the issue of buffalo decline in the middle and low Amazon region. In this context, the addition of value addition to buffalo milk is one of the key points to help buffalo farmers to conserve their herds. Livestock keepers are the responsible for their breeds and the related indigenous knowledge is the basis for sustainable husbandry practices. With the increased pressure on land use, both the keepers and ultimately the indigenous knowledge are under threat.

Thus the aim of the present report is to bring publicly to the local and international community the danger facing the buffalo herd in the region of the middle and lower Amazon, looking this way fully inform the aspects involved in this problem and seek initiatives to create effective measures steps towards with the commitment of persons, private and public institutions on the problem and look for a short and long-term aims to solve this problem.

BACKGROUND

The Brazilian Amazon region covers 57% of the territorial surface of the country, 65% of the Panamazonian continental area and 28% of the South American continent surface area. Besides, about 70 per cent of the area, or 3.37 million km², are forest ecosystems, areas of native grasslands and savannas of good and evil drained soils and alluvial floodplain, grasses and legumes. Only living in the Brazilian Amazon are 21 million of people or 12% of the Brazilian national population, of which four million developing activities in the primary sector and 40 % as manpower in animal production (IBGE, 2011).
In the Amazon region the floodplain, high and low, is a shaky ground, with recent origin, which owes its formation to a process of sedimentation of suspended particles, brought from other regions by the muddy waters of the rivers; and the process continues with full intensity. In this process, the soil is rejuvenated annually in cycle of ebb and flood of the Amazon River. The floodplain has a tendency to raising the ground level and thereby simultaneously reducing the annual flood period. The lowland terrain consists of a line level higher topographic accompanying the riverbank, the sandbank, and where focus riparian forests along slopes, and lower zones covered by the "grasslands", flood, formed excellent pastures (Sioli, 1951, McGrath et al., 2011). The soil studies conducted by Falesi and Silva (1999) in the floodplain lands demonstrated that chemical characteristics of the component units of the dominant soils, as Low Humic Gley, alluvial soils, and soils Umbraquults Halomorfics, these protruding up to the Solonetz-Solodizados are normal showing an interchangeable bases saturation above 50%, demonstrating its high fertility.

THE FLOODPLAIN ECOSYSTEM IN LOWER/MIDDLE AMAZON REGION

From the perspective of resource management by the smallholders, it can be distinguish four main elements of landscape: the major river channels, sandbanks with natural forests bordering the canals, permanent floodplain lakes that occupy much of the interior of the floodplain, and seasonally flooded fields that covering the transition zone between lakes and marshes.

In these regions, the flood period coincides with the higher intensity of rainfall and fewer rainy seasons with the ebb of the rivers. At this time, the lowland native grasslands have excellent conditions for livestock, which is evident from the abundance of native forage with high nutritive value. In flood season, the pastures are flooded, reducing the grazing area, which causes weight loss, and even death of animals, especially cattle, that may force to move their livestock to other areas, many of these with the need to rent pasture area, which can cost up to US$ 8,00 per animal per month. Figure 1 illustrates the seasonal variation in rainfall and river level of Amazon River and its tributaries (McGrath et al., 2011).

This system ensures that each home visit the four main areas of the floodplain. While private properties are recognized, there is a gradient of private property for collective ownership and use as one of the bar changes to inland lakes. The sandbanks, where virtually is all investments of households are concentrated and are clearly demarcated. Fields, although nominally privately owned, tend to be treated as common in which landowners can raise different domestic animals species mainly cattle, buffalo and pigs. Since there was no fence dividing the fields, it’s common that flocks from different owners feed together in same area. On the other hands, inland lakes are considered common property of those who own land around them, both communities as large farms. There is a strong seasonal dimension in the life of the floodplain resulting from the interaction between flood and precipitation. In the lower Amazon River fills gradually from December to June and then quickly dry from June to early November. The flood and ebb of the river coincide with precipitation patterns, resulting from two seasons: a dry season of low water levels
and lowering extending from July/August to November/December and a rainy season of high water levels and soaring December/January to May/June. Further the most intense period of drought occurs halfway through the season water levels lower, effectively cutting off the growing season into two periods. In terms of risk in cultivation, the main issue is the interaction between when land becomes available for planting in the dry season in October, and when the place is flooded again essential dynamic floodplain ecosystem is captured in the concept of flood pulse in which the high productivity of floodplain ecosystems is due to the flood pulse and its impact on ecological production in aquatic and terrestrial switch phases (Lima et al., 2001; Martínez. 2002; McGrath et al., 2011). Small properties in the floodplain employ diverse economic strategies involving various levels of emphasis in one of four major activities: fishing, farming and agriculture, with small livestock (chicken, duck and pigs) having a minor role, however large livestock animals, mainly cattle and buffaloes represents one of the most important socio-economic activities for smallholders and medium farmers. The most important gramineous stratum in terms of feed, according to Camarão and Souza Filho (1999), are: canarana verdadeira (Echinochloa polystachya), rat-tail (Hymenachne amplexicaulis and Hymenachne donacifolia Nees), Andrequicé (Leersia hexandra S.W.), uamã (Luziola spruceana Benth), mori grass (Paspalum fasciculatum Wild), wild rice (Oryza grandiglumis) and others. The use of pasture soils of flood plains is related the level of water in the rivers. In flood season, the pastures are flooded, grazing difficult, which causes weight loss and even death of animals, mainly cattle Camarão and Souza Filho (1999). At the peak of the dry season, depending on where they grow, the alluvial soils of pastures wetlands can produce more than 20 tons of DM/ha/year, (Serrão, 1986).

Notwithstanding, inadequate management and progressive increase of buffalo herds have generated controversy about the viability of this important economic sector in the region, case-control measures resource usage natural are not taken. The disorderly occupation of these areas and the lack of economic alternatives for the riparian "justify" the intense exploitation of natural resources because they depend for survival. .

Management strategies for smallholders take advantage of spatial and temporal variations in resource availability, since most farming occurs in higher sandbanks border the channels during the dry period. The smallholders begin land preparation in July or August, so the ground is dry enough to work, and reap the first harvest in October. They can plant a second crop when the rains begin in December to harvest in February or March, before the waters reach the sandbank. Internal fields are used for livestock during the dry season. When the waters cover the fields in March / April, the cattle are removed to corrals high in salt marshes or taken to pastures on land until the waters begin to recede in July / August. Most fishing occurs in the floodplain lakes. The river fishing is more important as the water level low and the shoals leave the floodplain and migrate upstream. At lower water levels, fish that remain are concentrated in even smaller water bodies, facilitating the capture (Homma, 1998; McGrath et al., 1998; Winkler-Prins and McGrath, 2000; McGrath et al., 2011).

Lowland communities range in size from 40 to 130 families, or
approximately to 200-650 people. Communities have a linear structure with houses extending along the sandbank overlooking the river. Usually a Catholic church, school and in some cases, a community center mark the center of the community. Most community organizations have their origins in the activities of the Catholic Church and include a group of catechists, a group of young mothers, one or more football clubs and a representation of municipal fishing colony. Community leadership is generally organized in one or two forums, a council composed of leaders of major community organizations or a presidential system with members elected for one or two years.

**A SHORT REVIEW OF BUFFALO BREEDING IN THE AMAZON**

Since long time, buffalo has played a fundamental role in agriculture in Asia like producer of milk, meat and work. In Latin American countries, especially in Brazil, its contribution to this aspect can be of great importance in small and medium-sized farms.

The history of domestic buffalo in Brazil, started with its introduction was in 1895, of the Mediterranean breed, originating from Italy, by the farmer Vicente Chermont Miranda in the Marajo Island, the big island located in the mouth of Amazon River. Thereafter, began several batches of imports of buffalo to different regions Brazil. In recent years, buffaloes have become an important alternative source of production of food to meet the demands of developing countries, for better exploit the low nutritional value of forage in areas difficult to use by other species and agriculture.

In spite of the all the characteristics and peculiarities buffalo lived and survived in the wild management on the island of Marajó and only after the fifties, the local farmers decided to explore them as productive activity. Moreover, some time later, the early sixties, were introduced animals and breeds Murrah and Jaffarabadi, in the North and Southeast of Brazil. These animals well adapted to tropical conditions found in the Amazon and its "habitat" ideal, which was considered by the eminent Dr. William Ross Cockrill, FAO buffalo expert as a “buffalo paradise” which has demonstrated excellent skills to produce meat, milk and work plus satisfactory reproductive rates without causing environmental damage. That being so buffalo found in Amazon region a natural "habitat" ideal. Thus the primacy of grouping animals of Mediterranean, Murrah, Jaffarabadi and Carabao breeds has provide to Brazilian primary sector a possibility to have such important form of commodities. They are excellent producers of meat, milk and work, and considered environmentally friendly animals, producing and playing areas and adverse idle native pasture land flooded, where cattle can barely survive.

The buffalo ranching in the Amazon in recent decades has experienced phenomenal growth, with surprising results, in view of its satisfactory profitability. This development raises increased income for the farmer, and jobs, in the various segments production chain. Conversely, in other regions of Brazil, expanding weakened, as areas that could be opened to livestock were running out and / or being used in agriculture. Furthermore, the accumulated scientific knowledge on buffalo production systems, has indicated these animals are the new ecological option for the occupation of vast areas of degraded pasture in the Amazon, producing and
reproducing in an exceptional manner, without harm to the environment and with positive effects partner for the local economy. According to the research results, the buffalo breeding is a viable alternative to the use of the wetlands due to their hard use by other species. Also, there are technologies for the development of buffalo in grassland ecosystems on land cultivated, through the recovery of degraded pastures.

**THREATS TO BUFFALO HUSBANDRY IN THE MIDDLE/LOWER AMAZON REGION, BRAZIL**

According to official data, in 2000 the population of buffalo in the middle/lower Amazons average was approximately 200 thousand head, but the current in 2013 is 106,839 heads (ADEPARÁ, 2013).

The low profitability of buffalo in traditional farming systems of the Amazon makes this activity economically unattractive, with the prediction that the medium and long term, there remain only those who are competent to adapt to the new reality of economic changes. This fact has induced the farmers to transfer part of the buffalo to areas already formed with pastures on land, or investing in the recovery of degraded areas, previous used for agriculture practices or pasture formation for buffalo production system, as a way to increase productivity and make this activity economic competitive. On the other hand, livestock pastures in the Amazon, has been stigmatized as responsible for the low efficiency of land use. The reduced use of inputs and technologies of the '60s and '70s have been replaced by more productive systems and perennial pastures, as a result of the use of technologies.

The profitability of livestock production depends on the effectiveness of the operation, the rational management of pastures, breeding of the herd, intensive production of meat and / or milk, high productivity, with economy and trade.

Small farmers adopt the buffalo as savings, because it is a strong currency and easily redeemable. The lucrative income from this activity becomes more palpable as the productive sector adopts new technologies and combines administration with maintenance costs of pastures. Clearly, reduction of deforestation is the key to the conservation of forest resources. Accordingly, the proper use of livestock in degraded pastures seems to be the obvious alternative to reduce deforestation. A pilot program of artificial insemination for the introduction of new genes regionally, was released by UFOPA and has shown satisfactory results. This provided for the extension of this program to meet a larger number of farmers. The semen breeding Murrah with milk production traits has been stimulated.

**BREEDING MANAGEMENT**

The breeding of buffaloes in floodplain use a primitive management and is based largely on native seasonal pasture, so the growth rate of the buffaloes follows the seasonal pasture growth. Errors in the management Heat stress represent a major factor for impairment of fertility in tropical and subtropical countries and need effective strategy for smallholders to overcome the harmful effect of heat stress on health condition and reproductive efficiency of buffalo cows as well as for the bulls.
HEALTH - INEFFICIENT SANITARY CONTROL HERDS

The primitive management methods used is combined with an inefficient sanitary control of infectious diseases, which affect every domestic species raised on the floodplain conditions. Diseases such as brucellosis, tuberculosis and others not yet described affect cattle and buffaloes. However when compared with other domestic livestock buffalo are generally a healthy animal. As the Foot and Mouth diseases (FMD) is now under control through compulsory vaccination program, the greatest buffalo losses are often among calves. Tuberculosis is the most important disease that affects directly the development of buffalo breeding in the meso region of the lower and middle Amazon. Neves et al. (2013) in a recent survey found that the prevalence of positive animals for TBC 20.48%. Furthermore, heavy mortality in calves is observed occasioned by Toxocara vitulorum, which if not controlled, causes high mortality rates. Moreover there is no committing enough time to observe, control and handle diseases by the smallholders. There is no appropriate program for vaccines and medication uses among the farm communities.

PROBLEMS OF INBREEDING

Whereas the genetic founding event (initial population) of buffaloes in Brazil in general, characterized by a reduced genetic variation of the invading population, especially in Murrah, Jaffarabadi and Carabao breeds, because there has been very few importation of animals selected and semen of animals tested and superior quality, is of course admit that the small number of founders, coupled with total disruption of genetic selection imposed, for example, in Marajó Island and in the Lower and Middle Amazon that contradict the Hardy-Weinberg, causes various problems of pathological heredity will be diagnosed in the regional herds where more than 25 diseases, mono-and polygenic have been diagnosed. Moreover, there has been a decrease in body weight and milk production in some affected herds of inbreeding. Uncontrolled mating leading to inbreeding due the gregarious behaviour that buffaloes have seems to be a serious problem at regional level.

MARKET PRICES FOR MILK AND MEAT

While in other regions of Brazil buffalo meat has a guaranteed market, with prices higher than in cattle, where regional meat is depreciated and the amount paid unreal, being more down about 20 percent compared to the bovine. This problem is accompanied by an economic exploitation of marchers, who pay the producer of buffalo a lower price, however when buffalo meat sold at retail, it is not sold as buffalo but as beef.

In regards to milk, although the sub-products of fresh buffalo milk, especially milk and cheese has a market price higher than beef, the demand for these products is high in the local market as well as throughout the state of Pará and Brazil.

It must be taken in account that a way out of the economic stagnation which is the production site of Buffalo is to establish farms nuclei of this species in areas outside the floodplain seeking to put the meat and milk of buffalo with forthcoming differentiated, regardless of what occurs in other regions of Brazil.
INDISCRIMINATE ANIMALS EXPORT AND SLAUGHTER

The large ruminant livestock, cattle and buffaloes in the study area represents one of the most important socioeconomic activities of small and medium farmers. With the current development process in the areas of wetlands in this region are in the process of change, where the buffalo are considered a problem and pointed out as a cause of progressive degradation of the ecosystem of the floodplain, farmers are turning to the sale of summary their flocks. Thus, within the context of this study, the indiscriminate slaughter of buffalo in all categories, especially cows seems to be one of the determining factors for the regional population decline of this species. Otherwise also selling arrays to other regions of Brazil and in particular for countries bordering such as Colombia and Venezuela, has been identified as a major responsible for the decrease of the local population. It is estimated that over 10,000 female buffalo have been exported in the last decade.

NEGLECT OF GOVERNMENT AGENCIES

There are several problems associated with the lack of attention of governmental agencies to small and medium farmers in the floodplain areas. Thus one of the main problems existing in the areas of floodplains seems to be the land, where the occupants / owners do not have outright ownership of land - tenure. Without the definitive document - land tenure, there is no possibility of financing by banks. This factor, limits the introduction of technology in the sector, leading to a cycle of permanent stagnation. Moreover small and medium farmers in the floodplain are totally underserved by funding agencies and government extension.

CONCLUSION AND RECOMMENDATIONS - OPPORTUNITIES FOR DEVELOPMENT

There is need to recognize that there are many constrains which are affecting the buffalo breeding at regional level. This implies that there is need to change many aspects involved in the actual buffalo production system in the floodplain in order to increase productivity of the regional herd and ensure efficiency in resource utilization. Although floodplain can be considered an adequate place to raise buffaloes economically, due the increased pressure on the land use associated to a campaign orchestrated by people and organizations opposing the presence of buffalo in the areas of floodplains, makes it seem more feasible from a technical and economic, foster and establish the breeding of buffaloes on small farms in upland areas, mainly taking advantage degraded areas where agricultural activity once existed or livestock. Thus deal with these problems already identified, we suggest the following measures:

- Preparation of a local development project (LDP) on buffalo production system to be promoted is promoted in order to produce milk for cheese production, milk preparations, yogurt and others;
- Establish with the future technology park created by UFOPA an experimental plant for processing of buffalo milk in the form of a company incubated
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**Figure 1.** The seasonal variation in rainfall and river level of the Amazon River and its tributaries. Adapted from McGrath et al. (2011).

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**Figure 2.** Presentation of buffalo population in the region of the middle/lower Amazon region (ADEPARÁ, 2012).
Figure 3. Estimate decrease of buffalo population between 2000 and 2012, in the middle/lower Amazon region, Pará State, Brazil. Adapted from ADEPARÁ, FAEPA & SAGRI (2012).
Buffalo Share in Small Farmer Welfare under Intensive Agricultural System: The Case Study of Egypt

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ABSTRACT

The study focused on the share of buffalo in generating income for small farm household, and its relation to crossing the poverty line. Such farm category represents the majority of farmers in the countries raising buffaloes in Asia and Africa. The small farmers in Egypt are the holders of less than 2 ha. Such category represents about 92% of the total agricultural holders. Even though, they hold only less than 50% of agricultural area, 72% of cattle and 87% of buffaloes. 10% of buffalo holders are without agricultural land holding and 14% of cattle holders are also without land holding. The study used a farm sample survey of 120 small farms in rural Egypt to achieve its objectives. The analysis of the sample survey data showed that the average land holding of the small farmer is less than 1 ha and the average household size is 5 persons. The average buffaloes stock on farm is almost one head and its followers. The main feed source is the cultivated winter green fodder “Berseem, which occupies on the average about 25% of the farm land. While 62% of the farm managers are fulltime males 20% are fulltime females. Whereas, 77% of wives share in farm labor 56% of them share in livestock operations.

The estimated total annual income of small farm's household is about US$ 4,170. Livestock generates more than one third of this income, poultry share is 1%, and crop income share is only 18%. i.e., around 52% of the small farm income is generated by agricultural enterprises. The rest, i.e. 48% is from off farm income. The sources of off farm income are, 3% from off farm agricultural work, 40% regular salaries, 5% from remittance of the farmer's work abroad. The study estimated the daily per capita income per household as US$ 2.3, which just passes the poverty line. Milking buffalo has a significant role in alleviation of poverty that might face small farm households. Without milking buffaloes holding on farm the household would suffer from being significantly below the poverty borders. To raise milk productivity is the proper approach to increase the income generated by buffalo, once there is no room for horizontal expansion. It requires an (A.I.) program using imported semen of potential high milk yield from e.g. Italy. Such program should be associated with reform of the institutional framework in the Egyptian village and establishment of an efficient marketing system, modern animals’ health care and a nonconventional feed regime.

Keywords: agricultural resources, equity, farm income, poverty alleviation
INTRODUCTION
The study focused on the share of buffalo in small farmer welfare. Such category represents the majority of buffalo holders in Asia and Africa (Nasser, 2009; Soliman, 2009). Therefore, they should be the target group of any proposed integrated rural development program in these countries.

The generated income from farming is a final proper indicator of welfare for small farm household. The higher the farm income, the most probable the small farm household will cross the poverty line. Employment of the household members in farming and buffalo production on farm is another criterion of such welfare, condition that the employment opportunity on farm is economically feasible, (Soliman, 2008). Even though the small farmers are willing to achieve welfare, the lack of equity in agricultural land holding and thereof, disability to acquire a satisfactory income enhanced a fast stream of migration to urban or even abroad (Soliman and Mashhour, 2002).

DATA AND ANALYTICAL METHODS
The socio-economic literatures on Egyptian rural had identified the small farm size of less than 5 feddans (Soliman, 2006). One feddan (Area unit in Egypt) = 4200m². The US$ exchange rate for one Egyptian pound was 6.07 in 2011 (Central Bank of Egypt, 2012). The study used a farm sample survey of 120 small farms in rural Egypt as a case study. They were collected from four villages. While two villages were close to urban market, the other two were relatively far from the urban market. The urban market was identified as the capital of the district. The selected district (Minia Al Kamh) occupied the first rank of livestock holdings in Sharkia governorate. Such governorate represents the rural community of Zagazig University (80 kilometers from Cairo). 30 small farms were randomly drawn from each village, according to three small farms categories (10 farms from each). These categories were (<2 feddan), (2-4 feddan), (4-5 feddan). The sample method was identified as cluster (village) stratified (farm size category) random (farms within each category). The appropriate analytical models were applied to achieve the study’s objectives. These included the farm income statement analysis and the estimation of some response functions (regression analysis). In addition, the GINI coefficient was estimated to quantify the magnitude of inequalities of the agricultural resources (land) distribution among farm holdings in Egyptian rural. The Gini coefficient is often calculated with the Brown Formula shown below.

\[
G = \left| 1 - \sum_{k=0}^{k=n-1} \frac{x_{k+1} - x_k}{x_{k+1} + x_k} \left( \frac{y_{k+1}}{y_k} + \frac{y_k}{y_{k+1}} \right) \right|
\]

Where;
- \( G \): Gini coefficient
- \( X \): cumulated proportion of the population variable
- \( Y \): cumulated proportion of the income variable

In order for \( G \) to be an unbiased estimate of the true population value, it should be multiplied by \( \{n/ (n-1)\} \), (The World Bank, 2004). The Gini coefficient is an index of wealth concentration. The coefficient represents the gap between the
perfect distribution of a country’s diagonal and a country's actual distribution curve of wealth. It is a score between zero and one, although it sometimes appears in percentile form. It represents the degree of inequality in the distribution of income in a given society. A Gini score would register zero (0.0 = no inequality) where each member has exactly the same share of the country’s wealth (resource). The Gini score would register one (1.0 = maximum inequality) if one citizen received all the wealth and the rest of society gets nothing.

RESULTS AND DISCUSSIONS

Lack of equity in agricultural resources distribution

About 92% of the farming households hold farms are less than 5 Feddan, even though they hold less than 50% of the arable land. Estimated Gini coefficient was 44% for the consistency between the number of holdings and arable land which implies poor equity of agricultural resources. On the other hand, the small farmers hold the majority of livestock assets, i.e. more than 87% of buffaloes and about 72% of cattle (Table 1). Due to limited area (the average farm size was 2.12 Feddan, the stocking rate of livestock was too high generating high demand for purchasing feeds as the average cultivated green fodder (Berseem) was 0.5 feddan (Table 2). Berseem is a winter legume crop. It is also called Egyptian clover. Thereof, the trend of feed prices goes up fast and also, there is no room for horizontal expansion in livestock assets. The only feasible option is the vertical expansion (to raise the productivity) (Soliman and Mashhour, 2011). In spite of limited land resources the small farmer has shown much capital accumulation, mainly due to investment in livestock over the last 20 years. The annual rate of increase in capital assets on farm was 10% (Table 2).

Human resources distribution

The estimated average household size of the small farm was 5 persons with 3.2 children. Among them about 60% were at the age of work, Table 2. Managerial-wise, 20% of full time farm managers were women, only 62% of the farmers were full time for farming and 18% were part time for farming, i.e. they have other jobs (Table 3). In addition, among the farmer’s children at the age of work about two thirds have nonagricultural jobs with salaries and 28% working off-farm on farms of others, the rest, i.e. 9% were unemployed (Table 4).

Farm household’s income sources

Surprisingly, the crops sale generates only 18% of the small farm household, while livestock generates 33% and poultry 1%. Even by adding up the off-farm income from seasonal hiring of the small farm household’s member for farming, the aggregate agricultural activities income was estimated as only 55%. 45% of the total income was from non-agricultural activities, where 40% from salaries and 5% from remittances of the farmers worked abroad (Table 5).

Role of buffalo enterprises in income generated from livestock activities

As shown from Table 6, the total herd size on a small farm was 3.5 heads, of which 31% milking buffaloes, generated 41% of livestock income, while milking cows share in the herd structure was 23% but generated only 20% of livestock income. The economic efficiency of a milking buffalo is two folds that of a milking cow on a small farming system. It seems that the small farmer raises cattle mainly
for meat production (fattening of males), while buffaloes are mainly for milk. While the Cattle feeder calves share in herd structure was 26% generated 33% of livestock income, buffalo feeder calves share in herd structure was 3%, generated only 2% of livestock income.

Role of buffalo enterprises in alleviation of farm household poverty

Going back to Table 5, the annual average total income (farm and off-farm income) provided US$ 2.3 per capita per day, i.e. 30% above the poverty line which was estimated as US$ 2.00 per capita per day (Chen and Ravallion, 2007). All agricultural sources of income generated only US$ 1.26, i.e., leaving a poverty gap of around 37% (the difference between the poverty line and the agricultural activities income). Without buffalo enterprising the poverty gap would have been deeper, reaching 55%. Therefore, buffalo enterprising decreased significantly the poverty gap impacts on the small farm household particularly that the dairy products income is almost daily.

Identification of the factors determining buffalo herd size on farm

It seems that the green fodder area is a main factor that determines the milking buffalo holding on the small farm. From the regression model in Table 7 if there is availability to allocate one more feddan of the farm area holding for Berseem the carrying capacity per one feddan two milking buffaloes (with their followers) will be added to livestock holding on the farm. The economic incentives were also of highly positive effect on the buffalo holding size on farm. These identified incentives were the farm price of milk and the volume of the milk processed on the small farm. The estimated response function (Table 7) showed that the farmer would be ready to add almost one more milking buffalo if either the farm price per kg of milk increased by L.E. 1 or if the value of sold dairy products, processed on farm increased by L.E. 100. It should be mentioned that the study did not find significant impact of these marketing incentives on the milking cows holding size on the farm. However, the response function of milking cows was omitted from the study as the focus was on buffalo’s role.

Identification of the factors determining berseem area on farm

As shown from the estimated model in Table 7 that the allocated area of Berseem is a highly significant factor that determines the buffalo holding size on farm, the study estimated the effect of farm size on determining the Berseem area. Table 8 presents the estimated response function. Such response implied that Berseem is determined by the farm acreage; in addition the income per feddan of Berseem is an economic factor that provides incentives to expand Berseem area.

The small farm size constrain has implication on the nature of the Berseem area and farm size relationship. It is a curvilinear relation rather than linear. Therefore, whereas the estimated response at the average farm size (2.12 feddan) estimated a Berseem area about 0.9 feddan, at the maximum small farm area (5 feddan), the Berseem area would be 2.1 feddan, i.e. the farmer would not allocate more than 42% of his land to Berseem in the winter season (Oct. – May) leaving the rest for subsistent competitor crops, mainly wheat.
CONCLUSIONS AND RECOMMENDATIONS

Milking buffalo has a significant role in alleviation of poverty that might face small farm households. A program to raise milk productivity is the proper approach to increase the income generated by buffalo, because there is no room for increasing, horizontally, the buffalo population due to limited agricultural land, water resources and competition between human food and animal fodders on such limited resources. Such program requires expansion in artificial insemination using imported semen carrying potential genetic makeup of high milk yield. Italy, probably, is the appropriate market to import such semen. Such program needs reform of the institutional framework in the Egyptian village to assure the success of such technology transfer package. It should be associated with establishment an efficient marketing system, animals’ health care and a nonconventional feed regime to overcome the scarce feed constrain.

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Table 1. Land and livestock holding structure in Egypt.

<table>
<thead>
<tr>
<th>Category of farm area</th>
<th>Holdings</th>
<th>%</th>
<th>Area (Feddan)</th>
<th>%</th>
<th>% of cattle</th>
<th>% of Buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1-Feddan</td>
<td>109307</td>
<td>34.72%</td>
<td>53360</td>
<td>6.17%</td>
<td>16.81%</td>
<td>21.54%</td>
</tr>
<tr>
<td>1 to less than 2 feddans</td>
<td>82199</td>
<td>26.11%</td>
<td>108079</td>
<td>12.49%</td>
<td>20.07%</td>
<td>26.92%</td>
</tr>
<tr>
<td>2 to less than 3 feddans</td>
<td>59405</td>
<td>18.87%</td>
<td>133786</td>
<td>15.47%</td>
<td>19.03%</td>
<td>23.67%</td>
</tr>
<tr>
<td>3 to less than 4 feddans</td>
<td>26300</td>
<td>8.35%</td>
<td>85143</td>
<td>9.84%</td>
<td>10.50%</td>
<td>9.32%</td>
</tr>
<tr>
<td>4 to less than 5 feddans</td>
<td>11455</td>
<td>3.64%</td>
<td>48726</td>
<td>5.63%</td>
<td>5.36%</td>
<td>5.71%</td>
</tr>
<tr>
<td><strong>Sub total</strong></td>
<td><strong>288666</strong></td>
<td><strong>91.68%</strong></td>
<td><strong>429094</strong></td>
<td><strong>49.61%</strong></td>
<td><strong>71.77%</strong></td>
<td><strong>87.16%</strong></td>
</tr>
<tr>
<td>5 to less than 6 feddans</td>
<td>12897</td>
<td>4.10%</td>
<td>70589</td>
<td>8.16%</td>
<td>6.32%</td>
<td>6.12%</td>
</tr>
<tr>
<td>6 to less than 7 feddans</td>
<td>4969</td>
<td>1.58%</td>
<td>39966</td>
<td>4.62%</td>
<td>2.84%</td>
<td>2.63%</td>
</tr>
<tr>
<td>7 to less than 10 feddans</td>
<td>3475</td>
<td>1.10%</td>
<td>39930</td>
<td>4.62%</td>
<td>1.97%</td>
<td>1.61%</td>
</tr>
<tr>
<td>10 to less than 15 feddans</td>
<td>1560</td>
<td>0.50%</td>
<td>25479</td>
<td>2.95%</td>
<td>1.00%</td>
<td>0.85%</td>
</tr>
<tr>
<td>15 to less than 20 feddans</td>
<td>1613</td>
<td>0.51%</td>
<td>36820</td>
<td>4.26%</td>
<td>0.85%</td>
<td>0.69%</td>
</tr>
<tr>
<td>20 to less than 30 feddans</td>
<td>1059</td>
<td>0.34%</td>
<td>39081</td>
<td>4.52%</td>
<td>0.77%</td>
<td>0.59%</td>
</tr>
<tr>
<td>30 to less than 50 feddans</td>
<td>494</td>
<td>0.16%</td>
<td>30634</td>
<td>3.54%</td>
<td>0.40%</td>
<td>0.28%</td>
</tr>
<tr>
<td>50 feddans and more</td>
<td>132</td>
<td>0.04%</td>
<td>153404</td>
<td>17.73%</td>
<td>0.11%</td>
<td>0.07%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>314865</strong></td>
<td><strong>100.00%</strong></td>
<td><strong>864997</strong></td>
<td><strong>100.00%</strong></td>
<td><strong>100.00%</strong></td>
<td><strong>100.00%</strong></td>
</tr>
</tbody>
</table>

(One Feddan = 4200 m²), Estimated GINI coefficient = 44.00% for the equity of the agricultural land distribution.
Table 2. Average resources on a small farm.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average farm area (Feddan)</td>
<td>2.12</td>
</tr>
<tr>
<td>Household size (Number)</td>
<td>5.0</td>
</tr>
<tr>
<td>Number of children (Total)</td>
<td>3.2</td>
</tr>
<tr>
<td>Number of children (At Work Age)</td>
<td>1.9</td>
</tr>
<tr>
<td>Berseem area (Feddan)</td>
<td>0.50</td>
</tr>
<tr>
<td>Beginning inventory (L.E./Farm)</td>
<td>7954</td>
</tr>
<tr>
<td>Farm End inventory (L.E/Farm)*</td>
<td>30300</td>
</tr>
<tr>
<td>Change in inventory (L.E/Farm.) over 20</td>
<td>22346</td>
</tr>
<tr>
<td>Annual growth in inventory</td>
<td>10.33%</td>
</tr>
</tbody>
</table>

One US$ = 6.10 L.E. (Egyptian Pound) in 2011
Source: Compiled and Calculated from the Sample survey of this Study in 2011.

Table 3. Type of small farm manager.

<table>
<thead>
<tr>
<th>Type of Farm Manager</th>
<th>% of farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full time male farmer</td>
<td>62%</td>
</tr>
<tr>
<td>Full time female farmer</td>
<td>20%</td>
</tr>
<tr>
<td>Part time male farmer</td>
<td>18%</td>
</tr>
<tr>
<td>Wives share in farm operations</td>
<td>77%</td>
</tr>
<tr>
<td>Wives share in livestock husbandry</td>
<td>58%</td>
</tr>
</tbody>
</table>

Source: Compiled and Calculated from the Sample survey of this Study in 2011.

Table 4. Employment of farm household children.

<table>
<thead>
<tr>
<th>Type of Employment</th>
<th>Number per farm</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of children at work age of which:</td>
<td>1.9</td>
<td>100%</td>
</tr>
<tr>
<td>Working in non-farming jobs</td>
<td>1.2</td>
<td>63%</td>
</tr>
<tr>
<td>Working on other farms</td>
<td>0.5</td>
<td>28%</td>
</tr>
<tr>
<td>Unemployed</td>
<td>0.2</td>
<td>9%</td>
</tr>
</tbody>
</table>

Source: Compiled and Calculated from the Sample survey of this Study in 2011.
Table 5. Sources of farm household income and daily per capita income.

<table>
<thead>
<tr>
<th>Source of income</th>
<th>Annual farm income</th>
<th>Daily/ Capita income</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L.E.</td>
<td>%</td>
</tr>
<tr>
<td>Crop Sale</td>
<td>4478</td>
<td>18%</td>
</tr>
<tr>
<td>Livestock Output</td>
<td>8296</td>
<td>33%</td>
</tr>
<tr>
<td>Poultry Output</td>
<td>236</td>
<td>1%</td>
</tr>
<tr>
<td>Working on Other Farms</td>
<td>876</td>
<td>3%</td>
</tr>
<tr>
<td>Total Agricultural Activities</td>
<td>13886</td>
<td>55%</td>
</tr>
<tr>
<td>Salaries</td>
<td>10145</td>
<td>40%</td>
</tr>
<tr>
<td>Remittances of the Farmer's work Abroad</td>
<td>1200</td>
<td>5%</td>
</tr>
<tr>
<td>Grand Total</td>
<td>25231</td>
<td>100%</td>
</tr>
</tbody>
</table>

One US$ = 6.10 L.E. (Egyptian Pound), in 2011 Source: Compiled and Calculated from the Sample survey of this Study in 2011.

Table 6. Role of buffalo in income generated from livestock on small farm.

<table>
<thead>
<tr>
<th>Livestock Category</th>
<th>Heard Structure Per Farm</th>
<th>Livestock Income/ Farm</th>
<th>Productivity/Head</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>head</td>
<td>%</td>
<td>L.E.</td>
</tr>
<tr>
<td>Milking Buffaloes</td>
<td>1.1</td>
<td>31%</td>
<td>3621</td>
</tr>
<tr>
<td>Milking Cows</td>
<td>0.8</td>
<td>23%</td>
<td>1725</td>
</tr>
<tr>
<td>cattle Male Feeder Calves</td>
<td>0.9</td>
<td>26%</td>
<td>2864</td>
</tr>
<tr>
<td>Buffalo Male Feeder calves</td>
<td>0.1</td>
<td>3%</td>
<td>194</td>
</tr>
<tr>
<td>Mature Sheep and Goats</td>
<td>0.3</td>
<td>9%</td>
<td>196</td>
</tr>
<tr>
<td>Lambs</td>
<td>0.3</td>
<td>9%</td>
<td>139</td>
</tr>
<tr>
<td>Total Herd</td>
<td>3.5</td>
<td>100%</td>
<td>8739</td>
</tr>
</tbody>
</table>

Source: Compiled and Calculated from the Sample survey of this Study in 2011.
Table 7. Factors determine milking buffaloes on small farm.

<table>
<thead>
<tr>
<th>Explanatory Variable</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>Significance Level</th>
<th>Goodness of Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.8172</td>
<td>0.1313</td>
<td>6.2245</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Farm Price per 1-Kg of Milk</td>
<td>0.3760</td>
<td>0.0581</td>
<td>6.4686</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Revenue of Dairy Processing on Farm</td>
<td>0.0037</td>
<td>0.0008</td>
<td>4.6223</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Berseem Area (Feddan)</td>
<td>0.8541</td>
<td>0.1329</td>
<td>6.4244</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

Source: Compiled and Calculated from the Sample survey of this Study in 2011.

Table 8. Factors determining the berseem area on farm.

<table>
<thead>
<tr>
<th>Explanatory Variable</th>
<th>Estimate</th>
<th>S.E.</th>
<th>t Stat</th>
<th>Significance Level</th>
<th>Goodness of Fits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0</td>
<td></td>
<td>0.97</td>
<td></td>
<td>Observations 120</td>
</tr>
<tr>
<td>(Farm Area/Farm)^2</td>
<td>0.428</td>
<td>0.058</td>
<td>7.338</td>
<td>&lt; 0.01</td>
<td>R Square 0.893</td>
</tr>
<tr>
<td>(Farm Area/Farm)</td>
<td>-0.035</td>
<td>0.013</td>
<td>-2.628</td>
<td>&lt; 0.01</td>
<td>Adjusted R Square 0.883</td>
</tr>
<tr>
<td>(Income from 1-Feddan of Berseem )</td>
<td>0.00007</td>
<td>0.00002</td>
<td>3.314</td>
<td>&lt; 0.01</td>
<td>F Ratio 325.35</td>
</tr>
</tbody>
</table>

The regression model was estimated under the assumption of zero intercept.
Source: Compiled and Calculated from the Sample survey of this Study in 2011
Plenary Session 3: Biotechnological for Efficient Buffalo Production
Latest Biotechnological Approaches for Efficient Buffalo Production

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ABSTRACT
The buffalo is imperative to the lives of small farmers and to the economy of the developing countries. Genome analysis of buffalo had advanced significantly in recent years. The completion of whole genome sequencing projects has brought a wealth of information about the structure of animal genome. High throughput technologies made feasible by sequencing and re-sequencing of some economically significant livestock species has led to the foundation of several thousand single nucleotide polymorphisms (SNPs), insertions/deletions (indels), or structural variants. All these biotechnological tools combine with bioinformatics tools and softwares would be of massive assist in understanding of buffalo genomes for efficient production. In the future, the proficient livestock production would be rely on even more profoundly on existing and promising biotechnological advances for better improvement in production efficiency.

Keywords: buffalo, genome, polymorphism, biotechnology, bioinformatics

INTRODUCTION
Buffalo is among the species of great economic importance in many parts of the world especially in developing countries. Buffalo is a multipurpose animal being a source of milk, meat, draft power, hide and employment people in several countries (Hussain et al., 2009). This article will inspect the role of modern and latest technologies that may applied for the efficient buffalo production. The future prospective of these technologies for identification of productive buffalo population is also discussed. All these technologies are rapidly growing and already available now for research in livestock field. The high throughput microarray technology combined with appropriate bioinformatics tool now available for microRNA (miRNA) and messenger RNA (mRNA) expression analysis. Epigenetics is an emerging field in genomics and has significant part in gene expression analysis. Advances in these technologies have led to the foundation of the high density single nucleotide polymorphism (HD-SNP) arrays as an up-to-the-minute implement for the genetic and genomic analysis of domestic animals (Fan et al., 2010). Now the new era of Next Generation sequencing (NGS) technologies make it possible to discover a large number of genetic polymorphisms at DNA level and currently represent the hottest topic in the field of animal’s genomic researches. Genotyping-by-sequencing is latest high throughput genotyping technique developed a
technically simple, highly multiplex and highly suitable for population studies, characterization, breeding and trait mapping in livestock species (Elshire et al., 2011). MiRNA is short, noncoding RNA molecule of ~22 nucleotides long control gene expression at sequence dependent manner and involved in many cellular pathways. The current progress in this novel field provided influential corroboration of association of miRNA in various diseases and use as potential biomarker for diseases prognosis and diagnosis in buffalo. Recent advances in the field of biotechnology has been directed primarily towards reproductive technology have been employed for genetic improvement of domestic animals which is leading concern over the years for scientist and researchers in developing countries. The applications of these technologies significantly have their impact for improvement of buffalo production.

APPLICATION OF GBS IN BUFFALO

For better understanding of complex traits the application of latest high-throughput genotyping techniques are preferred however high cost is a limiting factor. Genotyping-by-sequencing (GBS) is one of the most recent technology and is comparatively simple (Davey et al., 2011) and highly multiplexed technique based on the Illumina® sequencing platform. It was developed initially for plants (Gore et al., 2009), however it is equally suitable for population studies, characterization, genetic improvement and mapping of complex trait mapping in domestic animals. (De Donato et al., 2013). The use of methylation sensitive restriction enzymes in GBS helps to avoid repetitive genomic regions and simplifies further bioinformatics analysis burden.

This efficient and cost-effective technique can be adopted to use in buffaloes to identify large numbers of SNPs that may be used as markers for breed characterization, genetic selection, genetic mapping, and genome wide association studies (GWAS). This will enable us to identify the best buffalo for further breeding based on identified SNPs/markers across the entire genome. GBS technique is potentially applicable to other domestic animals as well like cattle, yak, camel, sheep and goat.

EPIGENETICS

Epigenetic has been deeming as an innovative challenge in the post-genomic era of livestock species. Epigenomic is the study of non DNA hereditable changes affecting the animal’s phenotype (Callinan and Feinberg, 2006). Epigenetic revealed that the genes as single are not the authors of inheritance, there are other characteristic such as DNA methylation, histone modification and noncoding RNA are common epigenetic features often impacted gene regulation and disease development. In livestock this emerging field has a significant part in understanding of the molecular basis of epigenetic mechanisms in gene expression studies. This is new field in research increasingly imperative and impacts several economically significant traits in livestock such as growth, development and breeding strategies. These traits reveal a complex inheritance due to both genetic as well as environmental factors such as diet, stress, drugs and pollution (Petronis, 2010). Some of these factors are involved in changing certain methylation patterns and
these blueprints would contribute to the phenotypic variations among species. Some studies also demonstrated that there are some genotypes detected are more susceptible to epigenetic changes methylation than others (Coolen et al., 2011). It will be increasingly significant for animal researchers to detect genotypes associated to unreceptive methylation affecting economically productive traits. Efficiently epigenome wide association analysis exercised towards detection of negatively affected methylation patterns on economically significant traits of interest. This is of huge challenge for breeding strategies to detect the correlation exist between methylation patterns for various traits. Buffaloes are economically important species, for the prediction of genetic merit providing privilege predictive accuracy, so epigenome wide association analysis is indispensable for the detection what methylation patterns negatively affect the traits of interest.

MICRORNAS EXPRESSION PROFILING

MicroRNAs (miRNA) are small regulatory molecules involved in the regulation of gene expression at post-transcriptional level and have prominent role in multiple cellular pathways mainly regarding with developmental and metabolic processes e.g. developmental timing, cell proliferation, cell differentiation and apoptosis (Garzon et al., 2009). This is an emerging field for animal scientist to tackle their role in biological process that affects the animal biology. Alterations in their expression levels were linked with several diseases (Ferdin et al., 2011). The deregulation of miRNA expression in various diseases by a variety of mechanisms including mutation, deletion, amplification and epigenetic silencing (Garzon et al., 2009). Mutations within miRNA sequences, targeted genes or coding genes involved in their biogenesis affects vital production traits and build susceptibility of the animals to diseases (Georges et al., 2007). This tiny non-coding RNAs are identified to regulate gene expression at post-transcriptional level binding to their complementary sequences of targeted messenger RNAs leads to translational repression. Recently several studies demonstrated that miRNAs expression is also regulated by epigenetic mechanisms including DNA methylation and histone modification and conversely miRNAs also involved in the regulation and expression of significant epigenetic regulators e.g. histone deacetylase, DNA methyltransferase and polycomb group genes (Sato et al., 2011). This makes a complicated feedback network and regulatory circuit between miRNAs and epigenetics in directive of whole gene expression profile.

With reproductive performance in buffalo, the evaluation of skeletal muscles and adipose tissue is of enormous interest, as profit margin in production affects carcass value including meat quality grade and yield. Therefore, acknowledgement of mechanisms that regulate cellular processes of skeletal muscles and adipose tissue development and growth is of immense interest. In progress miRNA research for economically imperative traits in livestock species including buffalo is of importance to establish our current standing and what route future research desires to take.
GENOME WIDE ASSOCIATION STUDIES (GWAS)

GWAS is an interesting approach have developed in last decade into a prevailing contrivance for the investigating the genetic architecture of livestock diseases. It revolutionized the livestock genetics and useful in detecting various genes affecting both normal variations among species and also susceptibility to diseases, and elucidated our understanding to studying economically significant traits. Researchers working on model organisms are being implemented both GWAS and other genomic technologies capturing genetic variations (Bush and Moore, 2012). These are the assembly of techniques used in genetic screening to identify the precise area of interest in genome. The purpose of genomic technologies is the characterization and mapping of the locus that affected these traits of interest (Koopaei and Koshkoiyeh, 2011). The current advancement in characterizing the genomes of animals, including the identification of large number of single nucleotide polymorphism (SNP) make a major impact on the identification of genes and mutation underlying this phenotypic diversity including diseases susceptibility, morphology and behavior. All these corresponding factors fetch the genetics community to reflect on a genome wide scale. With progresses in next generation sequencing and advance genotyping technologies and combined with innovative statistical genetics procedures floor the approach towards genomic selection. To exploit the genomic information e.g. sequences or DNA marker polymorphisms, for the selection of farm animals need the knowledge of the effect of physically mapped genes with effects on economically significant traits or quantitative trait loci (QTL) (Montaldo, 2006). Basically there are two QTL mapping strategies, association test using candidate gene and genomic scans based on linkage mapping in a cross population (Andersson, 2001). These two approaches have been used to identify the genes affecting trait of interest. Candidate genes are the genes with known biological function directly or indirectly regulating the development processes of the production traits, which could be confirmed by evaluating the effects of the causative gene variants in an association studies (Zhu and Zhao, 2007).

The digital candidate gene approach (DigiCGA) is recently the most outstanding improvement in this field, is a novel web resources based candidate gene identification approach (Glazier et al., 2002). The genome wide scan approach studies the relationship between a trait and markers selected across the genome to identify chromosomal locations associated with the trait (Andersson, 2001). Using the genome scan approach a huge amount of QTL can be achieved in farm animals that can provide a useful association to link genomic information with phenotype. The use of high through put genomic technologies for genetic based animal selection and understanding the basic principles and mechanisms of genes and their association with decisive expression into a particular phenotypes is deduced generally acceptable.

WHOLE GENOME SEQUENCING (WGS)

Whole genome analysis in buffalo has advanced extensively in last decade. High throughput DNA sequencing techniques have acquired revolutionary changes in the field of both basic and applied genetics, providing new and comprehensive genomic analysis with greater genetic resolution. The whole genome sequencing
Whole genome sequencing has been completed in cattle, dog, chicken and horse. It has been identified that in water buffalo more than ~66,935 nucleotide sequences have been deposited in the GenBank database (http://www.ncbi.nlm.nih.gov), whole genome shotgun sequences ~64,212, mitochondrial genome sequences ~974 and 1,748 of nuclear gene/genomic DNA sequences (Michelizzi et al., 2010). Previously two types of sequencing strategies were applied for whole genome sequencing BAC and shotgun sequencing employing sanger method which costly, time consuming and labor intensive (Metzker, 2005). Now the new era of demanding low cost sequencing technology led to the development of Next Generation sequencing (NGS) technologies make it possible to unearth a large number of genetic polymorphisms at DNA level and presently the hottest topic in the field of animal’s genomic researches. The technologies available from Roche/454 life sciences, Illuminia/Solexa and Applied Biosystem/SOLId. The high throughput Next Generation sequencing (HT-NGS) revolutionized the animal’s genomic research and has immense applications in providing a comprehensive knowledge on whole genome genotyping (Burgess, 2011), providing an inclusive analysis of epigenetic modifications furnishing a significant impact on epigenomic research (Meaburn and Schulz, 2012), targeting a functional genomic regions for massive screening and detection of mutations, deletion/insertion (Indel) (Senapathy et al., 2010), RNA sequencing and expression profiling to accurately determined the economically important genes expression, alleles specific expression of transcripts, differential splicing and providing method for preparing a small RNA libraries and measuring microRNA (miRNA) expression abundance (Morin et al., 2010; Buermans et al., 2010; Costa et al., 2010).

**MICROARRAY TECHNOLOGY**

A DNA microarray is a complex technology used in molecular biology and medicine. Various technologies have been accessible to identify differences in gene expression exist, including subtractive hybridization, differential display, serial analysis of gene expression and microarray hybridization. Microarray technology has become one of the significant tools, scientists and researchers used to monitor genome wide expression levels of genes in a given organism. In the field of transcriptomics, it is considered the most popular and efficient tool for RNA profiling, which is assembled by spotting a piece of each gene on a solid carrier. Through this exceptional tool we are able for handling and analysis of hundreds of thousands gene expression at one time. It is a novel technology has attracted a massive deal of attention from animal geneticists and breeders. It has been used for large scale gene expression studies and generated genotyping efficient and low cost, widely applied for SNP genotyping, indel (insertion/deletion) polymorphisms (Galbraith, 2006). The microarray used for such intention would allow large scale screening of many hundreds of markers in a sole experiments, allowing selection of
animals based on multiple traits. Genomic approaches such as microarray based SNP genotyping are hopeful tools for improving and advancing of animal production.

**NUTRIGENETICS AND NUTRIGENOMICS**

Different animals perform differently even with the same diet contained in animal feed. This is due to the effect of molecular pathways that result in differences in animal resistant to diseases and production performance. It is significantly apprehend to understand these effects, studies of differential genes function and expression are being undertaken to discern these effects in domestic animals. Novel and high throughput technologies, genomic resources and bioinformatics tools are now available for prominent domestic livestock species including buffalo. Nutrigenomics and nutrigenetics are the new research tools looking at genetic and epigenetic level to understand the effects of food (Neibergs and Johnson, 2012). Protein is a key factor involved in all biological processes in animal body. Proteomics is strappingly concerns with nutrigenomics, providing an insight into these mechanisms to understand how the animal genome is expressed as a response to diet (Ordovas and Corella, 2004). The field of nutrigenomics contains manifold disciplines in order to understand the nutrient-gene interaction and dietary effects on genome stability, RNA expression (transcriptomics), epigenetic alteration (DNA methylation and histone modification), metabolic changes (metabolomics) and protein expression (proteomics) (Fenech et al., 2011). All the today available tools such as genomics, proteomics, functional genomics, nutrigenomics, bioinformatics and epigenomics pledged new opportunities to investigate the complex interaction between genes and diet of domestic animals. Using these approaches in animal’s field provide new insight into genome-nutrition partnership would be resulting in improved efficacy of diets, improved sustainability of animals as a protein source and improved technique for preventing diseases.

**ASSISTED REPRODUCTIVE TECHNOLOGIES (ART)**

Buffaloes are important livestock resource for local economy because of its high milk production, highly demanded around the world. To rapidly propagate the superior germplasm, the traditional reproductive technologies have been performed in this species.

Biotechnology has been directed primarily towards reproductive technology have been employed for genetic improvement of domestic animals which is primary concern over the years for scientist and researchers. Advances in assisted reproductive technologies (ART) like Artificial insemination, Embryo transfer, In vitro Production/fertilization, Superovolution, transgenesis and cloning have become important in livestock breeding, have been introduced to overcome reproductive problems (Vikrama and Balaji, 2002). Artificial Insemination (AI) and embryo transfer (ET) are probably the most familiar techniques that have been implemented in developed and developing livestock production (Kahi and Rewe, 2008).

Transgenic animals and cloning are one of the fastest biotechnology growing areas. RT has prolonged effects on animal breeding in the future, as the increases the rate of reproduction and decrease the generation time (Abu et al., 2008). The most successful reproductive technologies like AI and ET necessitated applying on large
extent, some emerging biotechnologies such as Multiple Ovulation and Embryo Transfer (MOET), *in vitro* Fertilization (IVF) and cloning provides prevailing tool for rapidly changing the animal populations genetically. The cloning research in buffalo by somatic cell nuclear transfer is still in its early stages, but some indispensable understanding has been obtained regarding the parthenogenesis, *in vivo* and *in vitro* development of embryo reconstruction by transferring donor nuclei from foetal and adult fibroblast cells to enucleated buffalo oocytes (Meena and Das, 2006). When cloning technology does become ascertained in the buffalo, high incidence problems will need to be addressed before it can have wide practical applications. These technologies will absolutely play an imperative role in the future perspective and visions for efficient reproductive performance in livestock (Vikrama and Balaji, 2002).

**REFERENCES**


The Buffalo Genome and the Application of Genomics in Animal Management and Improvement

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ABSTRACT

The publication of the human genome sequence in 2001 was a major step forward in knowledge necessary to understand the variations between individuals. For farmed species, genomic sequence information will facilitate the selection of animals optimised to live, and be productive, in particular environments. The availability of cattle genome sequence has allowed the breeding industry to take the first steps towards predicting phenotypes from genotypes by estimating a “genomic breeding value” (gEBV) for bulls using genome-wide DNA markers. The sequencing of the buffalo genome and creation of a panel of DNA markers has created the opportunity to apply molecular selection approaches for this species.

The genomes of several buffalo of different breeds were sequenced and aligned with the bovine genome, which facilitated the identification of millions of sequence variants in the buffalo genomes. Based on frequencies of variants within and among buffalo breeds, and their distribution across the genome compared with the bovine genome, 90,000 putative single nucleotide polymorphisms (SNP) were selected to create an Axiom® Buffalo Genotyping Array 90K. This “SNP Chip” was tested in buffalo populations from Italy and Brazil and found to have at least 75% high quality and polymorphic markers in these populations. The 90K SNP chip was then used to investigate the structure of buffalo populations, and to localise the variations having a major effect on milk production.

Keywords: genetic association study, genomic selection, genome sequence, single nucleotide polymorphisms

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INTRODUCTION

Genetic improvement of buffalo for traits such as increased milk and meat production, health and efficiency would have a positive impact on agriculture in many regions of the world, and in particular to support poorer economies where buffalo production predominates with respect to cattle. Buffaloes are reared in 129 countries and contribute very significantly to the rural economy, especially in South and South-East Asia (Misra and Tyagi, 2007).

The world population of buffalo is about 158M in comparison with around 1.3B cattle, 1B sheep 500-600M goats. There are two types of domestic water buffalo, the River Buffalo (Bubalus bubalis) that are more widely spread globally, and are the predominant type found in the west from India to Europe. The second type, the Swamp Buffalo (Bubalus carabanensis), is found more frequently in the east from India to the Philippines.

Systems of buffalo production vary widely in different countries and are governed by several interacting factors, which include the climate, local geography, cropping systems, the size of farms and primary purpose for buffalo production: milk, meat or draught. In Italy, Mediterranean buffalo are kept on large commercial farms under modern intensive systems primarily for producing milk, which is mainly used for Mozzarella cheese making. In Latin America Murrah and Jaffarabadi buffalo were originally imported from India, between the 1940s and 1960s. More recently the Mediterranean buffalo has been imported. Today river, swamp and crossbred buffaloes are kept in systems varying from extensive beef production, through rural multi-purpose systems to intensively managed herds for milk production (Borghese, 2005).

The first application of genetics to buffalo improvement was carried out in Italy for the Mediterranean buffalo under the guidance of the Italian Buffalo Breeders Association (ANASB). Mediterranean buffaloes have been exported to many parts of the world. In addition to improved genetics, advanced reproductive technologies including artificial insemination and embryo transfer are used routinely in buffalo husbandry in Italy. At an international level, genetic improvement in production, health and reproductive success would help to sustain buffalo farming and could in part be achieved through molecular assisted genetic improvement.

In this study, the development of a genome-wide 90,000 SNP panel is described. The panel has been used for two practical applications on field data: comparing the efficiency of classical pedigree recording with pedigrees constructed from genomic data and a genome-wide association analysis to localise genes having a large effect on milk production.

MATERIALS AND METHODS

SNP discovery: Eighty-six river buffalo from 8 breeds (Italian Mediterranean, Murrah, Nili-Ravi, Jaffarabadi, Kundhi, Aza-Kheli, Egyptian and Swamp type from Philippines) were sequenced to a depth of between 5 and 12X by Illumina paired end reads, yielding a total of 470X genome coverage depth. Buffalo sequences were aligned to the bovine UMD3.1 genome using Burrows-Wheeler Alignment tool (BWA; Li and Durbin, 2009). Aligned sequences were processed with SAMtools (SourceForge.net; Li et al., 2009) and Picard tools (SourceForge.net) to format the
data for SNP calling with the unified genotyper of the Genome Analysis ToolKit (GATK, McKenna et al., 2010). Heterozygous SNPs were kept if they had a base pair quality score of Q>10 and did not have another SNP within 10bp. A total of 22,293,567 SNPs were discovered from four river breeds (Mediterranean, Murrah, Jaffarabady and Nili Ravi). The within and across breeds allele frequencies for these SNPs were calculated and the minor allele frequencies (MAF) were determined.

**SNP-chip design.** All SNPs that did not map uniquely, were too close together and had a low probability of being real SNPs were excluded. A total of 5,800,477 high quality SNPs were retained. The spacing of SNPs on the buffalo genome sequence was estimated by mapping them onto the bovine genome, and the probability of a SNP being polymorphic was weighted by breed: Mediterranean 30%; Murrah 30%; Jaffarabadi 20%; Nili-Ravi 20%. This identified 123,040 SNPs which probe design could be tested. Buffalo sequence was created around these SNPs by aligning the Buffalo sequence reads with the UMD 3.1 bovine sequence, and optimised 71mer probe sequences were identified for each SNP by Affymetrix using proprietary pipelines. The final Axiom® Buffalo Genotyping array design comprises 123,029 probes, which includes probes for 89,988 SNPs, 5,799 QC probes and 1,784 gender calling probes. The Axiom® Buffalo Genotyping array was tested by genotyping a total of 619 Italian Mediterranean buffalo and 282 Brazilian Murrah buffalos samples. The Affymetrix “Powertools” (APT) and “SNPolisher” R packages were used for quality control of the SNP. A total of 76,559 SNP probes validated when considering all the samples (see results for details).

**Genomic relationships on Brazilian dataset.** Classical recorded pedigree was compared with the genomic relationships for the 282 Brazilian Murrah animals by creating a genomic relationship matrix (VanRaden et al., 2008). Of the 78,136 SNPs validated only on Brazilian Murrah samples, monomorphic SNPs and those with MAF<5% were removed (10,556 SNP) leaving 67,580 SNPs which were used to create the G matrix.

**Genome-Wide Association analysis on Italian dataset.** Milk EBVs and lactation records for the 619 Italian Mediterranean buffalo coming from 4 farms in the Lombardy region (Italy) was provided by The Italian Buffalo Breeders Association (ANASB). After quality control to remove replicates, animals with missing data or failing pedigree checks, 529 records were retained. A total of 20,914 probes were discarded because of poor quality on these samples giving a final dataset of 78,137 SNP probes for these samples.

Associations between SNP genotypes and phenotypes were analyzed by fitting all SNP simultaneously using the GRAMMAR procedure (Genome-wide Rapid Association using Mixed Model And Regression: Aulchenko et al. (2007). The procedure involved two steps. First, an additive polygenic model was used to obtain individual environmental residuals using the polygenic function of the GenABEL package (Aulchenko et al., 2007). Then, association between residuals and genetic polymorphisms was tested using least squares methods.

The following model was used to estimate residuals:

\[
\text{LactRecord}_{ijkpqr} = \mu + \text{Farm}_i + \text{CalvYear}_j + \text{CalvSeason}_k + \text{Calvings}_p + \text{Age}_q + \text{Polygenic}_r + e_{ijkpqr}
\]
where \( LactRecord \) is a 270 DIM conventional lactation record, \( \mu \) is the general mean, \( Farm \) is a fixed farm effect (\( i=1,4 \)), \( CalvYear \) is a fixed effect for calving year (\( j=1,2 \) for pre and post 2010), \( CalvSeason \) is a fixed effect for season of calving (\( k=1,4 \)), \( Calvings \) is a fixed effect for the number of calvings (\( p=1,2 \) for primiparous and multiparous animals), \( Age \) is a covariate for age (in months), \( Polygenic \) is a polygenic effect for animal \( r \), and \( e \) is the random residual, with \( e \sim N(0, \sigma^2_e) \).

The polygenic effect was included to account for genetic sub-structure, as higher or lower degree of genomic relationship between animals can have a direct impact on estimates, increasing false positives and negatives.

**RESULTS**

**Axiom Genotyping.** All of the 901 samples belonging to Italian Mediterranean and Brazilian Murrah breeds genotyped using the Axiom array were female, hence the gender calling probes were not considered in the analyses. All plates had an initial call rate of 97% or greater and were considered to have passed the Affymetrix Quality Control (QC) parameters. Of the 89,988 SNP probes, 67,330 (74.8%) were polymorphic (PolyHighResolution) and 9,229 (10.3%) gave high quality signals but were monomorphic (MonoHighResolution) in the two breeds tested, but may be polymorphic in other breeds. Less than 0.1% (83 probes) gave spurious signals with variable intensity (Variable Intensity Non-hybridizing Oligo, VINO cluster), which most likely arise from variations within the target probe sequence that were not identified in the sequence set used for the probe design. About 1.7% of probes (1,494) were missing one of the homozygous genotypes (NoMinorHom). Only 4.1% (3,668) of the probes had a call rate below the threshold (0.15) and 9.1% were rejected for low quality genotypes. Considering the PolyHighResolution SNPs, the average sample call rate was 99.75% and the average sample reproducibility comparing the 26 replicate samples included in the sample set was 99.96%, demonstrating a high quality of the genotyping results.

**Identification of pedigree errors.** Recorded pedigrees for all populations contain errors, even when good systems to verify data are in place, with errors of up to 25% estimated for cattle populations (Matukumalli et al., 2009). A more accurate measure of relationship may be obtained considering the fraction of DNA in common between individuals, based on alleles shared at given loci. Genomic relationship measures are useful in selection and parentage testing (Dodds et al., 2005) and have been used to manage genetic diversity (Caballero and Toro, 2002).

The pedigree information on the Brazilian samples was available only as far as the sire and dam. Seven bulls in the sample had more than 10 daughters and were used to evaluate possible pedigree errors using genomic data. Clustering the daughters using a genomic relationship produced tight homogeneous groups of daughters for 5 of these bulls, while the daughters of the remaining 2 bulls fell into dispersed heterogeneous clusters. The heterogeneous clusters may be an effect of mendelian sampling, but are more likely to be caused by errors in the paternal assignment for the 2 bulls. Identification of potential pedigree errors to clean data sets prior to analyses is necessary in association studies to help reduce false positive associations, and to improve the accuracy of estimation of standard as well as genomic breeding values.
Genome-wide Association Study (GWAS) for milk production traits. Population structure was assessed using multi-dimensional scaling, which showed clear population structure clusters depending on the farm of origin. Clusters for Farms 1,859 and 26,225 overlapped, while clusters for Farm 71,801 and 61,207 were independent, showing only minimal overlap with each other and the other two farms (Figure 1). This genetic sub-structure is most likely due to a sire effect: while Farms 1,859 and 26,225 predominantly use bulls available for artificial insemination, and therefore have bulls in common, Farms 71,801 and 61,207 use mainly natural service bulls and only occasionally use the AI bulls in common with the other farms.

Heritability of the trait based on a classical animal model and recorded pedigree (additive relationship matrix) was 0.38, while it was 0.45 when using the genomic relationships matrix. This is similar to the results in cattle for the same trait (Interbull, 2009).

As the draft genomic sequence of the buffalo is currently based on the bovine genome, the results shown assumed the order of loci on the bovine genome, and reported chromosomes are bovine equivalents. Moreover, as the draft buffalo genome is not yet annotated, alignment with the bovine genome also facilitated the use of the bovine information to explore genes under the peaks identified.

Loci associated with milk production (genome-wide significance $P<10^{-04}$) were found on the chromosomes corresponding with bovine chromosomes 4, 11 and 19. Suggestive associations were also found on chromosomes 10, 14 and 23 (see Figure 2). The SNPs most significantly associated with milk yield are shown in Table 1.

The most convincing association with milk yield data was found on chromosome 11, with several significant SNPs (see Figure 2 and Table 1). In particular, three of the significant SNPs (AX-85041172, 85125077, 85114201) are located near two gene dense regions. The closest gene downstream is gene coding for the apolipoprotein B (APOB), a protein involved in the early lactation lipid metabolism in cow (Gruffat et al., 1997). This is probably the best functional candidate for the chromosome 11 QTL. Growth differentiation factor 7 (GDF7), and U4 small nuclear RNA genes are also present on this region. Upstream of these significant SNP are kelch-like gene 29 (KLHL29) and the U6 non-coding small nuclear RNA. The other two SNPs with strong association located on chromosome 11 (AX-85080229 and AX-85093842) are close the neurexin-1-alpha precursor gene (NRX1) and located upstream a dense gene region.

The most significant SNP on chromosome 19 (AX-85140457) is close to the short chain dehydrogenase/reductase SDR family member 11 gene (DHSR11), which codes for one of the enzymes involved in the metabolism steroid hormones, prostaglandins, retinoids, and lipids.

The SNP on chromosome 4 (AX-85143079) is located downstream the Collagen alpha-2 gene (COL1A2) which is associated with milk ability and more precisely the kinetics of milk emission in sheep (Dhorne-Pollet et al., 2012).

DISCUSSIONS

The data reported here describes the creation of a genome wide SNP panel for buffalo, which has been tested to analyse pedigree structure of a buffalo
population and to carry out a GWAS analysis for milk yield in buffalo. The GWAS identified SNPs strongly associated with milk yield on chromosomes 11 and 19. Further investigation of these regions with additional data is necessary to confirm the associations and fine-map the QTL regions.

In the future, breeding programs will be driven primarily by high-density SNP data (Eggen, 2012). Genomic information will be used both to identify the causal mutations to select for superior variants at loci having a major effect on desired phenotypes, or used to calculate estimated genomic breeding values to reduce the selection interval, thus boosting genetic improvement. The work presented here takes the first step towards these goals for the buffalo.

ACKNOWLEDGEMENTS

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**Table 1.** The 9 most significantly associated SNPs.

<table>
<thead>
<tr>
<th>SNP</th>
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<th>Position bp</th>
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<th>effB</th>
<th>se_effB</th>
<th>Pc1df</th>
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</tbody>
</table>

**Figure 1.** MDS plot representing the structure of the four farms used in the study.
Figure 2. Manhattan plot GWAS-Lactation.
Invited Papers
Buffalo Reproduction Symposium
Control of Buffalo Follicular Dynamics for Artificial Insemination, Superovulation and In Vitro Embryo Production

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ABSTRACT

Currently, timed ovulation induction and timed artificial insemination (TAI) can be performed in buffalo using GnRH or estradiol plus progesterone/progestin (P4)-releasing devices and prostaglandin F\(_2\alpha\) (PGF\(_2\alpha\)). The control of the emergence of follicular waves and of ovulation at predetermined times, without the need for estrus detection, has facilitated the management and improved the efficiency of AI programs in buffalo during the breeding and nonbreeding season. Multiple ovulations, embryo transfer, ovum collection and in vitro embryo production have been shown to be feasible in buffalo, although low efficiency and limited commercial application of these techniques have been documented as well. These results could be associated with low ovarian follicular pools, high levels of follicular atresia and failures of the oocyte to enter the oviduct after superstimulation of follicular growth. This review discusses a number of key points related to the manipulation of ovarian follicular growth to improve pregnancy rates following TAI and embryo transfer of in vivo- and in vitro-derived embryos in buffalo.

Keywords: artificial insemination, buffalo reproduction, embryo production

INTRODUCTION

The understanding of follicular dynamics in buffalo is necessary for developing new techniques and improving the currently used regimens for the manipulation of the estrous cycle. Ovarian follicular dynamics in buffalo are similar to those of cattle. Although the 2-wave cycle is the most common in buffalo (63.3%), both 3-wave and 1-wave cycles have also been observed. The number of waves in a cycle is also associated with the luteal phase and with the estrous cycle length. However, the number of follicles recruited per follicular wave is lower in buffalo than in cattle (Baruselli et al., 1997; Gimenes et al., 2009, Campanile et al., 2010). The success of several reproductive programs is closely related to ovarian follicular development and ovulation. In recent decades, several therapies have been proposed for manipulating ovarian follicle growth in buffalo. These hormonal manipulations have been successfully used to optimize the reproductive outcomes following the application of various biotechnologies.
Timed artificial insemination (TAI) programs provide an organized approach to the enhanced use of artificial insemination (AI), the progress of genetic gain and the improved reproductive efficiency of cattle in dairy and beef herds (Pursley et al., 1995; Baruselli et al., 2004). The success of biotechnologies such as TAI depends on the evolution of ovarian follicular manipulation techniques. In buffaloes, hormonal treatments have been designed to control both luteal and follicular functions, providing exciting possibilities for the synchronization of follicular growth and ovulation that can enable the use of timed artificial insemination (TAI) during the breeding and nonbreeding season (Singh et al., 1988; Baruselli et al., 1999a; Neglia et al., 2003a; De Rensis et al., 2005). Satisfactory pregnancy rates of approximately 40% to 60% (Baruselli et al., 1999b; Berber et al., 2002; Neglia et al., 2003a; Paul and Prakash, 2005; Ali and Fahmy, 2007) have been achieved with the Ovsynch protocol (Day 0, GnRH; Day 7, PGF$_{2\alpha}$; Day 9, GnRH; TAI 16 hours after the second GnRH injection; Pursley et al., 1995) in cycling buffalo synchronized during the breeding season. However, anestrous buffalo respond poorly to the Ovsynch protocol and have lower pregnancy rates after TAI during the nonbreeding season (Baruselli et al., 2003a; De Rensis et al., 2005; Ali and Fahmy, 2007; Baruselli et al., 2007). However, treatment with intravaginal P4 devices followed by eCG at device removal has been used to increase ovulation rates, CL growth rate, initial P4 concentrations, and pregnancy rates after TAI in buffalo during the nonbreeding season (Carvalho et al., 2013).

The use of superovulation (SOV) followed by AI is a technique that generates greater numbers of embryos per donor in cattle (Mapletoft et al., 2002). These techniques, which are associated with embryo transfer (ET) to recipients, are powerful tools that facilitate the dissemination of genetic material of high quality (Bó et al., 2002; Baruselli et al., 2011). However, buffalo donors generally have lower embryo recovery rates than bovines. While buffaloes have shown follicular responses after superovulation treatment (mean of 15 follicles > 8 mm), only a moderate ovulation rate (approximately 60%) and CL yield at the time of flushing (approximately 9 CL) and low embryo recovery rates (34.8%; Baruselli et al., 2000) have been obtained. The embryo recovery rate in buffaloes (approximately 20 to 40%) is lower than in bovines (63 to 80%; Boland et al., 1991; Adams, 1994; Vos et al., 1994; Shaw et al., 1995). This divergence in embryo recovery rates was hypothesized to be related to a failure of oocyte capture and/or of oocyte transport along the oviduct (Baruselli et al., 2000).

Ovum pick up (OPU), associated with in vitro embryo production (IVP), is another interesting technology that produces embryos from selected donors (Boni et al., 1996; Neglia et al., 2003b, Sá Filho et al., 2009). This technology has the potential to enhance genetic progression through the female lineage in buffaloes. The success of OPU–IVP is directly related to oocyte quantity and quality. The use of this technology in buffaloes has been limited by the innately low number of follicles and hence of cumulus–oocytes complexes (COCs) that can be recovered per ovary (Gasparrini, 2002; Gimenes et al., 2010), as well as by the seasonality of follicle production (Di Francesco et al., 2011).

This review discusses a number of key points relating to the manipulation of ovarian follicular growth to improve conception rates following TAI and ET of in
vivo- and in vitro-derived embryos in buffalo. The discussion focuses on the control of buffalo follicular dynamics for artificial insemination, multiple ovulation, embryo transfer (MOET), ovum pick up and in vitro embryo production.

CONTROL OF BUFFALO FOLLICULAR DYNAMICS FOR ARTIFICIAL INSEMINATION

Artificial insemination (AI) is one of the major biotechnologies used in domestic species and an important tool for the dissemination of superior genetic material of paternal origin. However, the use of this technique in the conventional manner (i.e., estrus detection following AI) presents two significant difficulties in buffaloes. The first is related to inefficient estrus detection due to discrete estrous behavior. The second is related to the seasonal and nutritional anestrous that lead to decreased reproductive activity in the species.

Hormonal protocols have been developed to control ovarian follicular dynamics and allow the use of AI without heat detection. In recent years, our research group developed studies to evaluate the efficiency of the Ovsynch protocol in buffalo (D0: GnRH; D7: PGF2α; D9: GnRH; TAI 16 hours after the 2nd GnRH injection; Baruselli et al., 1999a, 1999b; Berber et al., 2002; Baruselli et al., 2003a). In these experiments, we confirmed that buffaloes respond to hormonal treatment and that a new follicular wave emerges due to the ovulation of the dominant follicle present at the time of the first administration of GnRH. On day 7, buffaloes respond to PGF2α (luteolysis), and on day 9, approximately 80% of animals experience a synchronized ovulation within 12 hours. Additionally, a pregnancy rate (PR) of 50% can be obtained in cycling buffaloes during the breeding season. Nevertheless, PR is influenced by body condition score (BCS; good efficiency is achieved when BCS ≥ 3.5; 1 to 5 scale), parity (primiparous animals have lower PR than multiparous), and period of the year (higher PR is observed during the breeding season than in the non-breeding season).

Carvalho et al. (2007a) documented an increase in the PR and birth rates with the administration of GnRH 6 days after TAI in buffaloes on the Ovsynch protocol. This GnRH administration induced the formation of an accessory CL in buffaloes. The accessory CL increased the plasmatic concentration of progesterone (P4) and resulted in a positive effect on the PR and birth rates (Campanile et al., 2010; Marques et al., 2012). Studies have shown that P4 controls the function and secretion of the uterine glandular system (Spencer et al., 2004), which is important for the nutrition and development of the embryo (Mann et al., 1999). Therefore, the production of accessory CLs in buffalo represents an alternative method of increasing the efficiency of TAI.

When the Ovsynch protocol is used during the nonbreeding season (spring and summer; period of high anestrous incidence), lower PRs are obtained (approximately 7 to 30%; Baruselli et al., 1999b and Baruselli et al., 2002a). Therefore, studies have been conducted to develop the use of different hormonal protocols to increase PR in buffaloes submitted to TAI during seasonal anestrous. Various progesterone-releasing intravaginal devices impregnated with different amounts of progesterone (0.5 to 1.9 g) are commercially available. These devices contain natural progesterone, and blood levels of 4 to 5 ng/ml of progesterone are
reached during their use. Lower doses (3 to 6 mg) can be used with auricular implants of norgestomet, which is more potent than natural progesterone.

Progesterone protocol involves the insertion of an intravaginal progesterone device or a norgestomet implant and intramuscular injections of estradiol benzoate (EB) on a random day of the estrous cycle (day 0). Nine days later (day 9), the device/implant is removed and intramuscular doses of PGF$_{2\alpha}$ and eCG are administered. Forty-eight hours later (day 11), ovulation is induced by the administration of GnRH or hCG. TAI is performed 16 hours after the induction of ovulation (Baruselli et al., 2003b; Carvalho et al., 2007b).

The combination of progesterone/progestin and estradiol at the beginning of the protocol (day 0) is effective in inducing the emergence of a new follicular wave due to the suppression of both FSH and LH which promote the atresia of all follicles present in the ovary in cattle (reviewed by Bô et al., 2003) and in buffaloes (reviewed in Baruselli et al., 2007). Previous studies carried out in postpartum anestrous cows have demonstrated that P4 treatment stimulates an increase in LH pulse frequency during and following the treatment period (Rhodes et al., 2002). Treatment of anestrous cows with P4 results in greater follicular fluid volume and circulating concentrations of estradiol, increased pulsatile release of LH and increased numbers of LH receptors in granulosa and theca cells in preovulatory follicles (Rhodes et al., 2002). Furthermore, a short period of elevated P4 concentrations during the anestrous period is important for the expression of estrus and for subsequent normal luteal function (McDougall et al., 1992).

The use of equine chorionic gonadotropin (eCG) at the time of the removal of the progesterone-releasing device resulted in increased ovulation and pregnancy rates in suckled cows treated during postpartum anestrus (Baruselli et al., 2004). In buffaloes, treatment with eCG at the time of device removal increases the diameter of the dominant follicle at TAI (13.7±0.4 vs. 12.6±0.6mm, P=0.09) and the ovulation rate (66.7 vs. 44.8%, P=0.05); at the subsequent diestrus, treatment results in increased CL diameter (15.8±0.92 vs. 12.7±0.77 mm, P=0.03), increased P4 concentrations (0.59±0.08 vs. 0.27±0.05 ng/mL, P=0.01) and increased PRs (52.7 vs. 39.4%, P=0.03; Carvalho et al., 2013). These results confirm the necessity of eCG in ovulation synchronization protocols for TAI during the non-breeding season. In summary, eCG treatment stimulates the growth and maturation of the dominant follicle, resulting in increased ovulation rates and progesterone production by the subsequent CL and greater PR after TAI in anestrous buffalo.

Carvalho et al. (2012b) evaluated the possibility of replacing the ovulation inducer (GnRH) with EB in the buffalo TAI protocol. Treatment with BE resulted in a satisfactory follicular response, ovulation rate and PR in buffaloes synchronized for TAI during the nonbreeding season.

The use of the Ovsynch protocol during the breeding season and of P4+EB, PGF$_{2\alpha}$ and eCG during the nonbreeding season resulted in a PR of approximately 50% in a single TAI. Therefore, the TAI program can be used throughout the year to efficiently schedule conception and the calving period in buffalo.
CONTROL OF BUFFALO FOLLICULAR DYNAMICS FOR MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET)

Despite the progress achieved with TAI and the successful application of this technique in buffalo herds, the biotechnologies used to increase the dissemination of maternal genetic material still have low diffusion. This can be attributed to unsatisfactory numbers of embryos obtained per buffalo donor and reflect the only partial understanding of the events involved in the manipulation of the estrous cycle, follicular superstimulation and ovulation in this species.

Although births of buffaloes achieved using MOET have been reported in several countries (Drost et al., 1983; Drost et al., 1988; Misra et al., 1990; Zicarelli, 1992; Baruselli, 1994), the use of this technique still has limitations, mainly related to the low embryo recovery rate per donor (Misra et al., 1990; Ambrose et al., 1991; Baruselli, 1994; Zicarelli et al., 1994; Taneja et al., 1995; Madan et al., 1996; Zicarelli et al., 2000; Baruselli et al., 2000; Carvalho et al., 2002). Currently, superovulated buffaloes produce on average 1-3 viable embryos for harvest. This average remains lower than the mean number of recovered embryos in the bovine (10 total and 6 transferable embryos; Boland et al., 1991).

The advances provided by the MOET technique have revealed that buffaloes have satisfactory responses to superovulatory treatment (Baruselli, 1997), although embryo recovery in buffaloes is less efficient than in cows (Baruselli et al., 2000). A low number of embryos recovered in buffaloes has also been described by several authors (Karainov, 1986; Madan, 1990; Drost, 1996; Zicarelli, 1997). According to Baruselli et al. (2000), only 34.8% of buffalo ovulations obtained through superstimulation of follicular growth resulted in recovered embryonic structures, a percentage much lower than that found in bovines by Adams (1994) who recorded rates of 63% to 80%. This disparity between embryo recovery rates may be related to failures in the collection and/or transportation of oocytes in the oviduct.

According to Hunter (1988), the mechanisms involved in oocyte transportation (ciliary beats of the epithelium of the oviduct and waves of contraction of the myosalpinx) are controlled by ovarian steroids.

The low number of embryos obtained in buffalo MOET could be attributed to high estrogen (E2) levels during the superovulation treatment, as postulated by Misra et al. (1998). Prolonged exposure to elevated concentrations of 17β-estradiol may change the intrauterine and/or oviductal environment and, consequently, impair normal embryonic development and transport. It is also possible that buffalo are more sensitive to high 17β-estradiol levels during superstimulation treatments than bovines (Beg et al., 1997). To test this hypothesis, our group conducted experiments to reduce estradiol levels during superstimulation treatments (Baruselli et al., 2002b; Carvalho et al., 2002) using exogenous sources of progesterone during preovulatory periods or deslorelin bioimplants during superovulation. However, no increase in embryo recovery rate was observed.

To further test this hypothesis, our group performed a sequence of trials to investigate in detail the anatomical and physiological inter-relationships between ovarian steroids and the genital system of buffaloes and bovines (Carvalho et al., 2011; Carvalho et al., 2012a). We studied morphometric characteristics of females with single or multiple ovulations and we compared the direction of ciliar
movements in oviducts exposed or not exposed to estradiol in the culture medium. We observed no effect of estradiol on embryo transport or on ciliar movement of oviducts. However, we found that buffaloes have a higher number of anovulatory follicles, a more rigid ovary-mesovarium connection, and a thicker infundibulum muscle layer than bovine females. These factors could be partially responsible for the low embryo recovery rates in buffaloes.

As previously mentioned, low oocyte quality in buffaloes may be associated with a fragile connection between the oocyte and granulosa cells, in contrast to what occurs in bovine species (Gasparrini, 2002). Some studies infer that rbST can enhance this connection by a direct and/or indirect effect of IGF-1 and increase the population of small antral follicles (Pavlok et al., 1996; Lucy, 2000). Additionally, rbST can stimulate the expansion of cumulus cells (Izadyar et al., 1998), contributing to oocyte adhesion to fimbria and ciliated cells of the endosalpinx, which can improve embryo recovery in superstimulated animals. To confirm this hypothesis, studies were performed to investigate the effect of different doses of rbST (0, 250, or 500 mg) on embryo recovery in superovulated buffaloes (Baruselli et al., 2003c; Carvalho et al., 2007c). The results were mostly inconclusive; in the first study, 500 mg of rbST increased embryo recovery (50.0 vs. 33.3%; P=0.06) and the number of structures recovered (5.1±6.8 vs. 1.6±1.7; P=0.18); however, in the second experiment, none of the doses of rBST used had any impact on the efficiency of MOET in buffalo.

The low embryo recovery rate reported in buffaloes may be related to the failure of oocytes to enter the oviduct after superstimulation of follicular growth (Baruselli et al., 2000). In rabbits, the administration of sequential doses of PGF\(_{2\alpha}\) during the periovulatory period stimulated the contraction of oviduct smooth muscles, allowing the activation of the oviduct fimbriae to capture the oocytes (Osada et al., 1999). Based on this observation, our research group recently (Soares, et al., 2013) verified that the use of PGF\(_{2\alpha}\) during the periovulatory period increases the total number of structures (ova and embryo) recovered in superovulated buffaloes (buffalo treated with PGF\(_{2\alpha}\)= 3.5±0.6 vs. control group = 2.3±0.5; P=0.02). Furthermore, the treatment with PGF\(_{2\alpha}\) increased the number of transferable (1.8±0.5 vs. 2.7±0.6; P=0.05) and freezeable embryos (1.8±0.5 vs. 2.6±0.6; P=0.08; Figure 1). The results indicate that the administration of PGF\(_{2\alpha}\) during the periovulatory period was effective at increasing embryo production (total ova/embryos recovered) in superovulated buffaloes.

Generally, the reason for the low embryo recovery rate in superovulated buffaloes remains unknown, compromising the efficiency and the application of embryo transfer technology in this species. Further studies are needed to enable the use of MOET in buffalo, to allow this technique to be widely used by farmers and to accelerate genetic gain and productivity of buffalo herds.

CONTROL OF BUFFALO FOLLICULAR DYNAMIC FOR OVUM PICK UP AND IN VITRO EMBRYO PRODUCTION

Due the variable results of in vivo embryo recovery in superovulated buffaloes, the association of ovum pick up (OPU) with in vitro embryo production (IVP) represents an alternative method of exploiting maternal genetics. Historically,
OPU-IVP in buffaloes produced lower outcomes (Gasparrini, 2002; Ferraz et al., 2005; Sá Filho et al., 2009; Gimenes et al., 2010) than in bovines (Lonergan & Fair, 2008, Pontes et al., 2011). However, recent studies have demonstrated the commercial potential of these techniques in the buffalo species.

Two main biological problems seem to be related to the inefficiency of the OPU-IVP technique in buffaloes: low numbers of follicles on the ovary, which influence directly the numbers of oocyte recovery per OPU, and poor oocyte quality (only 27.3% to 31.3% of recovered oocytes are classified as viable; Campanile et al., 2003).

The first problem can be related to the lower number of follicles recruited per follicular wave (Baruselli et al., 1997), as observed in studies comparing buffaloes with *Bos indicus* cattle (Ohashi et al., 1998; Gimenes et al., 2010). Additionally, a higher level of follicular atresia was reported (Danell, 1987; Le Van Ty et al., 1989) and, consequently, a lower number of total recoverable and viable oocytes. Buffaloes and cattle raised with contemporary nutrition and management were compared post mortem by Ohashi et al. (1998), and in vivo by Gimenes et al. (2010; Table 1). In both studies, lower number of follicles (4.5±1.9 vs. 8.7±5.9; Ohashi et al., 1998) and viable oocytes (2.2±1.1 vs. 4.1±2.6; Ohashi et al., 1998) were observed in buffaloes than in *Bos indicus* cattle.

The second problem with the OPU-IVP technique in buffaloes can be attributed to a more fragile zona pellucida (Mondadori et al., 2010) and a more fragile bonding between cumulus cells and the oocyte (Ohashi et al., 1998; Gasparrini, 2002) in buffaloes than in cattle.

To improve oocyte quality and recovery, the following studies were conducted by our group. Initially, we tested the hypothesis that bST could elevate circulating IGF-1 levels, promoting recruitment of a greater number of follicles and enhancing oocyte quality (Sá Filho et al., 2009). OPU was performed twice a week for 10 sessions in control and bST (500 mg) groups. The bST treatment resulted in greater numbers of aspirated follicles (12.2 ± 0.1 vs. 8.7 ± 0.04) and retrieved oocytes per donor per session (5.2 ± 0.5 vs. 4.1 ± 0.5) than the control treatment. However, when oocytes were submitted to IVP procedures, a similar blastocyst formation rate was obtained for animals in the control (26.0%) and bST group (19.7%). In this study, 57 embryos were produced in vitro and transferred fresh (n = 7) or vitrified (n = 25) into 32 previously synchronized recipients (GnRH/PGF2α/GnRH; Sá Filho et al., 2005). Pregnancy rates obtained were 14.3% (1/7) for fresh embryos and 8.0% (2/25) for vitrified embryos. The association of OPU and IVP resulted in the first births from fresh (n=1) and vitrified (n=2) embryos reported in North or South America.

In a subsequent trial (Ferraz et al., 2007), the effects of the intervals between OPU (once a week or every 14 days) and the use of bST (control or treated with 500 mg of rbST) were evaluated. The treatment with bST increased (P<0.05) the number of visualized follicles (15.3 ± 0.5 vs. 12.1 ± 0.4), but reduced (P<0.05) the number of blastocysts (0.8 ± 0.1 vs. 1.4 ± 0.2) and the blastocyst formation rate on day 7 (10.9% vs. 18.9%). On average, OPU performed once a week reduced (P<0.05) the number of aspirated follicles (12.8 ± 0.4 vs. 15.6 ± 0.7) and the number of total (8.5 ± 0.3 vs. 10.0 ± 0.5) and viable oocytes (6.1 ± 0.3 vs. 7.2 ± 0.4).
To test an alternative approach, the effect of synchronization of follicular wave emergence on OPU-IVP in buffalo, Nelore (Bos indicus) and Holstein (Bos taurus) heifers raised under conditions of contemporary management and nutrition was evaluated (Gimenes et al., 2010). An important factor that is known to directly influence the quantity and quality of oocytes obtained by OPU and, consequently, IVP, is the phase of the estrous cycle (Machatková et al., 1996; Hendriksen et al., 2000; Machatková et al., 2000; Merton et al., 2003; Vassena et al., 2003). Oocytes from follicles with a mild degree of atresia have higher rates of embryonic development and require less time for in vitro maturation (Wit et al., 2000; Hendriksen et al., 2000; Vassena et al., 2003). In our study, animals were synchronized to be aspirated 1, 3 or 5 days after the follicular wave emergence. The results of this experiment show that OPU-IVP is less efficient in buffalo and Holstein heifers than in Nelore heifers (Table 1). However, contrarily to our initial hypothesis, the synchronization of follicular emergence for OPU did not affect IVP in any of the three genetic groups.

Recently, the influence of season (winter or summer) on oocyte viability (number of viable oocytes and mitochondrial DNA amount) was investigated in nulliparous (n = 8) and multiparous (n = 8) buffaloes (Macabelli et al., 2012). All animals had synchronized follicular wave emergences by hormonal treatment before OPU was performed five days after the beginning of the protocol (progesterone plus estradiol benzoate). Before OPU was performed, cutaneous and rectal temperatures and the respiratory frequency of all animals were measured. Oocytes (winter = 22 of nulliparous and 13 of multiparous; summer = 21 of nulliparous and 15 of multiparous) were processed for quantification of mitochondrial DNA (mtDNA). As expected, cutaneous and rectal temperatures and respiratory frequency were higher in summer than in winter (P> 0.05). Although the number of viable oocytes was greater in nulliparous animals than in multiparous animals (13.4±2.2 vs. 6.3±0.8), the percentage of viable oocytes was lower in summer than in winter (55.5±3.6 vs. 64.4±2.6). The number of copies of mtDNA is shown in Figure 1. Although mtDNA analyses do not suggest a negative effect of summer on oocyte viability, further conclusions on the effect of seasonality on oocyte competence are expected from ongoing gene expression analyses.

Finally, a partnership between a Brazilian private laboratory of IVP (Cenatte Embriões LTDA) and universities (UFMG, USP, and UNINA) proved that the production of buffalo embryos in vitro for commercial purposes was possible (Saliba et al., 2011; 2012; 2013a; 2013b). Briefly, buffalo donors were submitted to OPU sessions every 20 – 30 days from July to December, 2010. Viable oocytes (grade 1, 2 and 3) were subjected to IVP procedures, resulting in a blastocyst production rate of 44.9% (262/584). A portion of the embryos produced was transferred fresh or vitrified, resulting in pregnancy rates 30 days later of 43.5% (50/115) and 37.1% (26/70), respectively. Embryonic mortality 60 days after embryo transfer was 4% (2/50) and 5.7% (4/70), respectively. The results obtained in these studies were higher than those previously described in the literature and demonstrate the potential of OPU-IVP in buffaloes.
REFERENCES


Table 1. Effect of genetic group (Nelore – *Bos indicus*, Holstein – *Bos taurus* and Buffalo – *Bubalus bubalis*) on OPU-IVP (Gimenes et al., 2010).

<table>
<thead>
<tr>
<th>Metric</th>
<th>Nelore <em>(Bos indicus)</em> <em>(n=9)</em></th>
<th>Holstein <em>(Bos taurus)</em> <em>(n=9)</em></th>
<th>Buffalo <em>(Bubalus bubalis)</em> <em>(n=9)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of visualized follicles</td>
<td>$41.0^a \pm 2.1$</td>
<td>$22.1^b \pm 1.3$</td>
<td>$18.8^b \pm 0.9$</td>
</tr>
<tr>
<td>Mean number of total oocytes</td>
<td>$37.1^a \pm 2.5$</td>
<td>$15.4^b \pm 1.2$</td>
<td>$14.8^b \pm 1.0$</td>
</tr>
<tr>
<td>Recovery rate (%)</td>
<td>$89.4^a$</td>
<td>$73.3^b$</td>
<td>$82.8^{ab}$</td>
</tr>
<tr>
<td>Mean number of viable oocytes</td>
<td>$30.8^a \pm 2.4$</td>
<td>$10.7^b \pm 1.0$</td>
<td>$7.9^b \pm 0.7$</td>
</tr>
<tr>
<td>Mean number of structures in <em>in vitro</em> culture</td>
<td>$18.7^a \pm 0.8$</td>
<td>$8.0^b \pm 0.5$</td>
<td>$7.5^b \pm 0.4$</td>
</tr>
<tr>
<td>Mean number of cleaved structures</td>
<td>$15.4^a \pm 0.7$</td>
<td>$4.6^b \pm 0.4$</td>
<td>$4.4^b \pm 0.3$</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
<td>$81.8^a$</td>
<td>$59.1^b$</td>
<td>$62.3^b$</td>
</tr>
<tr>
<td>Mean number of blastocysts on D7</td>
<td>$5.1^a \pm 0.6$</td>
<td>$1.0^b \pm 0.2$</td>
<td>$0.6^b \pm 0.1$</td>
</tr>
<tr>
<td>Blastocyst rate (%)</td>
<td>$25.8^a$</td>
<td>$13.6^b$</td>
<td>$9.1^b$</td>
</tr>
<tr>
<td>Mean number of hatched blastocysts</td>
<td>$2.6^a \pm 0.4$</td>
<td>$0.3^b \pm 0.1$</td>
<td>$0.3^b \pm 0.1$</td>
</tr>
<tr>
<td>Hatching rate (%)</td>
<td>$50.6^a$</td>
<td>$23.2^b$</td>
<td>$31.9^{ab}$</td>
</tr>
</tbody>
</table>

$a \neq b$: superscript letters in the same row differ (P<0.05).
Figure 1. Total number of structures (ova and embryo recovered), transferable, and freezable embryos of buffalo donors (n=22) treated with sequential doses of PGF\(_{2\alpha}\) during the periovulatory period.

Figure 2. Number of copies of mitochondrial DNA (mtDNA) in oocytes of nulliparous and multiparous animals during winter and summer. *The asterisk indicates a significant difference (P <0.05) between seasons within the same animal category.
Effect of Use Pre-Synch + Ovsynch Protocols on the Pregnancy of the Buffalo Rodeo of the Argentinean NEA

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ABSTRACT

A low intensity of homosexual behavior in buffaloes causes difficulties in the heat detection, subsequently impedes the implementation of artificial insemination. In cattle, there are several hormonal treated protocols for synchronizing ovulation followed by fixed-time artificial insemination (FTAI). Although synchronizing ovulation protocols and FTAI were extensively used in cattle, but very few reports were found using this approach in Argentinean rodeo buffaloes. The aim of the research was to evaluate the efficiency of using two different protocols for synchronizing ovulation, followed by FTAI. Conception rates of the animals receiving different hormonally treated regimes were reported. 320 female Argentinean rodeo buffaloes were randomly divided into two treatment groups. In group I: 190 animals were subjected to the Pre-Synch protocol where 150 mg of PGF\textsubscript{2α} was injected intramuscularly twelve days prior to the first GnRH injection (50 mg synthetic GnRH) and designated as day 0, followed by IM injection of PGF\textsubscript{2α}, 7 day later (day 7), the second injection of GnRH was administered two days later (day 9). In group 2, 130 animals were treated with standard OvSynch protocol. All the animals received FTAI at 16 h before the last injections of GnRH. Pregnancies were confirmed by ultrasonography 38 days before FTAI. Conception rates for the animals in group I and II were 55.8% and 52.3% respectively with overall conception rate of 54.4%. No statistical differences were found between the treatment groups (p>0.05), however numeric differences were observed.

Keywords: Argentinean NEA, buffaloes, Ovsynch, Pre-Synch

INTRODUCTION

The artificial insemination (AI) still isn’t sufficiently rooted in Buffalo rodeos because of the difficulties in heat detection and appropriate time to inseminate (Crudeli et al., 2009). One characteristic of buffaloes is their low incidence of homosexual behavior during heat (Baruselli, 1994), which hinders its external observation and forces to use animal marker for the detection. This characteristic, associated at variations in the heat duration, becomes more laborious the heat detection and difficult the use of AI (Baruselli et al., 1996). In cattle, the heat and ovulation synchronization by hormonal methods submitted encouraging results for the use of fixed-time artificial insemination (FTAI) (Pursley et al., 1995).
Nowadays there are protocols that allow the ovulation synchronization to use the FTAI. In the Ovsynch protocol is used, the gonadotropin release hormone, prostaglandin and their analogues. Studying the follicular dynamic during this treatment was verify that after the first application of GnRH occurs the ovulation or the beginning the growth of a new follicular wave, which result in the presence of a dominant follicle after 7 days, the day of PGF$_{2\alpha}$ application; the luteólisis triggered by this makes that all the treated animals ovulate between 24 to 32 hours after the second dose of GnRH (Pursley et al., 1995). The injections of GnRH, in any stage of the estrous cycle, result in a LH peak causing the ovulation of the follicles $>9.0$ mm or the luteinization of the nonviable follicles, and a new follicular wave appears 2 or 3 days after (Twagiramungu et al., 1995). With the ovulation or luteinization of the dominant follicle, the progesterone levels remain high; hence the PGF$_{2\alpha}$ provide on day 7 induces luteolysis and promotes the ovulation of the follicle of the new wave (Bodensteiner et al., 1996). The second injection of GnRH is recommended 48 hours after the injection of PGF$_{2\alpha}$ for a good synchronization of the ovulation. In Buffalo rodeos of Argentina there are few reports about the use of this protocol (Crudeli et al., 2009).

This research aims study the response of the water buffaloes to the Ovsynch protocol of ovulation synchronization through the use of GnRH and prostaglandins for FTAI, taking into account for this the pregnancy rate.

MATERIALS AND METHODS

An experimental research was evaluated the efficiency of the ovulation synchronization method, through the Pre-Synch method against the traditional Ovsynch, comparing the efficiency of both methods through the pregnancy rate achieved in each.

320 multiparous buffaloes of Mediterranean breed, aged 5 to 12 years old with a good body score and without reproductive problems were used. All the animals were under the same management and feeding system with free access to the water, in the Empedrado department of the Corrientes province, Argentina.

Two groups of animals, one with 190 females for Group I, and 130 for Group II were formed. Subsequently, was assigned to each group the following treatments: Group I: the Pre-Synch protocol which consisted of an intramuscular injection of 150 mg of PGF$_{2\alpha}$ twelve days prior the application of the first dose of GnRH. After this, the animals of both groups received treatment by the Ovsynch method (GnRH/PGF$_{2\alpha}$/GnRH): intramuscular injection of 50 mg of synthetic GnRH (day 0). Intramuscular injection of PGF$_{2\alpha}$ (day 7). Intramuscular injection of 50 mg of synthetic GnRH (day 9). All the animals received FTAI at 16 hours after the last GnRH injection. The pregnancy diagnose by ultrasonography were performed at 38 days after the FTAI. The reproductive stage differences between groups (pregnant and nonpregnant) were performed through the Chi square test, through the InfoStat software InfoStat, student version (Di Rienzo et al., 2011).

RESULTS AND DISCUSSIONS

In the buffaloes the total pregnancy rate was 54.4% (174/320). The pregnancy rate for the animals of Group I was 55.8% (106/190) and for the animals
of Group II was 52.3% (68/130). Differences were not statistically significant between the pregnancy rates obtained with both treatments (p>0.05). Nevertheless, there was a numerical difference in favor of Pre-Synch, but this requires more movement of the animals.

Regarding the effectiveness of the protocol given by the ovulation rates, De Araujo Berber et al. (2002) found 86.6 % of ovulation 36.4 hours after the first dose of GnRH and 93.3% of ovulation after the second dose of GnRH, and Baruselli et al. (2003) reported 78.8% of ovulation at 32 hours after the second application of GnRH.

The results found in this research are superior to those obtained by Ramirez and Guarín (2003), which achieved 36% of pregnancy using the Ovsynch protocol for FTAI in buffaloes. Instead, Baruselli (1995), showed that buffaloes treated with this protocol during the favorable season present an average conception rate of 50.2%; with a marked influence of the body condition on this pregnancy percentages, similar results to those found in this research.

Although in this essay was not considered the body score as a variable of study, it’s clear that to increase the conception rates must be used animals with a body score >3.5 (scale from 1 to 5) as suggested by Geary et al. (1998) who studied the ovulation synchronization in 220 cows and showed that the conception rates are better in those animals with best body condition, since this greatly influence the ovarian cyclicity controlling the estrous cycle and the ovulation.

The results of the assay allow conclude that the hormonal treatments using PGF$_{2\alpha}$ and GnRH in the two variants used produce satisfactory responses in the ovulation synchronization in multiparous buffaloes in the breeding season.

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Sexed Semen and AI in the Mediterranean Buffaloes  
(*Bubalus bubalis*)

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**ABSTRACT**

The technology of Artificial Insemination (AI) was rudimentarily attempted for the first time in 1779 by L. Spallanzani in the dog, and it can be considered the very first applied reproductive technology which is nowadays employed worldwide in many animal species, both farm animals and endangered or wild species. Within the buffalo breeding management, young bulls are probably among the easiest mammals to be trained to serve an artificial vagina, although natural mating is still responsible for most of the pregnancies occurring among herds. Recently, an improvement in the efficiency of protocols for synchronization of ovulation has been reported in buffaloes, paving the road for a wider implementation of assisted reproduction in this species and particularly AI. In addition, since the very first report of buffalo calves born following use of sexed semen in conjunction to AI, the technology of sexing sperm cells has become an additional asset in the toolbox of reproductive technologies implemented within the most advanced buffalo farms. The optimization of protocols for synchronization of ovulation and the use of high quality frozen/thawed semen produced by truly superior bulls, in association to acceptable pregnancy rates and birth of live calves, will surely determine the wider diffusion of AI for both non sexed and sexed semen in the buffalo management worldwide for a faster enhancement of the genetic potential and its distinctive production.

**Keywords:** AI, buffaloes, sexed semen

**INTRODUCTION**

In the field of animal production, the use of AI has always brought within its application a number of advantages related to health, genetics and the overall zoo-economy. From a sanitary point of view, since its implementation this technology has made possible the halt to the transmission of some major venereal infectious diseases, such as *vibrio* and *trichomonas*, that were usually spread through natural mating. On a different hand, by using AI, one bull may produce hundreds of thousands of calves, taking into consideration the number of straws that can be obtained from a single ejaculate following dilution and freezing. In a more global picture, some of the advantages derived from the use of frozen/thawed semen through AI can be identified as: i) the availability of semen even after death of proven sires, and ii) the ease of moving semen to promote genetic improvement in other Countries. Another important aspect related to AI is the customization of the best sire to the recipient characteristics. This allows correction of some morphological and productive defective traits of recipients that will not be carried

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over to their progeny. Furthermore, AI can be a flexible tool to obtain calves with different characteristics, following insemination of recipients with semen from different sires. In the milk industry, the adoption of AI allows the evaluation of sires through progeny testing of daughters whose productive characteristics can be monitored and confronted. Until few decades ago the use of AI in the buffalo species was considered in its infancy and adopted only by few farmers, and therefore the implementation was only marginal for different reasons for both the river and more significantly for the swamp sub-species. In fact, the diffusion of AI in Swamp buffaloes has its drawbacks in the low number of heads per owner, lack of proper heat detection and the overall poor management. On the contrary, in intensive River buffalo breeding, the use of AI had been kept marginal, due mainly to lack of truly superior progeny tested bulls and to unacceptable low pregnancy rates when using both natural or synchronized estrus (Zicarelli et al., 1997). In some Countries like Italy, buffalo breeding is of particular competitiveness and importance and it has been on the rise in terms of population heads when compared to other more firmly established forms of livestock. Within the buffalo industry there is an ever increasing demand for milk, associated though with the need to cut production costs. In this scenario reproductive biotechnologies may become of stringent importance, by allowing the inclusion of specific selective programs within a more traditional framework of breeding strategies of which AI still retain its primary role. Following the first proven evidence of offspring derived from the use of sexed sperm cells and AI in rabbit (Johnson et al., 1989), the same technology, rooted in the differential amount of DNA content between X- and Y-bearing sperm cells (Garner, 2006), was used with success in cattle and in a number of other production animals (Rath and Johnson, 2008). More recently, artificial breeding in buffaloes has witnessed the introduction of the sexed semen technology, and following the birth of the very first buffalo calves through AI and sexed semen (Presicce et al., 2005a; 2005b), more available and published data has made evident the particular efficiency of such technology in this species (Campanile et al., 2012).

BUFFALO SEMEN QUALITY FOR AI WITH SEXED SEMEN

Buffalo bulls are characterized by a slower sexual development when compared to cattle bulls and, as with other animal species, gonadal sperm numbers are positively and significantly correlated with the weight of testicular parenchyma (Abdou et al., 1982; Pant et al., 2003). A number of factors such as hormonal fluctuations, are linked to the photo-neuroendocrine system influencing thus the male reproductive efficiency (Seren and Parmeggiani, 1997). The function of the male gonads too, is influenced by the melatonin hormone as the endocrine signal that marks the light and dark hour fluctuation of the day. Such multistep neural pathway is characterized by a domino effect cascade starting with a photoperiod sensitivity to the length and density of the light source, following involvement of the retina, the suprachiasmatic nucleus, the superior cervical ganglia and finally the pineal gland leading to incretion of melatonin. Such neuronal stimulus triggers the rhythmus of melatonin incretion which regulates hypothalamo-hypophysial activity, gonadal function and finally sperm composition and quality (Zicarelli, 1997). Interestingly, and in consideration of the seasonal fluctuation in reproductive
efficiency in buffalo bulls as well, both weight and size of scrotal circumference together with epididymal weight, are slightly but not significantly reduced in the course of the non-mating season when compared to the mating season (Ibrahim, 1985). In fact, some studies (Arrighi et al., 2010), suggest and confirm the potential of buffalo bulls to breed throughout the year, although reproductive function is somewhat compromised during the non-mating season, as confirmed by a large variability in semen quality reported among Nili-Ravi, Murrah and Mediterranean Italian buffaloes (Saeed et al., 1990; Kumar et al., 1993; Presicce et al., 2003). These considerable differences may be explained by the lack of long time selection for semen freezability in this species. In addition to semen quality and its freezability, photoperiod has also been reported to affect sexual activity and bull libido (Sansone et al., 2000). Furthermore, to underline the sensitivity to seasonality in the buffalo species and in particular in the bull, it has been reported a neuro-endocrine interaction between androgen hormones and the autonomic nerve supply in the regulation of male buffalo reproductive functions. In fact, during the mating period, dense noradrenergic innervations can be observed to supply the vas deferens as well as the accessory sex glands, whereas during the non-mating period the noradrenergic nerves are dramatically and significantly reduced (Mirabella et al., 2007). With regard to parameters usually taken into consideration for semen quality, minimal standards for a classification of “probably fertile” specimen of buffalo semen are: a) over 500,000/mm³ spermatozoa; b) more than 60% of motile sperm with forward progression and c) more than 70% of the spermatozoa conforming to normal morphology. Normal buffalo sperm concentration shows a wide range of variation (600,000 to 1,200,000 cells per mm³ i.e. 800,000 per mm³ on average) and this parameter is highly sensitive to seasonal and nutritional factors. Usually, first ejaculate contains higher number of spermatozoa per ml compared to second ones, and the seasonal and climatic factors have a strong effect on the morphological and chemical seminal characteristics. In temperate regions, it has been reported that better quality semen is produced during the winter and spring while it has been shown deterioration in semen quality during summer and autumn. On the other hand, in tropical regions the quality of semen is found to be good during the rainy season. Moreover, in warm and humid tropical Amazon region, the best time to work with semen freezing is between January and June. Buffaloes are sensitive to heat stress, thus decline in semen quality is a common finding during the hot season of the year. The best manner to overcome the problem of semen quality deterioration due to heat stress during summer is to sprinkle the animals with water during the hotter part of the day or allow the animals to wallow, protect the animals from radiation exchange and hot wind, and keep the animals in ventilated paddock. Free access of the animal to water and shadow is very important, since buffalo have a poorly-developed thermo-regulatory system, causing them to suffer of heat stress during summer. Semen with more than 30% initial dead spermatozoa may not to be good for freezing and semen samples with less than 50% of live spermatozoa may be of questionable fertility even throughout natural breeding. Complex double staining with Trypan Blu and Giemsa has been used in buffalo spermatozoa (Figure 2), to evaluate integrity of acrosome, tail and midpiece (Presicce et al., 2003; Boccia et al., 2007).
SEXED SEMEN AND AI

Following the first study on the detection of buffalo sex chromosomes in spermatozoa by fluorescence in situ hybridization (FISH) by Revay et al. (2003), where the evolutionary conservation of this locus in the water buffalo has been demonstrated, an increasing interest has been shown on the possibility to use sexed semen in the buffalo breeding strategies. A preliminary study was designed to show the possibility to use a significantly reduced number of spermatozoa, and at the same time to obtain acceptable pregnancy rates (Presicce et al., 2004). Subsequently, the same concentration was used to inseminate pluriparous buffaloes with sexed semen aided by a special catheter (Ghent device) designed on purpose to deposit such a reduced sperm number at the utero-tubal junction in order to allow the highest availability of sperm cells at the site of fertilization (Presicce et al., 2005a). This first study showed unequivocally that sexed semen technology in buffaloes allows sperm cell populations to be selectively sorted and enriched with purity similar to what previously reported for cattle bulls. Following these first encouraging results, the impact and feasibility of flow cytometric sorting of X- and Y- sperm cells of Murrah and Nili-Ravi buffalo bulls have been studied (Lu et al., 2006), leading to the born of the first buffalo calves produced by the combined use of \textit{in vitro} fertilization procedures (IVF) and sexed semen (Lu et al., 2007), showing that this new approach in the toolbox of reproductive technologies, can be used to efficiently exploit valuable sorted semen. The possibility to retrieve immature oocytes from preantral follicles in large ruminants by means of transvaginal guided puncture or ovum pick up (OPU), has open new possibilities for genetic improvement within herds and breeding schemes (Pieterse et al., 1991). Although the efficiency of late stage in vitro embryo production in buffaloes, following OPU and IVF can be considered similar to what reported in cattle (Neglia et al., 2003), the development to term following transfer of fresh or frozen embryos is still unsatisfactory (Neglia et al., 2004; Lu et al., 2007). Nevertheless, the combined use of IVF, OPU and sexed semen has been proven feasible and repeatable, mushrooming new research for a wider field application of these technologies in buffalo (Liang et al., 2008). More recently, very high pregnancy rates following AI with buffalo river type sexed semen into swamp and F1 (River x Swamp) recipients undergoing spontaneous estrus, have been reported (Lu et al., 2010). These last successful results indicate a powerful way to disseminate valuable semen for the acceleration of genetic gain in swamp buffalo too, bearing in mind the different breeding management and local conditions typical of Asian Countries. The first large trial on the use of sexed semen in Mediterranean buffalo heifers has confirmed the similarity of efficiency and pregnancy rates when compared to the use of conventional semen (Campanile et al., 2011). In this study, a modified Presynch-Ovsynch protocol for synchronization of ovulation was implemented. Additional variation within the protocol consisted of an adjunctive administration of GnRH and prostaglandin at the time of AI, in order to verify the beneficial combined effect on possible synchronous ovulation and enhanced internal progesterone production. In the same study, it has been shown that sexed semen deposition into the body of the uterus gives similar pregnancy rates if compared to the deep semen deposition into the uterine horn. This latter finding will facilitate the dissemination and use of sexed semen, since no special skills or
particular technique, as seen in Presicce et al. (2005a), are necessary to obtain acceptable pregnancy rates. More recently, on a similar trial on pluriparous buffaloes subjected to AI with sexed semen during the seasonal and transitional breeding periods, a conventional Ovsynch protocol was employed, reducing thus manpower on handling of animals and reducing the need of exogenous hormonal administration, and at the same time reporting good conception rates non dissimilar from the use of conventional semen (Campanile, 2012).

CONCLUSIONS
Normalcy of buffalo calves at birth, following use of sexed semen in AI schemes has been reported (Presicce et al., 2005b; Campanile et al., 2011). The efficiency of protocols for synchronization of ovulation, tied to good pregnancy rates following use of sexed semen will undoubtedly widen the opportunity of farmers into the selection and adoption of AI and other breeding strategies for improvement of production and reproduction traits.

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In Vitro Embryo Production in Buffalo: 
Yesterday, Today and Tomorrow

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ABSTRACT

In the last few years, there has been an increasing interest in the in vitro embryo production (IVEP) technologies for faster propagation of superior germplasm in buffalo, due to the low efficiency of multiple ovulation (MO) and embryo transfer (ET) programs. Early attempts to produce buffalo embryos in vitro have been made by using procedures that were proven effective in cattle. However, the acquisition of more specific information on oocyte and embryo culture requirements in vitro in this species has resulted in an improved efficiency over the years. The present review intends to describe the state of the art of IVEP in buffalo, emphasizing the advances achieved and the limitations still to overcome.

Keywords: buffalo, embryo, in vitro embryo production, oocyte, ovum pick up

INTRODUCTION

Interest in buffalo breeding has tremendously increased worldwide, due to the fundamental role played by the species in many climatically disadvantaged agricultural systems. Currently, the competitiveness of buffalo breeding highly depends on genetic improvement and, hence on the use of reproductive technologies, those allow to plan and accomplish selective programs in a shorter time. Due to the limitations of MOET programs in buffalo, the ovum pick-up (OPU) and IVEP technology is the best tool to speed up the genetic progress through the maternal lineage. First of all, OPU can be carried out on a wider typology of donors, such as non-cyclic animals, pregnant cows, subjects with patent oviducts or genital tract infections, and animals that are not responsive to hormonal stimulation, the last representing a high proportion in buffalo. Furthermore, the OPU combined with IVEP has greater potential than MOET to enhance the maternal contribution to the genetic improvement, allowing higher embryo yields on long term basis. In fact, it has been demonstrated that OPU can be performed for up to 6 months with high embryo production efficiency, without interfering with the reproductive activity of the donors (Neglia et al., 2011).

In the earlier studies dating back to the 1990s, the oocyte sources were abattoir-derived ovaries and the IVEP system was simply extrapolated from that used in cattle, without taking into account species-specific differences, resulting in poor efficiency. Ovum pick up was carried out in buffalo on deep anoestrous animals for the first time in 1994 (Boni, 1994). Since then, OPU trials have been performed several times (Boni et al., 1996; Neglia et al., 2003, 2004, 2011; Liang et al., 2008, Sá Filho et al., 2009). In the last 30 years the intensification of the

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studies over the world has led to the optimization of the sequential steps of in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC). Therefore, the IVEP efficiency has greatly increased, leading to high maturation (90%), cleavage (75-80) and blastocyst rates (30-40%), and to the production of offspring (Hufana-Duran et al., 2004; Neglia et al., 2004). The questions that arise are: “Is this technology commercially viable? Where are we today and where are we going to?”. The aim of this review is to highlight the major breakthroughs in the OPU and IVEP technologies in buffalo in the past 30 years, emphasizing the advances achieved in the sequential steps of IVEP, the limitations still to overcome and expected future improvements.

OOCYTE RECOVERY AND QUALITY

Since the earlier trials, it was evident that the major intrinsic limitation for the diffusion of IVEP in the field in buffalo is the low number of oocytes recovered per ovary, arising from physiological features of the species, such as the low number of primordial and antral follicles, as well as the high incidence of follicular atresia (Gasparrini, 2002). Although affected by various factors, such as ovarian status, days in milk, season, genetics, age, breeding system, environment, interval between sessions, the oocyte recovery is constantly poor, making the benefit cost ratio unfavorable. The scarce predetermined follicular reservoir of the species makes this factor difficult to improve. In fact, stimulation with FSH (Boni et al., 1994) or rBST (Sá Filho et al., 2009) have been successful to increase the number of gametes but only to a limited extend. A possible approach to increase the number of competent oocytes could be the selection of donors on the basis of their follicular population.

Oocyte quality, the major factor affecting IVEP efficiency in most species, plays a determining role in buffalo, further decreasing the availability of the oocytes suitable for IVEP. In fact, the percentage of good quality oocytes (Grade A and B) is lower in this species compared to others, not exceeding, in our experience 50% of the total oocytes. The oocyte quality may be affected by several factors, such as the aspiration pressure during collection, the source of gametes, the time between collection and processing, the temperature during transportation, season, etc. Despite their worse morphological appearance, OPU-derived buffalo oocytes have a higher developmental competence compared to abattoir-derived ones, as indicated by higher blastocyst rates (Neglia et al., 2003) and hatching rates following embryo vitrification (Manjunatha et al., 2008). At our latitudes season significantly affects oocyte competence, without affecting the follicular population and oocyte yield, with the highest blastocyst yields recorded in autumn, i.e. during short-day months (Di Francesco et al., 2011, 2012). This strongly suggests to restrict the gametes collection during autumn at our latitudes, in order to save resources and improve the benefit cost ratio. A seasonal effect on IVEP efficiency, mainly due, though, to decreased follicular population, was also reported by Indian authors (Manjunatha et al., 2009).

IN VITRO EMBRYO PRODUCTION: IVM, IVF AND IVC

As it is not here possible to cite all the studies carried out over a long time for lack of space, the focus will be here addressed to those that, in my opinion, had the
greatest impact on the IVEP efficiency. The significant increase of blastocyst rates has been achieved mainly through the optimization of the IVM system that led to improved oocyte developmental competence. In particular, a significant enhancement of the oocyte developmental competence has been obtained by increasing the oocyte antioxidant capacity during IVM, as the high lipid content (Boni et al., 1992) makes buffalo oocytes/embryos particularly sensitive to oxidative damages. The IVM medium has been enriched by thiol compounds, such as cysteamine (Gasparrini et al., 2003) and β-mercaptoethanol (Songsasen et al., 2002), known to act as antioxidants factors, by stimulating glutathione (GSH) synthesis. Lately, the IVM system has been further improved by adding cystine in the presence of cysteamine, leading to an additional increase of the GSH oocyte reservoir, as well as fertilization, cleavage and blastocyst rates (Gasparrini et al., 2006). Among other factors, the duration of IVM plays a critical role on embryo development in vitro, since an inappropriate timing of maturation may result in abnormal chromatin, oocyte aging and reduced development. Although the majority of oocytes completes nuclear maturation between 20 and 24 h, a progressive decrease of cleavage and embryo development after both IVF and activation, together with an increased incidence of degenerated oocytes, have been recorded as the IVM duration increases from 18 to 30 h (Gasparrini et al., 2008). This indicates an earlier aging of the buffalo oocyte that imposes to perform IVF in a narrow time window, ideally at 18 h post-IVM or, in any case, not later than 24 h.

In the past fertilization was considered the most critical step of the IVEP procedures in buffalo, as cleavage rates were lower than those obtained in other domestic species (Neglia et al., 2003). In earlier times, the quality of the frozen semen was considered the major factor impairing in vitro fertilization (IVF), as indicated by severe sperm damages (Meur et al., 1988) and poor cleavage rates (Totey et al., 1992) recorded after cryopreservation. Currently, the quality of frozen semen has greatly improved, whereas the “bull effect”, i.e. the high degree of variation of the in vitro fertilizing capability among buffalo bulls, is still a serious limitation (Gasparrini, 2002). It follows that an accurate screening of sperm is required before enrolling a bull in IVF programs. Furthermore, the great variability in the penetration speed among buffalo bulls and its correlation with blastocyst yields (Rubessa et al., 2009), suggested to include this assessment in the fertility test. This also allows to adapt the gametes co-incubation time during IVF in relation to the bull used. Among many other studies, it is worth reporting the improvement of cleavage and blastocyst yields (Di Francesco et al., 2009) obtained by supplementing the IVF medium with osteopontin, a male fertility marker that was found to be greatly reduced by cryopreservation (Pero et al., 2007). Finally, it is worth emphasizing that the fertilization efficiency has at last improved, reaching approximately 80% of cleavage rate, by enriching the IVM medium with thiol compounds (Gasparrini et al., 2006), indicating that the poor cleavage rate of this species so far recorded was mainly related to an inappropriate maturation of the female gamete.

The great improvement of blastocyst yields (35-40%) achieved in recent years is mainly due to the optimization of the IVM and in part of the IVF systems rather than to modifications applied to the IVC system. In fact, despite many efforts
to improve the IVC, at present the original version of SOF remains the most suitable medium. Despite high blastocysts rates, pregnancy to term following ET of cryopreserved buffalo embryos up to recently has been very poor. This is likely due in part to the poor viability of IVP embryos, resulting from suboptimal culture conditions. It has been demonstrated that oxidative stress determines DNA damage in buffalo embryos during IVC, that can be partly ameliorated by cysteamine supplementation in the medium (Mukherjee et al., 2010). The enrichment of the culture medium with either taurine or melatonin has been shown to improve blastocyst yield (Manjunantha et al., 2009b). A beneficial effect of vitamins A, E and C on blastocyst production has been recorded in the presence of high O2 concentration (Thiyagarajan and Valivittan, 2008; Saikhun et al., 2008). We demonstrated that relatively high concentrations (1.5 mM) of glucose are required for in vitro culture of buffalo embryos during early embryonic development (up to Day 4), whereas glucose withdrawal/reduction during late culture is not detrimental. Recently, much higher concentrations of glucose (5.6 mM) during both IVM and IVC have been proven beneficial on blastocyst development (Kumar et al., 2012). It follows that energy requirements of buffalo embryos are different from those of sheep and cattle, highlighting the importance of identifying species-specific differences. However, glucose can be removed from SOF medium if replaced by myo-inositol and citric acid (unpublished data).

It is known that blastocyst quality more than blastocyst production has an impact on pregnancy outcome. A recent study demonstrated that the addition of leukaemia inhibitory factor to the IVC medium improves blastocyst development and quality (Eswari et al., 2012). An improvement of embryo quality, indicated by faster development and increased cryotolerance, was determined by high concentration of hyaluronic acid during late culture (Boccia et al., 2012). Finally, we recently observed that L-carnitine supplementation of culture medium also improves the resistance to cryopreservation of IVP buffalo embryos, likely facilitating the utilization of the endogenous lipid stores (Boccia et al., 2013).

**PREGNANCIES TO TERM**

It is known that buffalo IVP embryos are very sensitive to cryopreservation, due to their high lipid content (Boni et al., 1992), and that cryotolerance may be increased by in vivo culture in sheep oviducts (Galli et al., 1998), but this system is unpractical and expensive. Nevertheless, buffalo embryos entirely produced in vitro have been successfully cryopreserved by vitrification, as demonstrated by their in vitro survival and development to term after ET (Hufana-Duran et al., 2000; Neglia et al. 2004). In recent times, the ET of buffalo IVP embryos vitrified by Cryotop (n=20) in Italy gave high pregnancy rate (50%) on day 30 but, due to a high incidence of embryonic mortality (EM) between day 25 and 50, development to term was strongly reduced (10%). At our latitudes during the long-day length period EM is also observed after natural mating, to further increase when reproductive biotechnologies, such as AI and ET of IVP embryos are used (Campanile et al., 2010). This phenomenon is due to both a poor quality of the oocytes and embryos (Di Francesco et al., 2011, 2012), and to the reduced corpus luteum function, leading
to lower progesterone secretion (Campanile et al., 2010) during the unfavorable season, when we are obliged to carry out ET to meet the market demand.

Finally, unprecedented results came from an OPU trial carried out in Brazil, where 70 vitrified embryos were transferred into recipients by fixed time embryo transfer (FTET), based on the use of eCG in oestradiol/progestin-based protocol (Saliba et al., 2013). With regard to the embryo-recipient synchrony, day 5, 6 and 7-expanded blastocysts were transferred into recipients on day 6 after ovulation. Pregnancy rates were 37.1% on day 30 and 31.4% on day 60, with 15.4% EM. Development to term was higher than ever reported in literature, with 12 female and 10 male healthy calves born. These results completely changed the scenario, opening opportunities for the diffusion of OPU-IVEP in the field. The question that arises is how this was possible. It is known that the final pregnancy outcome is in part due to the viability of the embryo, determined by culture conditions, and in part to the status of the recipient and the perfect embryo-recipient synchrony. Therefore, encouraged by the excellent results, we planned an OPU trial and subsequent ET last autumn, to start with a better status of both donors and recipients, and we used FTET, with eCG in GnRH based protocol (as oestradiol is not allowed in our country). We transferred 46 blastocysts (25 fresh and 21 vitrified). The ET of fresh blastocysts resulted in 32% and 24% pregnancy rates on day 25 and 45, respectively. However, faster developing embryos, i.e. expanded and hatched, gave higher pregnancy rates than slower developing embryos, i.e. early blastocysts and blastocysts (40 vs 26.7% on day 25 and 30 vs 20% on day 45, respectively). Pregnancy rates after ET of vitrified embryos were 23.8% on day 25 and 19.0% to term. However, the difference related to the chronology of development was greater for vitrified embryos: in fact, faster embryos gave 45.5% pregnancy rate on day 25 and 36.4% to term, whereas no pregnancy was obtained from slower embryos. In contrast with the other trial (involving higher numbers), in this study the sex ratio was skewed towards males (66%). The overall improvement of pregnancy rate and the higher efficiency of faster embryos suggest that both the embryo viability and the recipient management play an important role. It is well known that the main factor affecting the cryopreservation efficiency is undoubtedly the embryo quality and that it is current practice in cattle and other domestic species to operate a strict selection of the embryos prior to cryopreservation. In buffalo, however, a strict selection of superior quality embryos would further limit the number of embryos, affecting the benefit cost ratio. Therefore, as the embryo quality is in part due to the oocyte quality but is also affected by the culture conditions, the optimization of culture system is still required.

CONCLUSIONS

The recent results are unprecedented in science and open possibilities for the commercial use of OPU and IVEP in buffalo. Therefore, the major current limitation is the low number of oocytes recovered that depends on the scarce follicular reservoir of the species. In future perspective, the optimization of the oocyte cryopreservation efficiency may lead to increased oocyte availability for IVEP, allowing better planning IVF trials and saving resources. Furthermore, the use of sexed semen for IVF may further optimize the competitiveness of this technology.
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Vitrification of Buffalo Oocytes: Current Status and Perspectives

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ABSTRACT

During the past decade vitrification has been acknowledged as a viable alternative to traditional slow-rate freezing in both human and animal embryology. The buffalo is the major milk and meat producing farm animal in many developing countries. Buffalo oocytes obtained from slaughterhouse ovaries and matured in vitro are useful sources. Cryopreservation of buffalo oocytes is very important in preserving this endager species for future use. This review presents the recent buffalo oocytes vitrification procedures, the principle of vitrification, protocols for buffalo oocytes vitrification and the future of buffalo oocytes vitrification.

Keywords: buffalo, oocyte, vitrification

INTRODUCTION

Water buffalo are divided into two main types: river buffalo and swamp buffalo, which have 50 and 48 chromosomes respectively. Nowadays, the buffalo is the major milk and meat producing farm animal in many developing countries. Buffalo oocytes obtained from slaughterhouse ovaries and matured in vitro are useful sources for reproductive procedures such as somatic cell nuclear transfer, IVF and intracytoplasmic sperm injection (ICSI), in which mainly cryopreserved spermatozoa are used. Yet the limited number of buffalo oocytes made it difficult to assess the success rate of these reproduction technologies. From this problem, the topic of “How to store the oocytes for further used?” has been raised several years ago.

Although, the biotechnologies of reproduction have been used in this special, most of them are not as efficient as in bovine. Hence, it is imperative to study the factors necessary to improve the success rate of the application for reproductive biotechnologies in this species.

Cryopreservation of oocytes is very important in preserving female gametes for future use. Efficient oocyte cryopreservation protocols will widen and improve the strategic implementation of reproductive technologies in the buffalo species. After cryopreservation, the oocytes presents compromised developmental competence. Optimal cryopreservation protocols should be adapted to individual

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species requirements, which present large variation in gamete size, permeability and sensitivity to cryoprotectant (CPA).

In assisted reproductive technology (ART), cryopreservation of embryos has become important for the best use of supernumerary embryos. During the cryopreservation of embryos, various types of injury may occur. Among the most damaging is the formation of intracellular ice. The first strategy to prevent intracellular ice from forming was to use a lower concentration of CPA and a long slow-cooling stage. This slow-freezing method has proven effective for embryos of a wide range of mammalian species. Unlike embryos of laboratory animals and domestic animals, in which dimethylsulphoxide (DMSO), glycerol or ethylene glycol (EG) are commonly used as the CPA. With slow freezing, however, it is difficult to eliminate injuries occurring from ice formation completely. Furthermore, the slow-freezing method requires a long period of time before embryos are stored in liquid nitrogen (LN₂).

An alternative form of cryopreservation is vitrification. Vitrification is defined as “the solidification of a solution brought about not by crystallization but by extreme elevation in viscosity during cooling” (Fahy et al., 1984). The rapid cooling process can minimise chilling injury and osmotic shock to the embryos. With recent improvements in past decades, vitrification has become the most reliable strategy because it is technically simple, and can lead to high survival and implantation rates.

Several advances have taken place in vitrification technologies over the past decade. It is proposed that vitrification will definitely become the most suitable method for cryopreservation of any cells and tissues in the near future. Therefore, vitrification technologies applied on buffalo oocytes and embryos has become more successful as an alternative to slow cooling methods.

**PRINCIPLES OF VITRIFICATION**

Vitrification is the alternative method of cryopreservation which uses an ultra rapid cooling rate, eliminating the need for programmable freezing equipment. Furthermore, the vitrification technique uses high concentrations of CPA which avoids water precipitation, preventing intracellular ice crystal formation.

The basic procedure for vitrification is simple. Embryos or oocytes are suspended in a vitrification solution and then plunged in LN₂, or super-cooled air. Embryos are warmed rapidly and diluted quickly with a sucrose solution. The most important stage is the exposure of embryos to the vitrification solution before rapid cooling. In order to prevent intracellular ice from forming, a longer period of exposure is desirable. If the exposure is too long, however, embryos suffer from the toxicity of the CPA solution. Therefore, the optimal exposure time for successful vitrification must be a compromise between preventing the formation of intracellular ice and preventing toxic injury. Ironically, embryos may be injured by the toxicity of the cryoprotectant before enough cryoprotectant can permeate inside the embryos. To prevent this, a two-step procedure is commonly used, in which embryos are first equilibrated in a dilute (e.g. 10%) CPA solution, followed by a brief (30–60 s) exposure to a vitrification solution before embryos are cooled with LN₂. The optimal exposure time in the vitrification solution depends on the CPA solution and the
temperature, as both the permeability of embryos and the toxicity of the CPA are largely influenced by the temperature (Rall and Fahy, 1985; Menezo et al., 1992). In vitrification, the selection of CPA requires extreme care because their concentration can be as high as 6 M, which can make the toxicity of these compounds a key limiting factor in cryobiology. The most appropriate characteristics of a penetrating CPA are low toxicity and high permeability. As a less toxic CPA, EG is commonly and widely used (Kasai, 2002).

PROTOCOLS FOR OOCYTES VITRIFICATION

Several protocols have been applied on buffalo oocytes vitrification. In those protocols, however, the basic concept is similar, and the differences between the protocols are related to the vitrification container, the type and concentration of CPA and duration of exposure of CPA. Protocols and outcomes are presented in Table 1.

Because of the high intracytoplasmic lipid content, buffalo oocytes are supposed to be particularly sensitive to chilling injuries (Boni et al., 1992). Slow freezing results of immature and matured buffalo oocytes demonstrated that slow freezing is not suitable for immature buffalo oocytes, as proven by both poor maturation rates and development to morulae (Gautam et al., 2008a), and that vitrification is more effective than slow freezing for the cryopreservation of in vitro-matured buffalo oocytes (Gautam et al., 2008b).

A two-step protocol for vitrification with French straw as a container using EG and DMSO as base-CPA for GV stage oocytes have been described at the beginning of the buffalo oocytes vitrification research (Dhali et al., 1999; Dhali et al., 2000). In 2004, the first successful production of a buffalo blastocyst derived from IVM and IVF of vitrified-warmed oocytes was reported (Wani et al., 2004), although the efficiency remained low. Insufficient cooling rates of oocytes were considered one of the principal obstacles in vitrification technology. In order to overcome this problem, several methods have been proposed using very small amounts of solution. Some improved vitrification methods have been successfully used for buffalo oocyte cryopreservation, including solid surface vitrification (SSV, Gasparrini et al., 2007; Boonkusol et al., 2007; Liang et al., 2012), cryoloop (Gasparrini et al., 2007), cryotop (Muenthaisong et al., 2007), open pulled straw (Mahmou et al., 2008), microdrop (Liang et al., 2011).

In 2007, Sharma and Loganathasamy proved that the meiotic stage affects survival rates of buffalo cumulus–oocyte complexes (COC) submitted to vitrification/warming, with higher values for those with 24 h maturation compared to those with a shorter one. One of the most successful ultra-rapid vitrification techniques is the Cryotop method that has resulted in excellent survival and developmental rates with human and bovine oocytes (Kuwayama et al., 2005). Cryotop (Muenthaisong et al., 2007; Attanasio et al., 2010; Liang et al., 2012) has been successfully utilized to cryopreserve buffalo oocytes, which retain the capability to develop into blastocyst following parthenogenetic activation, nuclear transfer, and IVF.

CPA mixtures may have some advantages over solutions containing only 1 permeable CPA (Vajta et al., 1998; Chian et al., 2004), and a mixture of EG and DMSO has been widely used for oocyte cryopreservation (Muenthaisong et al.,
2007, Morato et al., 2008). Recent progress in the establishment and improvement of oocyte cryopreservation methods in swamp buffaloes shown that mixture of EG and DMSO has gained popular in this species.

THE FUTURE OF BUFFALO OOCYTES VITRIFICATION

With the aim of improving survival rate and quality of oocytes after vitrification, different approaches have been developed pointing to the use of different vitrification container, type and concentration of CPA.

The major damaging factors, which occur during cryopreservation, are associated with chilling injury, osmotic stress, CPA toxicity, and ice crystallization (Mazur et al., 1972). In general, we are trying to reduce these damages by increasing cooling and warming rates using vitrification. In the past, vitrification was based on the combination of a high cooling rate and high concentration of CPA, which caused breakthrough in the field of vitrification came when sample volume was reduced to a level that permitted lowering the CPA concentration. Success has been reported in a handful of mammalian species, but differences between species make cryopreservation techniques’ dissemination difficult.

Some attempts to improve cryopreservation outcome through manipulations to germplasm have been reported, but more studies are needed to identify the more promising ones, which will be incorporated into routine oocytes vitrification protocols.

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<th>Author and reference</th>
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<th>Equilibration step</th>
<th>Vitification step</th>
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<td>Dhali et al., 1999</td>
<td>straw</td>
<td>GV</td>
<td>2.25M EG+1.7M DMSO 1 or 3 min</td>
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<td>Dhali et al., 2000</td>
<td>straw</td>
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<td>I: 2.25M EG+1.7M DMSO 1 or 3 min II: 1.75 M EG 1 or 3 min</td>
<td>I: 4.5M EG + 3.4M DMSO 2 min II: 3.5 M EG 2 min</td>
<td>0.5M S. 5min</td>
</tr>
<tr>
<td>Wani et al., 2004a,b</td>
<td>straw</td>
<td>GV</td>
<td>1.5 M of DMSO, EG, PROH and glycerol, respectively, 5 min</td>
<td>3.5,4,5,6,and 7 M of DMSO, EG, PROH and glycerol, respectively, 5 min</td>
<td>0.5M S. 5min+ 0.25M S. 5min+ 0.1M S. 5min</td>
</tr>
<tr>
<td>Gasparrini et al., 2007</td>
<td>SSV, CLV</td>
<td>MII</td>
<td>SSV: 4%EG 12-15min CLV:7.5%EG+7.5% DMSO 3min</td>
<td>SSV:35%EG+5%PVP+0.4M trehalose 25-30s CLV:16.5%EG+16.5%DMSO 25s</td>
<td>SSV: 0.3M trehalose 3min CLV: 1.25M S. 1min+0.62M S. 30s+0.42M S. 30s+0.31M S. 30s</td>
</tr>
<tr>
<td>Sharma et al., 2007</td>
<td>straw</td>
<td>MII, GV, 6h IVM, 12h IVM 18h IVM</td>
<td>40%PROH+0.2M trehalose 3 min</td>
<td>1 M S. 15min</td>
<td></td>
</tr>
<tr>
<td>Boonkusol et al., 2007</td>
<td>SSV, straw</td>
<td>MII</td>
<td>SSV: 4%EG 5-10min Straw: 4%EG 5-10min</td>
<td>SSV: 35% EG+ 5%PVP+ 0.4M trehalose 25-30 s Straw:40%EG+5% PVP+0.4M trehalose 1 min+ LN2 vapor 3min</td>
<td>SSV: 0.3M trehalose 1min+0.15M trehalose 2min+0.075M trehalose 2min Straw: 0.3M trehalose 1min</td>
</tr>
</tbody>
</table>

### Table 1. Protocols, containers, CPA concentration, time and properties of the buffalo oocytes vitrification (Continue).

<table>
<thead>
<tr>
<th>Author and reference</th>
<th>Type of container</th>
<th>Oocytes stage</th>
<th>Equilibration step</th>
<th>Vitrification step</th>
<th>Warming step</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muenthaisong et al., 2007</td>
<td>cryotop</td>
<td>MII, enucleated MII</td>
<td>7.5% EG+7.5%DMSO 4, 7 or 10 min</td>
<td>15%EG+15%DMSO +S. 1 min</td>
<td>0.5M S. 5min</td>
</tr>
<tr>
<td>Mahmou et al., 2008</td>
<td>Straw, open pull straw</td>
<td>GV</td>
<td>1: 3M EG 2: 1.5M EG+1.5M DMSO 3: 1.5M EG+1.5M glycerol 4: 1.5M DMSO+1.5M glycerol 45 s each</td>
<td>1: 6M EG 2: 3M EG+3M DMSO 3: 3M EG+3M glycerol 4: 3M DMSO+3M glycerol 25 s each</td>
<td>0.5M galactose 5 min</td>
</tr>
<tr>
<td>Gautam et al., 2008b</td>
<td>straw</td>
<td>MII</td>
<td>1: 10%, 25%, 40% EG each 1 min 2: 10%, 25%, 40% DMSO each 1 min 3: 1.10%EG+10%DMSO 1 min 4: 10%EG+10%PROH 1 min</td>
<td>1: 40%EG 1 min 2: 40%DMSO 1 min 3: 20%EG+20%DMSO 1 min 4: 20%EG+20%PROH 1 min</td>
<td>0.5M S. 1 min+ 0.33M S. 1 min+ 0.17M S. 1 min</td>
</tr>
<tr>
<td>Attanasio et al., 2010</td>
<td>cryotop</td>
<td>MII</td>
<td>10%EG+10%DMSO 3 min</td>
<td>20%EG+20%DMSO 20-25 s</td>
<td>1.25M S. 1 min+ (0.62, 0.42, 0.31) M S. 30 s each</td>
</tr>
<tr>
<td>Liang et al., 2011</td>
<td>microdrop</td>
<td>MII</td>
<td>10%EG+10%DMSO 1 min</td>
<td>20%EG+20%DMSO 30 s or 45 s</td>
<td>0.5 M S. 5 min</td>
</tr>
<tr>
<td>Liang et al., 2012</td>
<td>cryotop, SSV</td>
<td>GV</td>
<td>1: 7.5 μg/mL CB 15 min +10%EG+10% DMSO 1 min 2: 10%EG+10% DMSO 1 min</td>
<td>20%EG+20%DMSO 30 s</td>
<td>0.5 M S. 5 min</td>
</tr>
</tbody>
</table>

Fuel Sensor PPARγ: a Potential Gateway for Fertility Regulation in Buffalo

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ABSTRACT

The mechanisms controlling the interaction between energy balance & reproduction are still a subject of intensive investigation in mammals. Animal diet is usually supplemented with polyunsaturated fatty acid to combat energy imbalance. It has been found, particularly in cattle and buffaloes, dietary fats influence reproductive function as it has been observed that fatty acid supplementation in the diet increases the follicle number & stimulates the growth of preovulatory follicle i.e. follicular maturation. The integrated control of folliculogenesis is probably a multifaceted phenomenon involving an array of signals governing energy homeostasis, metabolism & fertility. The levels of various molecules, including metabolites (glucose, fatty acids, amino acids) and hormones (Adiponectin, insulin, leptin, ghrelin, etc.), are modulated by nutrition and energy supply. Most of these molecules are known to be directly involved, through fuel sensors, in the regulation of fertility at each level of the hypothalamo-pituitary-gonad axis. One such fuel sensor, PPARs -a superfamily of nuclear receptors, expressed in ovary, has been found to be involved in various aspects of ovarian function and hence raising the question about biological actions of PPARs in fertility. In general, PPARs mediates their effect through their ligands (Endogenous ligands- PUFA and Exogenous ligands- Herbicide, Thiazolidinediones, plasticizers) and alter target gene expression. In the present study CLA and rosiglitazone has been chosen as endogenous and exogenous ligands, respectively. Recently, we cloned, characterized and identified a novel PPARγ transcripts in buffalo ovary and elucidated molecular mechanism how PPARγ could influence the buffalo fertility under different pathophysiological conditions.

Keywords: conjugated linoleic acid, CYP19, peroxisome proliferative activated receptor, polycystic ovarian syndrome, post-partum anestrus

INTRODUCTION

Reproductive function is regulated by the interplay of the hypothalamus, pituitary and gonads, which form gonadotropic or reproductive axis (Tena-Sempere & Huhtaniemi, 2003). Proper function of the gonadotropic axis, and hence reproductive capacity, is gated by metabolic and nutritional factors. Although experimental evidence suggested that common regulatory pathways are involved in the joint control of reproduction and energy balance, the neuroendocrine and molecular signals responsible for such a concerted control remained poorly understood for decades. A major breakthrough in the characterization of the Accepted April 10, 2013; Online November 11, 2013.
mechanisms for the integrated control of reproduction and metabolism took place long time back, with the origin of a word “fuel sensors”. Fuel sensors including glucose, insulin, leptin, and nuclear receptor-PPARγ are known to be directly involved in the regulation of fertility at each level of the hypothalamo-pituitary-gonad axis (Poretsky et al., 1999; Froment et al., 2001). But in the present review, the discussion is focused on the credentials of PPARγ in relation to fertility regulation. Since the discovery of PPARs in 1990 (Issemann & Green, 1990), numerous functions have been attributed to these receptors. Among three isoforms of PPARs, PPARγ stands out to be the critical for normal ovarian functions. PPARγ is a well-established nuclear receptor and is activated after the binding of natural ligands such as polyunsaturated fatty acids and prostaglandin metabolites. It can also be activated by synthetic ligands such as thiazolidinediones (TZDs), which are also known as glitazones (rosiglitazone, pioglitazone or troglitazone) (Lehmann et al., 1995). The binding of TZDs to their receptors primarily to PPARγ in adipose cells increases insulin sensitivity. TZDs are frequently administered to patients with insulin resistance associated with type II diabetes (Houseknecht et al., 2002; Gurnell et al., 2003; Staels & Fruchart, 2005). Activation of PPARγ is known to be directly involved in the regulation of fertility at each level of the hypothalamo-pituitary-gonad axis. For example deletion of PPARγ in mice affected the ovulation in mice (Lee et al., 1995; Peters et al., 2000; Barak et al., 2002) and the polymorphisms in PPARγ were associated with PCOS in humans (Gu & Baek, 2009). PPARγ activation modifies the transcription and/or activity of different key regulators of energy homeostasis (Desvergne & Wahli, 1999) like stimulation of several glucose regulators (glucose transporters, insulin receptor, IRS, etc.) (Picard & Auwerx, 2002). Hence, it can be hypothesized that PPARγ can also act as a fuel sensor in reproductive compartments to inform cells about the energy status such as glucose or fat utilization. In this case, PPARγ may be a link between energy metabolism and reproduction, as in polycystic ovary syndrome (PCOS), which is frequently associated with insulin resistance. Numerous PPARγ dependent and independent pathways have been claimed to play important role in maintaince of the link between energy homeostasis and fertility. Considering the complexity of these pathways, this review aims to throw some light on some of the possible pathways opted by PPARγ in response to ligand activation by focusing on buffaloes, which have poor reproductive potential due to several reproductive problems.

1. EXPRESSION OF PPARγ AND ITS ISOFORM IN OVARY

PPARγ has been reported to be expressed in ovarian tissue from humans (Lambe et al., 1996), cattle (Sundvold et al., 1997; Lohrke et al., 1998) and adult rat (Braissant et al., 1996). Among three isoforms, PPARα deletion has no apparent effect on the fertility of mice, but the deletion of PPARγ and PPARβ/δ affected fertility (Lee et al., 1995; Peters et al., 2000; Barak et al., 2002). In the ovaries of rodents and ruminants, PPARγ is expressed strongly in the granulosa cells, and less strongly in the theca cells and corpus luteum (Gasic et al., 1998; Komar et al., 2001; Froment et al., 2003). PPARγ is detected early in folliculogenesis, at the primary/secondary follicle stage (Komar, 2005). PPARγ expression is more in small antral follicle (Froment et al., 2003; Komar, 2005), and decreases after the LH surge.
The studies conducted in our lab have also extended these initial findings by characterizing the expression of PPARγ and LHR throughout follicular development in buffaloes. We have shown that PPARγ expression decreases as the follicle approaches the stage lutenization (Sharma et al., 2012). It is well established that PPARγ1 had a broad range of expression than PPARγ2 which is primarily confined to adipose tissues (Tontonoz et al., 1994; Zhu et al., 1995). Although there are reports claiming the involvement of PPARγ2 protein in gonadal steroidogenesis in comparison to PPARγ1, its mRNA expression was found to be negligible in Leydig tumor cell line and constant in immortalized mouse granulosa cells (Kowalewski et al., 2009). In buffalo ovary, PPARγ1 was highly expressed than PPARγ2 (Sharma et al., 2012). Additionally, we reported ovary specific novel isoform of PPARγ in buffalo and is named as PPARγ1b. The PPARγ1b was not only highly expressed in ovary but also contributing to the predominant expression of PPARγ. It has been named ovary specific because its expression was found to be nil in other buffalo tissues. So, there can be reasonable speculation that it is the novel isoform PPARγ1b, which is responsible for the significant expression of PPARγ1 in buffalo ovary and most likely PPARγ1 is responsible for downstream signaling effects of PPARγ ligands.

2. EFFECTS OF PPARγ LIGANDS ON STEROIDOGENESIS AND PROLIFERATION DURING FOLLICULOGENESIS

Together with expression of PPARγ itself, availability of ligands is a primary regulating factor determining the ability of PPARγ to influence target gene expression. Ligands can be produced endogenously, providing physiological significance, or sourced exogenously, as therapeutic factors given to target specific metabolic and reproductive symptoms. All PPARs bind to naturally occurring fatty acids and their metabolites (Xu et al., 2001), thus acting as fatty acid-activated receptors that function as key regulators of glucose and cholesterol metabolism. The precise nature of endogenous PPARγ ligand binding and activation remains poorly defined and more research is needed in this area. However, the potential for important physiological ovarian PPARγ activation is considerable as many natural ligands have been shown to be present within the ovary and produced locally by ovarian cells. Included in this list of ligands are ω3- and ω6-polyunsaturated fatty acids (PUFAs) such as the essential fatty acids linoleic acid, linolenic acid, arachidonic acid and eicosapentanoic acid (Berger et al., 2002). Additional PPARγ agonists such as prostaglandin metabolites of these substances and immunologically-derived eicosanoids are also produced within the ovarian environment in a hormonally regulated manner with elevated production as ovulation progresses (Tsafiriri et al., 1995; Evans et al., 1983). It is possible that PPARγ may have a role in the feed-forward production of eicosanoid ligands based on identification of a PPRE in the prostaglandin-endoperoxide synthase 2 promoter (Pontsler et al., 2002), which would facilitate amplified production of pre-ovulatory prostaglandins. However, progress has been made regarding the exploration of exogenous ligand for PPARγ. The first evidence of PPARγ involvement in ovarian function comes from reports utilizing synthetic PPARγ ligands, specifically, administration of TZDs to women diagnosed with PCOS. TZDs have shown lots of potential benefits in
improving reproductive outcomes during PCOS (Aziz et al., 2001). Since then, treatment of PCOS patients with the TZDs, rosiglitazone or pioglitazone, have been shown to not only improve insulin action in peripheral tissues, attenuate hyperinsulinemia, and lower circulating levels of lipids (Ehrmann et al., 1997; Picard et al., 2002), but also to improve a range of reproductive outcomes particularly circulating sex hormone levels, and ovulation rate. But the major focus has been subjected to direct action of PPARγ on synthesis of ovarian steroid hormone and expression of many rate limiting steroidogeneic enzymes. Several studies have shown that TZDs present contradictory actions on secretion of steroids (inhibition or stimulation of progesterone and estradiol production) in granulosa cells (Figure 1).

Considering the foremost step of steroidogenesis, steroidogenic acute regulatory protein (StAR) plays pivotal role in mobilization of cholesterol for initial catalysis to pregnenolone by the P450-side chain cleavage enzyme located within the mitochondria. It has been recently reported that both rosiglitazone and pioglitazone significantly up regulate StAR protein synthesis in vitro in human granulosa cells (Seto-Young et al., 2007). Studies conducted in our lab have also shown that natural and synthetic ligands of PPARγ upregulate StAR mRNA levels, respectively, in buffalo granulosa cells (Sharma, 2012). The next important enzyme in steroidogenesis is 3β-hydroxysteroid dehydrogenase (3β-HSD), which catalyses the conversion of pregnenolone to progesterone in luteal cells. TZDs did not affect messenger RNA concentrations of CYP11a1 and 3β-HSD by using standard Northern methodology (Gasic et al., 1998). On the other hand, in media from porcine granulosa cells, TZD treatment increased the release of pregnenolone, a precursor of progesterone and substrate of 3β-HSD, but decreased the release of progesterone. These results suggest that TZDs decrease the activity of the 3β-HSD enzyme. However, we have also observed conflicting results regarding expression of these two genes in buffalo ovary. Rosiglitazone has shown to decrease of CYP11a1 and 3β-HSD level, whereas conjugated linoleic acid has increased the level of both these enzymes in buffalo granulosa cells. These reports hint about the use of some PPARγ independent pathways also. While TZDs treatment had no effect on the amount of CYP11a1 and 3β-HSD proteins in ovine granulosa cells (Froment et al., 2003) or of CYP17 protein in porcine theca cells (Shoppee et al., 2002), their mRNA concentration was increased. But some other reports indicate suppression of these enzymatic expression and/or activity in primary porcine thecal cells or human cell lines (Arlt et al., 2001; Veldhuis et al., 2002; Kempna et al., 2007) by TZDs treatment. Most important enzyme of steroid biosynthesis i.e. CYP19, which is called as candidate fertility gene, has been found to be down regulated in human ovarian cancer cells and same has been observed in buffalo granulosa cells (Sharma et al., 2012). Taken together, these findings provide strong evidence for the direct effect of TZD and CLA administration and PPARγ activation on ovarian hormonal synthesis and secretion.

Concerning the important steroids, TZDs found to inhibit LH- and insulin stimulated androgen biosynthesis in purified porcine thecal (Veldhuis et al., 2002), and mixed human ovarian (Seto-Young et al., 2007) cells. TZDs also reduced plasma testosterone levels in women with PCOS (Romualdi et al., 2003; Baillargeon
et al., 2004). While it is accepted that TZDs indeed influence estrogen secretion, estrogenic responses to TZDs appear to be dependent on confounding factors such as species, age, and endocrine setting. For instance, TZDs have been found to increase estradiol secretion, and decrease estradiol production (Lovekamp-Swan et al., 2003). Downfall in estrogen goes well along with our studies in buffalo granulosa cells. PPARγ activation by TZDs and phthalate toxins are believed to mediate the antiestrogenic effects of these agents in cultured rat granulosa cells (Lovekamp-Swan et al., 2003), and TZDs have also been found to suppress stimulated estradiol secretion in human granulosa cell cultures (Seto-Young et al., 2005). As for estrogens, progesterone responses to PPARγ activation via natural or endogenous ligands are unclear. Perhaps, the progesterone responses could be regulated by species and stage of folliculogenesis. Most publications investigating a range of species including bovine, ovine, porcine and rodent cell cultures reported increases in progesterone secretion following administration of PPARγ activators in vitro (L¨ohrke et al., 1998; Komar et al., 2001; Schoppee et al., 2002; Froment et al., 2003) whilst some others suggested inhibition of stimulated progesterone secretion by porcine granulosa cells (Gasic et al., 1998). We reported a contradictory result as a response between rosiglitazone and CLA treatments in buffaloes. CLA showed an increased trend in progesterone synthesis, which was not statistically significant, whereas rosiglitazone has been found to decrease progesterone synthesis.

The net influence of natural and synthetic ligand on ovarian PPARγ activation and subsequent steroidogenesis in vivo remains poorly defined across all species investigated. The most evident advantage was on hormone profile during PCOS in women as a result of TZDs treatment and during postpartum period when diets are blended with natural ligand of PPARγ i.e. CLA that has shown to improve reproductive outcomes in dairy animals (de Veth et al., 2009). Concerning the effect of PPARγ ligands on steroid secretion, increasing attention is being paid which can be used for application of these ligands in significant reproductive problems like PCOS and postpartum anestrus.

3. POSSIBLE DIRECT MOLECULAR MECHANISM OF ACTION

A significant research has been carried out in this area and still molecular mechanisms of PPARγ in ovarian function are not fully understood. Comparisons with other cell models suggest that PPARγ may regulate the expression of genes required for follicular development, ovulation, oocyte maturation and maintenance of the corpus luteum. For example, the genes encoding cyclooxygenase-2 (COX-2) and nitric oxide synthase (NOS) are implicated in ovulation and oocyte meiotic maturation, as attested by the invalidation of these genes in mice (Lim et al., 1997; Jablonka-Shariff et al., 1999). Our lab has tried to figure out the direct possible routes taken up by this nuclear receptor in regulating steroidogenesis in buffalo.

Anti-IGF activity of PPARγ: In adrenocortical cancer cells, PPARγ ligands can rapidly interfere with the Akt phosphorylation/activation, which mediates IGF-I-stimulated proliferation (Giulia et al., 2008). These evidences support the anti IGF-I role of PPARγ agonists observed in a wide variety of tumor cancer cell lines and tissues. A possible mechanism for this antagonistic effect is the increase in PTEN expression. In line with this hypothesis, in human anaplastic cancer cell lines,
Rosiglitazone, via PTEN upregulation, inhibited IGF-I mediated biological effects such as cell migration, survival and anchorage-independent growth (Aiello et al., 2006). Similar results have been reported in human hepatocarcinoma cell lines (BEL-7404 and Hep3B) (Zhang et al., 2006; Cao et al., 2007), colon cancer cells (Caco2) (Patel et al., 2001), breast cancer cells (MCF-7) (Patel et al., 2001), nonsmall cell lung carcinoma cells (H1792, H1838 and A549) (Han et al., 2006; Lee et al., 2006) and in pancreatic cancer cells (AsPC-1) (Farrow et al., 2003). Studies conducted in buffalo granulosa cells has shown that natural and synthetic ligands of PPAR\(\gamma\) increase PTEN level and then dephosphorylate Akt that lead to reduced cell proliferation and steroidogenesis (Figure 3a) with a special impact on CYP19 gene (Sharma et al., 2012). Infertility after postpartum period is a daunting problem in dairy animals and it has been found that it is not the compromised growth of ovarian follicles but actually ovulation from dominant follicle. For that it is mandatory that CYP19 level should go down as a result of LH surge. When follicle fails to do so, anovulation occurs resulting in postpartum infertility. The proposed pathway revealed that the exact molecular mechanism behind PTEN induction due to PPAR\(\gamma\) agonists has yet to be fully understood. Two hypotheses have been proposed to explain the interaction between PTEN and PPAR\(\gamma\): PPAR\(\gamma\) directly impact PTEN transcription and/or regulating secondary unknown factors that in turn regulate its expression. Furthermore, because PTEN expression can be regulated by interfering with its transcription activity or by inducing posttranscriptional modifications, it is also possible that PPAR\(\gamma\) agonists induce PTEN overexpression by decreasing its degradation (Vazquez et al., 2000; Waite et al., 2003). This serial connection among PPAR\(\gamma\), PTEN, and Akt provides a powerful mechanism to shut down the basal or stimulated signals of PI3K cascade, and it could be exploited for future treatment of tumors in which PTEN is downregulated /lost and IGF1-mediated signals are amplified. And also there are reports that PPAR\(\gamma\) is known to decrease the bioavailability of IGF1 by increasing the expression of IGFBP (Yi et al., 2005). The variety of the effects induced by PPAR\(\gamma\) ligands on components of IGF1 system is still under investigation and may be due to the multiple and alternative signaling pathways they stimulate or inhibit.

Changes in post-translation modification of histone: The modification of the lysine groups of core histones by multiple post-translational events including phosphorylation and acetylation coincident with activation of mitogenic signaling (Fu et al., 2002). It is well established fact that H3 acetylation and phosphorylation increased markedly in cancerous cells. In case of rosiglitazone treated rats, there are decreased levels of histone H3 acetylation and phosphorylation. Rosiglitazone pre-treatment showed maximum decrease in H3 acetylation and phosphorylation in breast cancer animals. This decrease in histone H3 acetylation and phosphorylation clearly indicates that there was decreased cell proliferation and increased differentiation, which can be well correlated with maximum tumor reduction in combination therapy. These effects are PPAR\(\gamma\) dependent as rosiglitazone treatment has increased the PPAR\(\gamma\) expression, which was well correlated with maximum percentage of tumour inhibition (Tikko et al., 2009). CLA is also a potent natural agonist of PPAR\(\gamma\), so there is reasonable possibility CLA could also play a role in regulating histone modification. Preliminary studies conducted in our lab showed...
that CLA affected acetylation of histone. Western and ChIP analyses have shown that overall level of histone H3 acetylation was decreased in buffalo granulosa cells. ChIP assay results showed that CLA and rosiglitazone decreased the chromatin accessibility for ovary specific promoter II, which is known to be the proximal promoter for the expression of CYP19 gene (Figure 3b). This data indicated that regulation of histone modification could be a probable reason for decreased proliferation of buffalo granulosa cells as increased level of histone acetylation is directly associated with proliferation potential of cell. To best of our knowledge, this is the first report which shows that rosiglitazone and CLA decreased the acetylation of histone H3 and also decreased the chromatin accessibility for proximal promoter II in any of the species (Sharma et al., 2012). These effects are PPARγ dependent as both the ligands have shown to increase the expression of PPARγ, and an antagonist GW9662 showed to increase the level of acetylated histone H3 and the chromatin accessibility for proximal promoter II.

Inhibiting the translocation of p65 subunit of NF-kB: Nuclear factor-kB binds in the proximal region of ovary specific promoter II (Figure 3c) of the CYP19 gene. In human granulosa–like tumor KGN cells, the activation of PPARγ was recently shown to downregulate aromatase gene expression via the NF-kB pathway (Fan et al., 2005). It was found that a combined treatment of TGZ+LG decreased aromatase promoter II (ArPII) activity in both ovarian KGN cells and fibroblast NIH-3T3 cells in a PPARγ-dependent manner. Furthermore, the inhibition of both aromatase activity and the transcription of ArPII by TGZ+LG was completely eliminated when nuclear factor-kB (NF-kB) signaling was blocked by specific inhibitors, suggesting NF-kB, which is endogenously expressed in both fibroblast and granulosa cells, might be a mediator of this inhibition. Interestingly, activation of NF-kB by either forced expression of the p65 subunit or NF-kB-inducing kinase upregulated ArPII activity. Positive regulation of aromatase by endogenous NF-kB was also suggested by the fact that NF-kB-specific inhibitors suppress basal activity of the aromatase gene. A concomitant formation of high-order complex between NF-kB p65 and ArPII was also observed by chromatin immunoprecipitation assay. Although activation of PPARγ and RXR affected endogenous expression levels of neither inhibitory kBα nor p65, it impaired the interaction between NF-kB and ArPII and the p65 based transcription as well. This is probably further explained by the finding that activated PPARγ can physically interact with p65 and results in inhibition of NF-kB (Chung et al., 2000; Chen et al., 2003). And probably as an outcome of the impaired transcription factor-promoter association, it was found that PPARγ activation by TGZ+LG suppressed the transactivation ability of NF-kB. Altogether, these results indicate that activation of a nuclear receptor system constituted by PPARγ and RXR, downregulates aromatase expression through the suppression of NF-kB-dependent aromatase activation and thus provide a new insight in the mechanism of regulation of the candidate fertility gene in farm animals.

CONCLUSIONS AND FUTURE DIRECTIONS

In the recent years, a major progress has been seen in our understanding of the molecular signals and physiological mechanisms responsible for the
collaborative control of reproduction and energy balance as illustrated in Figure 4. Since the discovery of PPARγ, its role has been implicated in multitude of cellular functions. This review tried to discuss on the fertility regulatory functions of PPARγ in buffalo ovary in comparison with other species. Reproductive effects mediated by this nuclear receptor PPARγ had a multifunctional phenomenon transduced at different levels of hypothalamus-pituitary-gonadal axis. Although a lot of advances have been made regarding the role of PPARγ in fertility regulation, additional experimental work is still needed to decipher the physiological relevance and molecular mechanisms responsible for the reported effects of PPARγ. In this context, it will be important to characterize the role of PPARγ on reproductive axis in different experimental settings by administration of PPARγ ligands under different pathophysiological conditions (postpartum infertility and nutritional development). PPARγ is found to be expressed in different isomeric forms. Studies must be directed in such a way so that they can demonstrate the differential usage of different promoters under different pathophysiological conditions. For instance, studies from one of our groups have reported a novel ovary specific isoform of PPARγ in buffalo and this isoform is preferentially used for PPARγ expression (Sharma et al., 2012).

Researchers have reported several molecular mechanisms by which PPARγ regulates its downstream signaling effects on fertility. Some of them are PPARγ dependent and some are independent.

Some of the possible PPARγ dependent pathways have been discussed above and our lab is also working in this direction. A serial connection among PPARγ, PTEN and Akt has been established for fertility regulation in buffalo granulosa cells (Sharma et al., 2012). Decrease in post-translational modification of histones as a result of PPARγ agonist treatment emerges as a novel potential mechanism for fertility regulation in any of the species, which may lead to epigenetic regulation of ovarian genes. However, PPARγ independent effects cannot be denied completely as PUFAs are known to take PPAR independent pathways (Derecka et al., 2008) and also PPARγ inhibitor (GW9662) inhibited the cell growth of human tumor mammary cell line, which supports the existence of PPARγ independent pathways (Seargent et al., 2004). In addition, studies conducted in our lab on buffalo granulosa cells demonstrated that rosiglitazone, a synthetic ligand of PPARγ used as a potential drug to treat PCOS, was found to increases PTEN expression through the activation of PPARγ, which in turn inhibits the PI3K/Akt pathway and cell growth. These observations may explain the underlying mechanism for PPARγ agonist including the effects of rosiglitazone in granulosa cell, and constitute a potential novel mechanism to treat anovulation. Though, it cannot be stated directly, but it can be speculated and suggested that CLA can also be used as a substitute to these synthetic ligands to treat anovulation during PCOS, as rosiglitazone are currently listed as a Pregnancy Category C drug (i.e., not tested for use during human pregnancy) as TZDs administration is characterized by some side effects, such as weight gain, fluid retention and possible bone demineralization.

However, further experimental work is needed to fully elucidate the mechanisms and physiological relevance of such functions. It is anticipated that future efforts in this field will be devoted to unravel the epigenetic or post-
translational modification of target genes affected by PPARγ ligands. Moreover, further studies are required to achieve detailed functional analysis of CLA in ovary during different patho-physiological conditions in vivo, particularly during postpartum infertility.

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**Figure 1.** The schematic representation of steps leading to progesterone, estradiol, and testosterone production. The name of the enzymes involved in steroid biosynthesis and regulated by PPARγ are underlined.
Figure 2. Possible direct molecular mechanisms of action by PPARγ. (a) Presents the intervening effect of PPARγ in IGF1 signaling, (b) presents the epigenetic regulation of gene expression mediated PPARγ ligands, and (c) depicts inhibition of translocation of important subunit of NF-κB inside nucleus. These pathways finally merge to decrease gene expression inside ovarian granulosa cells.

Figure 3. Collaborative control of energy balance and reproduction by PPARγ. Activation of PPARγ either by endogenous (e.g., PUFA) or exogenous (e.g., TZDs, Bisphenols etc.) ligands stimulates its fuel sensor attribute by promoting carbohydrate and fat metabolism. Eventually, it regulates reproductive functions by decreasing aromatase gene expression, estradiol levels and proliferation of granulosa cells. As PPARγ is a key regulator in energy balance and fertility regulation, it could be a potential drug target for reproductive problems.
Invited Papers
Buffalo Genetics and Breeding Symposium
Breeding Strategies for Genetic Improvement in Buffaloes

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ABSTRACT

The paper gives an account of the buffalo population trend over the last one decade highlighting the growth pattern on buffalo population in India and in different states. Breeds of buffaloes in different States and the breeding policy on selective breeding of the buffalo breeds for milk production and grading up of the non-descript and low producing animals with breeds of superior genetic merit preferably Murrah is generally the accepted guideline. Results of the selective breeding and progeny testing towards improving milk yield at various institutional herds, Military dairy farms, AICRP Associated Progeny Testing under the Network Project on Buffalo Improvement have been discussed highlighting the details of top ranking bulls from nine sets for progeny testing. Associated method of progeny testing increased population size, intensity of selection and number of daughters record available for testing. Field progeny testing undertaken to substantiate the progeny testing at institutional herds needed to undertake atleast 30 AI for making available one recorded daughter. ONBS with embryo transfer, sexed semen and molecular breeding using MAS has been emphasized to facilitate early selection of individuals with desired characteristics in order to improve productivity.

Keywords: buffalo breeds, progeny testing, genetic response, crossbreeding, marker selection.

INTRODUCTION

The world buffalo population is estimated to be approximately 185.3 million spread in some 42 countries of which 179 million (97%) of them are found in Asia, while approximately 5.54 million (3%) are found in rest of the world (Fao.org/stat, 2008). India has 105.1 millions and they comprise approximately 56.7 percent of the total world buffalo population. The percent increase in India was about 1.5% as compared to 1.45% in Asia and 2.67% in rest of the world. Buffaloes in India are spread over almost all parts of the country with varying density of population in different states and union territories. The majority of the population (72%) is concentrated in the north and western states where most of the milch breeds of buffaloes are found. During the last 10 years there has been continuous growth of this species in this region at the rate of about 2.1% per annum as against the average growth rate of approximately 1.0% in the country. Recent trends indicate that the landless farmers are losing out, while medium and big farmers / entrepreneurs are consolidating ownership over milk animals especially high milk producing buffaloes. Breeds and Breeding tracts: India possesses the best milk buffalo breeds of the world namely Murrah, Nili Ravi, Surti, and Jaffarabadi, which originated in the north-western states of India. These breeds have high potential for milk and fat.
production besides being used for work and surplus stock used for meat production. The number of pure bred animals of specified breeds is expected to be about 25 to 30% of total population. About 55% of the total milk produced in the country is contributed by buffaloes inspite of the number of adult buffaloes being only 60% to the total milk cattle population indicating higher production potential of buffaloes as compared to cattle.

MATERIALS AND METHODS

All India coordinated Research Project (AICRP) on buffalo breeding was launched in 1970 at four centers namely PAU, Ludhiana and NDRI, Karnal each for large sized buffaloes, comprising mainly Murrah breed and two centres for small/medium sized buffaloes comprising mainly Surti and Mehsana breed. The objectives of the project were to improve the production potential of buffaloes through assessment of genetic merit of sires and to increase the production by breeding, feeding and management. Buffalo improvement programs through bull selection on the basis of progeny performance and distribution in the field were taken up at several state and central government farms.

Network Project on Buffalo Improvement was initiated during 1993 under ICAR at CIRB with the objective to increase the intensity of selection of bulls from large population and increased number of progeny per bull for testing, by associating the buffalo herds of Murrah breed at various centres with the objective to evaluate the sires on the basis of as many daughters as possible involving six centres namely GADVASU Ludhiana, HAU Hisar, CIRB Hisar, NDRI Karnal, IVRI Izatnagar and CCBF Alamadi. Subsequently NDUAT Faizabad, KAU Manuthy, and SVU V, gudam were added so as to have common technical program for the associated progeny testing.

Field progeny testing under Network Project on Buffalo Improvement was initiated to strengthen the ongoing sire evaluation program of associated herds progeny testing at institutional herds by involving performance recording on farmers animals using the semen of bulls selected under the Network Project.

RESULTS AND DISCUSSIONS

All India Coordinated Research Project on Buffalo Breeding

Results of progeny testing under the AICRP show that number of bulls in each set ranged from 4 to 8 and the number of daughters from each bull ranged from 2 to 23 at various centres. The superiority of the top ranking bull over the contemporary daughters varied from 7 to 99% from the 6 sets as reported by Nagarcenkar and Sethi (1988) at NDRI and 12.1 to 42.4% in 5 sets by Tiwana et al. (1985) at PAU. Chadha et al. (2004) estimated breeding value of 36 bulls used from 1976 to 1996 by 3 methods namely best linear unbiased predictor, least square mean and contemporary comparison method and found that ranking of top 20% bulls was almost similar by all the three methods. The breeding value of bulls by contemporary comparison method ranged from 1431 to 2205 kg. These estimates were also close to the breeding value estimated by Vij and Tiwana (1988) from the same herd.

Progeny tesing on Surti buffaloes at MPUAT show that progeny per bull
varied from 8 to 17 in first 4 sets of bulls and superiority of the selected bulls over contemporary daughters ranged from 22.62 to 2.71. Number of daughters per bull in the field ranged from 7-44.

**Network Project on Buffalo Improvement**

Progeny test evaluation of 9 sets of bulls under the Network Project has shown considerable increase in the number of daughters per bull which ranged from 6 to 27 daughters on the basis of which bulls were progeny tested. Percent superiority of the top ranking bulls in the sets ranged from 9.37% to 24.89% in the various sets (Table 1) which have been used for nominated mating.

The surplus genetically superior germplasm is disseminated to state governments, village Panchayats and other developmental agencies for breeding in the field. Furthermore, this institute had supplied more than 442 Murrah buffalo breeding bulls of superior genetic merit throughout the country for improving local /non- descriptive buffaloes. The 305 day or less lactation milk yield has increased from weighted average of 1,550 kg during 1992-1993 to more than 2,300 kg during 2011-2012 in the participating herds (Figure 1).

**Field Progeny Testing**

Average CR from artificial insemination in the field has been estimated as 41.68% based on 82,604 inseminations spread over 3 locations for murrah breed. It is estimated that at least 8 to 10 AI are needed for each daughter born in the field while number of AI for each daughter recorded is likely to be very high (approx. 25-30) due to frequent movement of animals in the breeding tract as well as outside the catchment area. More than 18,000 superior animals have so far been produced under the field progeny testing project with a conception rate of about 42% in the field animals. Records of 477 daughters in the field indicate average age at first calving of 45.2 months and average record day yield of 7.57 kg (Table 2) in their first lactation and the trend of improvement in age at first calving and milk yield in field recorded daughters (Table 3) at centres of Network Project on Buffalo Improvement.

**Farmers participation in improvement program**

CIRB has initiated the exercise of conservation and propagation of superior bulls belonging to the farmers through semen collection at the farmers doorstep and propagation in the farmers buffaloes in the field. After having the pedigree, breed characteristics, health examination and semen quality, the semen is collected at the farmers doorstep, examined for its normalicy and diluted appropriately with semen extender. The diluted semen is transported to the semen freezing laboratory, frozen and stored in the state of the art semen freezing laboratory at the institute. The frozen semen straws are made available to the farmers.

**ONBS / SOET-MOET for faster multiplication of superior germplasm**

A single herd open mixed MOET program was planned by NDDB (Trivedi, 1992) to evaluate bulls on two Dam’s records, 4 full sibs records and 12 half sibs records from 8 families and select one young bull per family for the next cycle. An initiative has been taken in this regard by the CIRB, Hisar and application of this technique in buffaloes resulted in 13 pregnancies with embryos obtained from 8 superior buffaloes inseminated with semen from top-ranking progeny tested Murrah bulls with the result of about 1.5 calf per buffalo per reproductive cycle. There is need to implement this technique to include more elite females so that more
progenies are obtained, including bull calves, for supplementing frozen semen production from high merit bulls.

**Meat Production**

In India, meat production from buffaloes is largely a by-product of livestock production system utilizing spent animals at the end of their productive life and surplus males. Buffalo meat production accounts for about 25% of the total 6.5 million tons meat production in the country. During the last 25 years meat production has increased indicating average growth rate of about 3 to 4%.

Dressing percentage is low and varies between 40 to 45% with average carcass weight as low as 138 kg. However, dressing percentage in these animals can be substantially increased by proper feeding prior to slaughter and in such animals growth rate as high as 1000 gm per day has been reported under feed lot system (Ranjhan, 2007). Dry matter intake of such animals is generally more than 3% of body weight and dressing percentage as high as 55 to 60% can be achieved. A strong need has been felt to establish a production base around each modern abattoir to produce quality disease free animals as per the sanitary and phytosanitary (SPS) requirement of OIE standards (Qureshi and Ranjhan, 2004). In experimental trials on daily body weight gain up to 541 gm per day has been achieved under conventional concentrate feeding system. Weight gain during summer months is higher than in hot humid months. (Bharadwaj and Sethi, 1994).

**Newer Technologies which needs standardisation**

*Sexed semen production:* semen sexing technique can help to quickly address the requirement of superior germplasm through programmed birth of male calves to elite females so that these can be used extensively in AI programme to cover the vast population of low-producing indigenous and non-descript cattle and buffalo for faster upgrading. Progeny testing can also be accelerated through controlled births of required numbers of daughters within short period. Recent development of modern cellular methodologies has led to development of a flow cytometric system capable of differentiating and separating living X- and Y- chromosome with about 90% accuracy. The speed of sorting is low (~15 million/hour). The technology needs to be standardised under Indian conditions.

*Cloning:* The production of first buffalo clone in the world GARIMA (www.ndri.res.in), its normal pregnancy and the birth of its calf MAHIMA by the scientists of NDRI karnal is a major landmark in developing the niche technologies for achieving higher success and a step forward for faster multiplication and production of superior germplasm.

*Molecular breeding and MAS:* Allelic variation ranging from 9 to 36% was observed by RAPD primers in high and low yielding buffaloes. Sequence, showing the highest polymorphism in buffaloes associated with high and low lactation milk yield was identified. (Sethi and Sikka, 2004). Buffalo genomics work has been initiated at NBAGR and CIRB for identification of molecular markers associated with performance traits. Six grand sire families have been identified phenotypic data compiled for undertaking linkage studies (Tantia et al., 2011).
REFERENCES
Table 1. Top ranking progeny tested bulls, % superiority of various categories of bulls selected for elite mating under network project on buffalo improvement.

<table>
<thead>
<tr>
<th>Set No.</th>
<th>Bull No.</th>
<th>Location</th>
<th>Dam No.</th>
<th>Sire No.</th>
<th>Dam’s best lact. 305 day yield (kg)</th>
<th>Daughter’s first lact 305 day or less av. yield (kg)</th>
<th>No. of daughters</th>
<th>Sire index</th>
<th>% superiority over contemporary daughters</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>392</td>
<td>CIRB</td>
<td>238</td>
<td>PQ1</td>
<td>2594</td>
<td>2074</td>
<td>13</td>
<td>2118</td>
<td>22.80</td>
</tr>
<tr>
<td>III</td>
<td>1354</td>
<td>PAU</td>
<td>762</td>
<td>989</td>
<td>3088</td>
<td>2072</td>
<td>6</td>
<td>1975</td>
<td>13.11</td>
</tr>
<tr>
<td>IV</td>
<td>1506</td>
<td>PAU</td>
<td>-</td>
<td>988</td>
<td>3018</td>
<td>2065</td>
<td>12</td>
<td>2089</td>
<td>18.81</td>
</tr>
<tr>
<td>V</td>
<td>4393</td>
<td>NDRI</td>
<td>2762</td>
<td>1908</td>
<td>3898</td>
<td>2143</td>
<td>13</td>
<td>2187</td>
<td>22.29</td>
</tr>
<tr>
<td>VI</td>
<td>1153</td>
<td>HAU</td>
<td>618</td>
<td>759</td>
<td>2675</td>
<td>2022.8</td>
<td>21</td>
<td>2121</td>
<td>13.31</td>
</tr>
<tr>
<td>VII</td>
<td>4915</td>
<td>NDRI</td>
<td>3521</td>
<td>2921</td>
<td>3437</td>
<td>2038</td>
<td>17</td>
<td>2116</td>
<td>17.26</td>
</tr>
<tr>
<td>VIII</td>
<td>1875</td>
<td>GADVASU</td>
<td>1669</td>
<td>558</td>
<td>2714</td>
<td>2357</td>
<td>8</td>
<td>2300</td>
<td>24.89</td>
</tr>
<tr>
<td>IX</td>
<td>1994</td>
<td>GADVASU</td>
<td>1884</td>
<td>392</td>
<td>2938</td>
<td>2432</td>
<td>18</td>
<td>2487</td>
<td>11.73</td>
</tr>
</tbody>
</table>
Table 2. Total AI, calving, PD, conception and daughter’s milk recording in field units up to March 2012.

<table>
<thead>
<tr>
<th>Duration</th>
<th>Total AI</th>
<th>Pregnancies</th>
<th>CR%</th>
<th>Calving Females born</th>
<th>Daughters recorded</th>
<th>Av. AFC (mts)</th>
<th>Av. Milk Yield (kg/day)</th>
<th>Daughters to be recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIRB Hisar</td>
<td>20931</td>
<td>10469</td>
<td>50.02</td>
<td>6201</td>
<td>3022</td>
<td>205</td>
<td>42.07</td>
<td>7.96</td>
</tr>
<tr>
<td>GADVASU Ludhiana</td>
<td>40550</td>
<td>14448</td>
<td>35.60</td>
<td>8854</td>
<td>4138</td>
<td>121</td>
<td>47.50</td>
<td>8.10</td>
</tr>
<tr>
<td>NDRI Karnal</td>
<td>21123</td>
<td>9509</td>
<td>45.01</td>
<td>6366</td>
<td>3005</td>
<td>151</td>
<td>46.18</td>
<td>6.88</td>
</tr>
<tr>
<td>Total</td>
<td>82604</td>
<td>34426</td>
<td>41.68</td>
<td>21421</td>
<td>10165</td>
<td>477</td>
<td>45.22</td>
<td>7.57</td>
</tr>
</tbody>
</table>

Table 3. Trend of improvement in age at first calving and milk yield in field recorded daughters at centres of network project on buffalo improvement.

<table>
<thead>
<tr>
<th>Year</th>
<th>GADVASU Ludhiana</th>
<th>NDRI Karnal</th>
<th>CIRB Hisar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. AFC</td>
<td>Av. Milk Yield (kg./days)</td>
<td>Av. AFC</td>
</tr>
<tr>
<td>2002-03</td>
<td>56.1</td>
<td>8.1</td>
<td>42.02</td>
</tr>
<tr>
<td>2003-04</td>
<td>50.3</td>
<td>8.0</td>
<td>46.61</td>
</tr>
<tr>
<td>2004-05</td>
<td>50.3</td>
<td>8.1</td>
<td>53.9</td>
</tr>
<tr>
<td>2005-06</td>
<td>46.5</td>
<td>7.9</td>
<td>43.8</td>
</tr>
<tr>
<td>2006-07</td>
<td>46.6</td>
<td>8.1</td>
<td>45.6</td>
</tr>
<tr>
<td>2007-08</td>
<td>39.2</td>
<td>8.1</td>
<td>41.4</td>
</tr>
<tr>
<td>2008-09</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Overall</td>
<td>47.5</td>
<td>8.1</td>
<td>46.18</td>
</tr>
</tbody>
</table>
Figure 1. Weighted average 305 day or less lactation milk yield of participating herds (Murrah).
Buffalo Genetic Resources of India and Their Conservation

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ABSTRACT

The contribution of buffalo (\textit{Bubalus bubalis}) to the Indian agrarian economy is considerable by way of milk, meat and draught power production and as a source of security that requires minimum inputs. The domesticated buffaloes in Indian are mainly of river type with small number of swamp buffaloes present mainly in north-eastern part of India. India is having 56.70 percent of the world buffalo population and they supply 68.21 percent of the total milk produced around the world. The river buffaloes of the Indian sub-continent are maintained chiefly for milk production, but all of them are also dual purpose animals, exhibiting good meat characteristics. The swamp buffalo is more or less a permanent denizen of marshy lands, where it wallows in mud and feed on coarse marsh grass. India has rich repository of buffalo breeds with 13 recognized breeds and the best known breeds of buffaloes are Murrah, Nili-Ravi, Jaffarabadi, Surti and Mehsana. The germplasm of such well-defined breeds constitute a valuable genetic resource which needs to be conserved on priority basis. The rich biological diversity of this species is progressively being eroded due to unplanned breeding. Except in few organized farms which maintain small herds of pure breed, there is almost unrestricted interbreeding among different breeds and there is a marked decline in the availability of unique animals conforming to the attributes of defined breeds, particularly in their native breeding tracts. The situation is further complicated by the fact that there exists no breed societies or breed registration/improvement societies to register animals of specific breeds, maintain herd books and ensure the purity of the breeds. Hence, proper conservation measures have to make to preserve the valuable buffalo genetic resources of India for the sustainable utilization.

Keywords: breeds, buffalo, conservation, India

INTRODUCTION

Livestock sector plays a vital role in the economy of many developing countries including India. It provides food (more specifically animal protein in human diets), income, employment opportunity, draught power, means of transport and organic fertilizer for crop production. In fact, livestock are considered as financial assets to farmers, since they serve as an insurance against the risk of crop failure due to drought and other unfavourable climatic conditions. Dairy industry in India has made significant progress in the last few decades. Today, India is the largest producer of milk in the world and the India’s contribution to the total world milk production is about 15.8 percent. In India, the buffalo is the principal dairy animal and occupy an important place in the agricultural economy of India because of their adaptability to harsh climatic conditions, tolerance to tropical diseases and survival under poor feeding and management practices. Although, the breedable buffaloes are almost one-third in number as compared to cattle, buffaloes contribute in

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excess of 50 per cent of the total milk produced in the country. Their contribution in terms of meat is also significant. The organized dairy sector in India is largely dependent on buffalo milk because of their contribution to total milk production, rich fat and total solid content. The genetic diversity in buffaloes of the country is represented by thirteen recognized breeds of buffaloes besides several lesser-known breeds/strains comprising about 25 to 30 per cent of the total bovine population in India. Buffaloes in India have a place of pride in all respects: production, productivity, population in the world, diversity/ breeds, service to the underprivileged, clean draft power, sure-footed transport, rich food/nutrition, support to a host of industries and livelihoods. For the purpose of a continued and sustained use of this important species, it is necessary to recognize and enhance its role and give higher attention to conserving and improving the diverse breeds as a global resource. Already, the world is looking at this Black Gold as a hope for food in the near future, especially in the face of imminent global warming. This paper gives an overview of buffalo genetic resources of India, improvement programmes followed over the decades and measures of conservation adopted for sustainable utilization (Shrestha and Shrestha, 1998; Balain, 1999; Resali, 2000; Das et al., 2008; Report, 2010).

BUFFALO POPULATION AND PRODUCTION DETAILS IN INDIA AND THE WORLD

The number of the world buffalo population is estimated to be approximately 185 million spread in some 42 countries around the world (FAO, 2008), of which 97 per cent of them are found in Asia and the remaining are found in rest of the continents. India has over 105 million buffaloes, which is 56.7 percent of the total world buffalo population (Table 1). India is the highest producer of buffalo milk in world with the total share of 68.21 per cent. Among the different countries, a total of 91.72 per cent of the milk is obtained from India and Pakistan alone. The milk production particulars of major buffalo rearing countries are as follows in Table 2.

BUFFALO GERMPLASM OF INDIA

The buffaloes are normally classified into river (2N=50) and swamp (2N=48) types though both are called *Bubalus bubalis*. Most of the animals in India are river type and are found throughout India where clean water of rivers, irrigation canals and ponds are available to wallow. The swamp buffaloes are found only in small areas in the north-eastern part of the country. Swamp buffaloes are used mainly for work and a very small amount of milk. Swamp buffalo is more or less a permanent denizen of marshy lands, where it wallows in mud and feeds on coarse marshy grass. There are no distinguished breeds in swamp buffaloes.

India possesses the richest source of germplasm of buffalo with 13 recognised breeds of riverine buffaloes. These include breeds like Murrah, Nili-Ravi, Jaffarabadi, Marathwadi, Mehsana, Nagpuri, Pandharpuri, Bhadawari, Surti, Banni, Kalahandi, Toda and Chilka. There also exist a number of buffalo populations, known for their adaptability to harsh climatic conditions, tolerance to tropical diseases and survival under meager feeding and poor management practices. These local varieties have not been defined as breeds and hence they have to be assessed, defined and recognized. The buffalo breeds habituated in different agroclimatic zones of India have evolved themselves more through genetic isolation and natural selection than through deliberate intervention by man. They have been distributed in extremes of climates i.e., from saline condition (i.e., Chilka buffalo of Orissa) to high altitude mountain areas up to 2,000 m.s.l (i.e., Toda buffaloes). Besides the cultural and religious considerations, buffaloes are contributing significantly to food and agriculture in terms of milk, meat, manure, fuel and draft power. Variations in regional demand for
animal products have influenced the use of different buffalo genetic resources. The major buffalo breeds are classified into five distinct groups according to their phenotypic similarity and the breeding tract and are as follows:

- Murrah group: Murrah, Nili – Ravi, Kundi
- Gujarat group: Jaffarabadi, Surti, Mehsana, Banni
- Uttar Pradesh breeds: Bhadawari, Tarai
- Central India group: Nagpuri, Pandharpuri, Kalahandi, Manda, Jerangi, Sambalpur
- South India group: Toda and South Kanara

The breeding tract, morphological characters and performance of important breeds of buffaloes are presented in Table 3. The buffalo breeds and their home tracts are stable, have a well-defined place in the local natural cycles; consume the remnants of the crops, yet produce valuable milk and provide draft-power and dung/urine as organic manure. With excellent ability to convert poor quality feed to fodder, buffaloes provide milk that is rich in nutrition and quality, meat that is known for flavor and marbling and steady but sure-footed draft power in rural/remote areas and a host of other services, including Mozzarella cheese. All the recognized breeds and local populations have unique qualities for meeting local requirements of food, energy and livelihoods in an environmentally suitable manner.

The number of purebred animals of above specified breeds and lesser known breeds is expected to be about 25 to 30 per cent of the total buffalo population in the country. Rest of the buffaloes are non–descrip in type and have extremely variable composition being either non-descript or crosses among various breeds and cannot be categorized in any other well-established breed. There is general concern that the genetic variation within the few domestic animal species is disappearing through breed substitution and inter-breed crossing. Any reduction in the diversity of genetic resource narrows the scope to respond to selective breeding (Sethi and Kala, 2005).

**INTERVENTIONS TOWARDS GENETIC IMPROVEMENT**

All types of breeding structures are being used in India for genetic improvement of buffalo population. Selective breeding is the main program for recognized breeds of buffaloes and the non-descript buffaloes are improved through grading up program with Murrah and Surti breeds to augment the milk production potential. Under the various schemes of ICAR [Indian Council of Agricultural Research] and SAUs [State Agricultural Universities], improvement of defined breeds and also some of the strains has been taken up in the recent past. Progeny-testing scheme was started in the Third Five-Year Plan to ensure identification of superior Murrah bulls tested on the basis of performance of their progeny rather than only the dam’s yield. The tested bulls were used through AI for achieving higher genetic gain. Subsequently, field progeny testing programs for Murrah and other indigenous breeds of buffaloes supported by the Government, Cooperatives dairies, Research Institutes and NGOs have also been made to select the superior bulls for the production and supply of semen throughout India. Under the AICRP/Network Project approach, genetic improvement and conservation of indigenous AnGR is being undertaken at a number of species specific institutes of ICAR and in collaboration with the SAUs. Such activities have also been taken up by State and Central Government Farms, NDDB and some NGOs including Gaushalas. Recently a state sponsored scheme ‘National Project on Cattle and Buffalo Breeding’ envisaged raising AI facilities for buffalo breeding in the country. A field oriented approach ‘Central Herd Registration Scheme’ implemented at some of the locations undertook earmarking of elite buffaloes at the farmers level with regular recording and genetic selection.
APPROACH TO CONSERVATION OF BUFFALO BREEDS IN INDIA

Conservation is the act or process of protection, preservation, management or restoration of wildlife, livestock and natural and cultural resources and management of human use of bio-sphere so that it may yield the greatest sustainable benefit to present generation. Conservation of live specimens of buffaloes consumes sizable manpower, valuable space and costs besides demanding proper planning skills. However, the buffalo needs to be conserved for the following reasons in brief:

1. They possess adaptive characteristics to thrive in the stressful environment which could be lost through dilution and intensive selection for production traits.
2. They also possess the ability of converting poor quality feed resources into meat, milk and working capacity in the field.
3. The genetic variability should be maintained which is the basis for genetic improvement for the future.
4. The future expectations of our buffaloes are unknown or unknowable. Selection goals in cattle breeding in the past have changed. From single trait selection, we have turned to selection on total merit, when we may have lost some of the negatively correlated valuable genes. Selection for conformation traits are beginning to show their importance in relation to lifetime stability and production. With raising production costs, we are now looking at yield per unit dry matter or energy input. In this respect the buffalo has an important role. The future spectrum of diseases and feed availability is unknown. Therefore we need to maintain the present variability or even increase it.
5. Finally, we ask ourselves, do we have the right to destroy or even neglect our indigenous germplasm collection which rightfully belongs to our children and grandchildren who may find greater uses for them.

The general approach to be followed for conservation of buffalo genetic resources is Live animals

An actively breeding population should be maintained, perhaps, each line or variety in a different farm to reduce costs. The major advantages of live animal conservation are:

1. They are always available for immediate utilization in the event of any setbacks in the upgraded population;
2. They are constantly exposed to new strains of diseases and their resistance evaluated.
3. Such live animals would also contribute to education and to community awareness of the indigenous fauna. Cost of maintenance is often argued to be high. This may be an exaggeration overlooking their cheap maintenance costs, better longevity, lower veterinary costs besides revenue from milk and meat.

A major problem that needs to be defined is the type of selection to be practiced without altering the genetic variability. It is suggested here that both random selection as well as overall merit (with equal weighting for each trait) should be practiced with minimum intensity.

Cryogenic storage

This is convenient and cheap and further work needs to be done. Another advantage is that the genotype will not be subjected to genetic drift. A disadvantage is that the animals, especially in the case of females, have a time lag when live adults are urgently required.

DNA genetic material storage

This is a useful tool but certainly not an immediate task (FAO, 1987).

CHARACTERIZATION OF INDIAN BUFFALO BREEDS AND POPULATIONS

The buffaloes of India have evolved within their ecosystem over several centuries and have thus acquired adaptive characteristics and still remain useful in food production.
However, they have been subjected to genetic manipulation such as selection and crossbreeding only during recent decades. By virtue of the fact that it is often considered a neglected species, much of its genetic variation may not have been lost except through natural selection in the domestic environment. However, the main areas of concern are that a complete documentation and evaluation of the variability and characterization of the various breeds and strains are lacking. Such information is vital in agricultural planning strategies and allocation of animals and breeding programs to various farmers and farming systems. Hence, phenotypic and molecular characterization of the recognized and lesser known buffalo breeds have been taken up and the breed descriptors have been in the recent past prepared. The breed descriptors contain detailed information on the geographic distribution, population status, morphological characters, physical traits and production and reproduction performances. At present, most of the breeds have been identified on the basis of morphological characters; information on genetic architecture is available for few breeds. The molecular characterization of AnGR for establishing genetic distances among various breeds has been undertaken using microsatellite based DNA markers.

CONSERVATION MEASURES ADOPTED IN INDIA

The rich biological diversity of this species is progressively being eroded due to unplanned breeding. Except in few organized farms which maintain small herds of pure breed, there is almost unrestricted interbreeding among different breeds and there is a marked decline in the availability of unique animals conforming to the attributes of defined breeds, particularly in their native breeding tracts. There has been a non-judicious utilization of buffalo genetic resources in the country. The males are only partially utilized in the form of bulls and bullocks. There is always a scarcity of breeding bulls of superior genetic merit. Above all, the high producing milk buffaloes from the breeding tract, representing the best germplams, are taken to metropolitan cities in large numbers for milk production. After completion of lactation, these buffaloes are slaughtered, causing a serious erosion of elite germplasm. Based on the population the major breeds are classified into two groups and the group-1 comprises of stable breeds viz. Murrah, Mehsana, Jaffarabadi, Nagpuri and Pandharpuri, since they do not face reduction in their numbers over the decades. The group-2 comprises of the breeds facing dilution and reduction in numbers; the breeds are Nili-Ravi, Bhadawari, Surti, Marathwadi and Toda.

Both in-situ and ex-situ conservation efforts have been made under different schemes at the institutional level to conserve these breeds for sustainable utilization. Conserving the live animals that exist in nature is in-situ conservation. The animals are maintained in their original habitats under native conditions with no interference in their mode of management, feeding and other conditions. The main problem of in situ conservation is inbreeding and genetic drift typical of small populations. The ex-situ conservation is used when the endangered population is dismally low in numbers, as this process has its own innate problems. It may suffer from spread of disease or neglect during periods of institutional weakness, besides being costly in long term preservations and losing the relatedness of current genotype with environment, when one of these is preserved for long time (Singh et al., 2004).

Under the in-situ conservation scheme, a set of 150 buffaloes have been identified on the basis of their peak yield and then the milk production is recorded at monthly interval. The owners are provided an incentive for two years so that the animals are retained and kept in good health. The tagged females are inseminated with the semen/ bull of the same breed of higher genetic merit. The farmers rear the male progeny from these females up to six months; incentives are provided to retain the calf. As these calves grow, a total of 50 unrelated males are selected as future bulls. Under the ex-situ conservation program, live
animals are maintained at the different government institutional farms and semen samples of the superior bulls (3000 doses/animal) are collected and stored in the liquid nitrogen container for future revival (Sadana, 2010).

CONCLUSIONS
It is evident that the buffalo plays an integral part of our farming system and its numbers have been maintained or increased in some breeds and erosion is observed in some breeds. As a part of conservation measures, various river breeds have been documented and evaluated. Strains of swamp buffalo have been observed but attempts to characterize them genetically have yet to be made. Conservation measures should begin with a proper sampling technique to represent the existing variability and in sufficient numbers. In these populations for conservation, selection should be minimal and to maintain population size constant, either random culling or culling based on total merit should be practiced. However, for the national herds, genetic improvement could be achieved through selection and grading up with Murrah and Surti breeds. For both, germplasm collection and national herd, data banks are essential to monitor their genetic progress.

REFERENCES
Table 1. Buffalo population (millions) of India and the world (FAO, 2008).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country</th>
<th>Population</th>
<th>Per cent to world population</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>105</td>
<td>56.7</td>
</tr>
<tr>
<td>2</td>
<td>Pakistan</td>
<td>29</td>
<td>15.7</td>
</tr>
<tr>
<td>3</td>
<td>China</td>
<td>23</td>
<td>12.6</td>
</tr>
<tr>
<td>4</td>
<td>Nepal</td>
<td>4.5</td>
<td>2.4</td>
</tr>
<tr>
<td>5</td>
<td>Egypt</td>
<td>4.1</td>
<td>2.2</td>
</tr>
<tr>
<td>6</td>
<td>Philippines</td>
<td>3.3</td>
<td>1.8</td>
</tr>
<tr>
<td>7</td>
<td>Myanmar</td>
<td>2.9</td>
<td>1.6</td>
</tr>
<tr>
<td>8</td>
<td>Vietnam</td>
<td>2.9</td>
<td>1.6</td>
</tr>
<tr>
<td>9</td>
<td>Indonesia</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>Thailand</td>
<td>1.7</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Total world population</td>
<td>185</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Production of buffalo milk (million tonnes) by major countries (FAO, 2008).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country</th>
<th>Milk production</th>
<th>Per cent to world production</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>60.90</td>
<td>68.21</td>
</tr>
<tr>
<td>2</td>
<td>Pakistan</td>
<td>20.99</td>
<td>23.51</td>
</tr>
<tr>
<td>3</td>
<td>China</td>
<td>2.90</td>
<td>3.25</td>
</tr>
<tr>
<td>4</td>
<td>Egypt</td>
<td>2.64</td>
<td>2.96</td>
</tr>
<tr>
<td>5</td>
<td>Nepal</td>
<td>0.99</td>
<td>1.11</td>
</tr>
<tr>
<td>6</td>
<td>Iran</td>
<td>0.25</td>
<td>0.27</td>
</tr>
<tr>
<td>7</td>
<td>Myanmar</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Italy</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>8</td>
<td>Iraq</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Other countries</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Total world production</td>
<td>89.28</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3. Description of important buffalo breeds of India (Source: http://www.cirb.res.in/).

<table>
<thead>
<tr>
<th>Buffalo breeds</th>
<th>Home tract (Name of the State)</th>
<th>Morphological characters</th>
<th>Production and reproduction performances</th>
</tr>
</thead>
</table>
| Murrah         | Central Haryana & Delhi       | • Large sized animal and Jet-black in colour with white markings on tail.  
• Horns are short and tightly curved in a spiral form.  
• Tail is long reaching up to the fetlocks with white switch | Average 305-day milk yield (kg): 2000  
Age at first calving: 44  
Calving Interval (days): 453  
Fat percent: 7.3 |
| Nili-Ravi      | Western Punjab                | • Medium sized animal.  
• Colour is black-brown or fawn with white markings on forehead, face, muzzle, legs and tail and the peculiarity of the breed is the *walleys*.  
• Horns are small, tightly curved and circular in cross section. | Average 305-day milk yield (kg): 1950  
Age at first calving (months): 45.3  
Calving Interval (days): 487  
Fat percent: 6.8 |
| Jaffarabadi    | Southern Gujarat              | • Large sized and heaviest Indian buffalo and the colour is usually black.  
• Horns are heavy, emerge out by compressing the head, inclined to droop at each side of the neck and then turning up at points (ring-like).  
• Forehead is very prominent, broad and convex. | Average 305-day milk yield (kg): 1850  
Age at first calving: 50.7  
Calving Interval (days): 440  
Fat percent: 7.7  
• Bullocks are heavy and are used for ploughing and carting |
| Mehsana        | Northern Gujarat              | • Developed by crossing Murrah and Surti breeds of buffaloes  
• Medium sized animal and the colour is usually black  
• Horns usually sickle shaped with curve more upward than in Surti breed and less curved than in Murrah breed but are longer and could be of irregular shape. | Average 305-day milk yield (kg): 1700  
Age at first calving (months): 42.2  
Calving Interval (days): 476  
Fat percent: 7.0 |
| Surti          | South Western Gujarat         | • Medium sized and the oat colour is rusty brown to silver-grey.  
• Peculiarity of the breed is that there are two white collars, one around the jaw and the other at the brisket.  
• Horns are sickle shaped, moderately long and flat. | Average 305-day milk yield (kg): 1400  
Age at first calving (months): 56.4  
Calving Interval (days): 535  
Fat percent: 7.5-8.3 |
Table 3. Description of important buffalo breeds of India (Source: http://www.cirb.res.in/) (Continue).

<table>
<thead>
<tr>
<th>Buffalo breeds</th>
<th>Home tract (Name of the State)</th>
<th>Morphological characters</th>
<th>Production and reproduction performances</th>
</tr>
</thead>
</table>
| Bhadawari     | Uttar Pradesh and Madhya Pradesh | - Medium sized with wedge shaped body  
- Body is light or copper coloured  
- Tail is thin, long with black or white markings | Average 305-day milk yield (kg) : 1100  
Age at first calving (months) : 50  
Calving Interval (days) : 478  
- Bullocks are reputed good draught animals. |
| Nagpuri       | Vidarbha region of Maharashtra. | - Horns are long, flat, curved and carried backwards on each side of the neck nearly to shoulders.  
- Coat colour is black with white patches on face, legs and tail tip. | Average 305-day milk yield (kg) : 1200  
Age at first calving (months) : 55.8  
Calving Interval (days) : 430  
Fat percent : 7.0-8.5 |
| Toda          | Western Tamil Nadu             | - Medium sized animal and the colour is fawn and ash-grey in adults and the calves are usually fawn and rarely grey.  
- Horns: typical, set wide apart, curving outward, slightly downward and upward with the points being recurved inward, forming a crescent shape. | Average 305-day milk yield (kg) : 700  
Age at first calving (months) : 47  
Calving Interval (days) : 480 |
Breeding Program of the Bulgarian Murrah Buffalo

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ABSTRACT
The aim of Bulgarian National Association for Development of Buffalo Breeding (BNADBB) is to work out an effective breeding scheme (BS) for fast genetic progress and improved profitability of production for Bulgarian Murrah (BM) buffalo through application of the latest achievements at the field of breeding and use of modern selections methods. Basic principles for the development of the breeding program is based on the most important phenotypic and genotypic parameters of the selection traits; fixed factors of the breeding policy, selection intensity (I), duration of the generation interval (L), breeding value (BV), optimization of selection by selection index (SI) including age at first calving (AFC), calving interval (CI) and milk yield for first lactation (MYFL); estimation of genetic progress (ΔG).

Keywords: AFC, breeding value, Bulgarian Murrah buffalo, genetic gain, MYFL, selection index

INTRODUCTION
The main objective of the Breeding program of the Bulgarian Murrah (BM) breed, created on the basis of crossing between the native Bulgarian buffalo and the Murrah breed from India, is to work out an effective breeding scheme (BS) for fast genetic gain and improved profitability of the farms through application of modern methods of selection.

Selection in buffalobreeding is a more difficult and complex process in comparison with cattle, resulting from the differences between the species.

The difficulty of applying these methods of selection originate from the fact that it is to be applied in smallholders in the private sector. The realized genetic progress is lower than the theoretic one because of the various factors reducing the efficiency of theoretical models. These limitations are much more present in buffaloes.

Independently of this difficulty our efforts should be directed towards more effective methods of selection and breeding systems to increase the genetic progress in the buffalo population.

KEY STEPS IN THE BREEDING PROGRAM

Number of buffaloes
According to "Agrostatistics" program of the Ministry of Agriculture (2012) dated 01.11.2011, the number of buffaloes was 9,900, of which 6,300 dairy
buffaloes (Table 1). Compared to the same time in 2010 it increased by 7.6 and 11.7 % for total number and dairy buffaloes, respectively. Roughly 70% of the total number of buffaloes are reared in herds with more than 20 heads. The average size of the herds is 12.8 heads. The smallest buffalo population in Bulgaria was registered in 2002.

**Genetic Progress (ΔG)**

The value of the realized genetic progress determines the selection efficiency in the Bulgarian Murrah breed. Despite of the high phenotypic performance of the trait, in practice it is possible to have a negative progress. This is due to ill-organized selection and inaccurate methods of genotype evaluation. According to Alexiev (1979), Vankov (1980) and Peeva (2000), the predicted genetic progress of milk yield is from 1.069 to 3.2%.

**Breeding value**

*Breeding value on the basis of the pedigree.* Selection based on pedigree is the earliest evaluation of animals. Selection can be based either on the information about one parent or on the basis of information about the two parents, or the grandparents. In buffalo farms this kind of selection is used very often. Selection of bulls based on their mothers is less effective. Relatively high genetic progress (ΔG=1.78%) can be attained when the selection of bulls is based on their fathers.

*Breeding value on the basis of the phenotypic performance of the selection trait.* This selection is too much time consuming. The animal recording is the most important task of the breeding work in the Association. The selection of the buffaloes was done on the basis of the information from milk recording. The application of AI with frozen semen is highly recommended for the genetic improvement of the buffaloes. About 70% of the buffalo cows are under milk recording (A4).

*Breeding value on the basis of half and full sibs.* The coefficients of determination (R²) show that only 6.42 and 7.63% of the expected genotype is formed by the productivity of the half and full sister and 93.58 and 92.37% by other factors. The analysis of the many our researches shows that the relative accuracy of the selection by using the information from the lateral relatives would not have a significant effect on the magnitude of the genetic progress in the population. Despite of the low accuracy of the method, it allows early prediction of breeding value of the bulls.

*Proreny testing.* Progeny testing has been proposed persistently in the recent three decades as a method to accurately estimate breeding. Progeny testing consists in taking into consideration the performance of the offspring as a criterion for selection among the parents. The principles of the progeny testing come from the sampling nature of inheritance. Sire index is used in the selection of best sires among those subject to testing. In Bulgaria the most commonly used was the daughter-dam comparison representing the difference between the production levels of a sire’s offspring and their dams. Another method of evaluating sires is the daughter-herdmate comparison practised in Bulgaria as well.

The implementation of AI in buffaloes significantly increased the bulls’ influence on the creation of the dairy population. This estimation was and remains a basic method of evaluation of the additive genotype. The large size of the farms (100 – 500 buffalo cows) allowed to accomplish the progeny testing of the bulls. The milk
recording (A4) and the well organized information system in the Bulgarian National Association for Development of Buffalo Breeding are the basis for realizing the progeny testing program. Over 20 recorded daughters per sire were used in order to increase the accuracy of progeny testing.

**SELECTION METHODS USED IN THE BREEDING PROGRAMME**

**Selection index**

The method of selection indices, as more effective than the tandem selection and independent culling levels, has been applied during the last decades in the countries with developed cattle breeding. Most of the theories of the quantitative genetics and the selection criteria are based on the individual’s phenotype, but a problem appears in their practical application for simultaneous improvement of a couple of traits.

In buffaloes the method of selection index is introduced 20 years ago. Some authors (Gajbhiye, 1987; Sharma & Singh, 1988; Gajbhiye & Tripathi, 1991) reported significant effect of multiple selection.

For first time in Bulgaria the method of selection indices was used by Peeva (2000) in buffalo breeding.

The selection index was expressed by the following equation:

\[
I = b_1 P_1 + b_2 P_2 + \ldots + b_n P_n = \sum_{i=1}^{n} b_i P_i,
\]

where: \( P_1 \) – phenotypic value of the economic traits, included in the index (\( P = 1\ldots n \))

\( b_1 \) – regression coefficient (\( b = 1\ldots n \))

Selection indices have been developed combining the traits: milk yield at first lactation (MYFL), fat (F), protein (P), body weight of the buffalo cows (BW), age at first calving (AFC) and calving interval (CI) as most important for the selection in Bulgarian buffalo population. Fifty-seven selection indices are constructed with different combination of the traits. Their relative effectiveness depends on the number of the included selection traits.

The index combining milk yield for 305-day first lactation, calving interval, and age at first calving:

\[
I = 0.332 \text{ MYFL} + (-4.81 \text{ CI}) + (-62.92 \text{ AFC})
\]

proves to be most efficient. If the selection uses this selection index, the genetic merit for MYFL, CI and AFC will be 36.8 kg, -10.7 days, and -120.5 days, respectively.

**Identification and recording**

The main objectives of identification and recording are:

- identification of high productive animals;
- optimization of nutrition and management;
- estimation of Breeding Value;
- optimization of the genetic progress.

The well-organized buffalo farms created good conditions for milk recording, which comprised all buffalo cows on the large farms. Another reason for establishing a well-organized recording system was the implementation of machine milking on buffalo farms and AI.
Body weight of young animals is:
- at 6 months of age – 140 kg
- at 12 months of age – 290 kg
- at 22 months of age – 400 kg

Body weight of breeding animals is given in Table 2. Body measurements taken at 3, 6, 12, 18, and 24 months.

Age and body weight at first insemination are:
- body weight – 390-400 kg
- age – 22-24 months

Selection criteria for bull-mothers

Average values of the milk production:

<table>
<thead>
<tr>
<th></th>
<th>I- lactation</th>
<th>II- lactation</th>
<th>III- lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, min, kg</td>
<td>1900</td>
<td>2600</td>
<td>3500</td>
</tr>
<tr>
<td>% fat, minimum</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>% protein, minimum</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Body measurements and body weight
- height at withers – 140 cm
- body length – 146 cm
- rump width – 58 cm
- chest girth – 220 cm
- body weight – 550-600 kg

Type rating
In all countries with traditions in buffalo breeding no proper attention is paid to this type of selection, as to the selection on productive and reproductive traits. Only in the recent decades does it attain definite importance. Depending on breed, type of productivity, and region of breeding, clearly are outlined the features of the buffalo that form the ratings of the different traits to determine the overall score. Studies of Bulgarian (Peeva, 1981, Peeva & Alexandrov, 1993) and other authors (Velea, 1991, Velea et al., 1991, Bingzhuang et al., 2002) show that body measures, type and conformation are more substantially affected by the environmental than by the additively determined factors. On the basis of the relationships between the body measures and the milk production traits Peeva (1981) and Saini and Gill (1984) have, independently for Bulgaria and India, developed 100-score cards involving four main groups of traits.

Hundred-score cards were used for estimation including four groups of traits – general appearance (30 scores), dairy character (20 scores), body capacity (20 scores), mammary system (30 scores) and overall rating in Breeding Programme (Peeva, 1981).

OPTIMIZATION OF THE BREEDING PROGRAMME
The optimization of the breeding program is based on the most important phenotypic and genetic parameters of productive and reproductive traits (Penchev,
1999; Peeva, 2000; Ilieva, 2006) (Table 3) and fixed factors of the breeding policy (Table 4).

The phenotypic parameters of the selection traits, including in Breeding Programme were determined by LS-analysis as follow:

$$Y_{ijmnp} = \mu + a_i + b_j + l_m + f_n + g_p + E_{ijmnp}$$

Where:

- $Y_{ijmnp}$ – observation vector
- $\mu$ - overall mean
- $a_i$ – fixed effect of $i$-th farm in $j$-th season, from $m$-th line, on $n$-th lactation, during $p$-th period ($i = 1...10$)
- $b_j$ – fixed effect of $j$-th season in $i$-th farm from $m$-th line, on $n$-th lactation, during $p$-th period ($j = 1...4$)
- $l_m$ – fixed effect of $m$-th line in $i$-th farm during $j$-th season for $n$-th lactation, during $p$-th period ($m = 1...6$)
- $f_n$ – fixed effect of $n$-th lactation in $i$-th farm during $j$-th season for $m$-th line during $p$-th period ($f = 1...3$)
- $g_p$ – fixed effect of $p$-th period in $i$-th farm during $j$-th season for $m$-th line of $n$-th lactation ($p = 1...4$)
- $E_{ijmnp}$ – residual error

Based on the optimization and reporting the impact of various factors on the genetic progress for milk yield, age at first calving and calving interval as optimal, the following criteria and parameters were suggested:

- Sires per stud for AI – 3
- Preliminary selected bull mothers – 135
- Actually selected bull mothers – 45
- Young tested bulls – 15
- Tested sires – 11
- Number of stored doses from a tested sire – 20 000
- Number of daughters per bull – 15

REFERENCES


240

**Table 1.** Number of buffaloes according to Agrostatistics, Ministry of Agriculture, 2011.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9000</td>
<td>7500</td>
<td>8000</td>
<td>8200</td>
<td>9200</td>
<td>9200</td>
<td>9900</td>
<td>+7.6%</td>
</tr>
<tr>
<td>Buffalo cows</td>
<td>5200</td>
<td>3900</td>
<td>4100</td>
<td>4800</td>
<td>5300</td>
<td>5400</td>
<td>6300</td>
<td>+11.7%</td>
</tr>
</tbody>
</table>

**Table 2.** Body weight of breeding animals.

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight</td>
<td>Daily gain</td>
</tr>
<tr>
<td></td>
<td>kg</td>
<td>g</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>650</td>
</tr>
<tr>
<td>12</td>
<td>280</td>
<td>680</td>
</tr>
<tr>
<td>18</td>
<td>360</td>
<td>600</td>
</tr>
<tr>
<td>24</td>
<td>470</td>
<td>600</td>
</tr>
<tr>
<td>36</td>
<td>600</td>
<td>520</td>
</tr>
</tbody>
</table>
Table 3. Phenotypic and genetic parameters of the selection traits.

<table>
<thead>
<tr>
<th>№</th>
<th>Traits</th>
<th>Values of parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>305-day milk yield at 1-st  lactation, kg</td>
<td>1700</td>
</tr>
<tr>
<td>2.</td>
<td>Fat content in milk,%</td>
<td>7.55</td>
</tr>
<tr>
<td>3.</td>
<td>Average number of lactations</td>
<td>4</td>
</tr>
<tr>
<td>4.</td>
<td>Maximum number of lactations</td>
<td>10</td>
</tr>
<tr>
<td>5.</td>
<td>Calving interval, days</td>
<td>420</td>
</tr>
<tr>
<td>6.</td>
<td>Inseminations per conception</td>
<td>2.3</td>
</tr>
<tr>
<td>7.</td>
<td>Daily gain of female calves from birth to weaning, kg/day</td>
<td>0.600</td>
</tr>
<tr>
<td>8.</td>
<td>Daily gain of female calves from 6 months to conception, kg/day</td>
<td>0.550</td>
</tr>
<tr>
<td>9.</td>
<td>Daily gain of young bulls, kg/day</td>
<td>0.700</td>
</tr>
<tr>
<td>10.</td>
<td>Age at first calving, days</td>
<td>720</td>
</tr>
<tr>
<td>11.</td>
<td>Proportion of live borne calves of the total number of borne calves</td>
<td>0.97</td>
</tr>
<tr>
<td>12.</td>
<td>Proportion of calves at 6 mo of the total number of borne calves</td>
<td>0.95</td>
</tr>
<tr>
<td>13.</td>
<td>Proportion of calved heifers of the number of female calves at 6 mo, %</td>
<td>45</td>
</tr>
<tr>
<td>14.</td>
<td>Proportion of culled calves of the total number of borne calves, %</td>
<td>15</td>
</tr>
<tr>
<td>15.</td>
<td>Phenotypic standard deviation for lactation milk yield, kg</td>
<td>420</td>
</tr>
<tr>
<td>16.</td>
<td>Heritability of milk yield</td>
<td>0.25</td>
</tr>
<tr>
<td>17.</td>
<td>Heritability of age at first calving</td>
<td>0.50</td>
</tr>
<tr>
<td>18.</td>
<td>Heritability of calving interval</td>
<td>0.13</td>
</tr>
<tr>
<td>19.</td>
<td>Heritability of daily gain</td>
<td>0.50</td>
</tr>
<tr>
<td>20.</td>
<td>Repeatability of milk yield</td>
<td>0.52</td>
</tr>
<tr>
<td>21.</td>
<td>Selection intensity in bull dams</td>
<td>2.50</td>
</tr>
<tr>
<td>22.</td>
<td>Selection intensity in buffalo cows</td>
<td>1.10</td>
</tr>
<tr>
<td>23.</td>
<td>Selection intensity in sires of bulls</td>
<td>2.70</td>
</tr>
<tr>
<td>24.</td>
<td>Phenotypic standard deviation for daily gain (SD), g</td>
<td>100</td>
</tr>
<tr>
<td>25.</td>
<td>Numbers of buffalo cows for 1 effective daughter</td>
<td>5</td>
</tr>
<tr>
<td>26.</td>
<td>Stored semen doses per year per bull</td>
<td>6000</td>
</tr>
<tr>
<td>27.</td>
<td>Generation interval in sires of bulls, years</td>
<td>7.5</td>
</tr>
<tr>
<td>28.</td>
<td>Generation interval in dams of bulls, years</td>
<td>8.3</td>
</tr>
<tr>
<td>29.</td>
<td>Live weight of young breeding bulls, kg</td>
<td>420</td>
</tr>
<tr>
<td>30.</td>
<td>Live weight of mature bulls, kg</td>
<td>800</td>
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Table 4. Fixed factors of the breeding policy.

<table>
<thead>
<tr>
<th>№</th>
<th>Factors</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total number of buffaloes (year 2011)</td>
<td>9300</td>
</tr>
<tr>
<td>2.</td>
<td>Size of active breeding population, heads</td>
<td>1350</td>
</tr>
<tr>
<td>3.</td>
<td>Number of potential bull mothers</td>
<td>135</td>
</tr>
<tr>
<td>4.</td>
<td>Bull mothers per 1 selected young bull</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>Proportion of culled bulls after semen production</td>
<td>0.10</td>
</tr>
<tr>
<td>6.</td>
<td>Proportion of culled bulls after sexual activity</td>
<td>0.15</td>
</tr>
<tr>
<td>7.</td>
<td>Proportion of heifers at first calving</td>
<td>0.30</td>
</tr>
<tr>
<td>8.</td>
<td>Sires per stud for AI</td>
<td>3</td>
</tr>
<tr>
<td>9.</td>
<td>Storing period of frozen semen, years</td>
<td>12</td>
</tr>
</tbody>
</table>
Evolutionary, Clinical and Molecular Cytogenetics in Water Buffalo: an update

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ABSTRACT

Cytogenetics represents one of the modern biotechnologies applied to the genetic improvement of livestock, including the water buffalo (Bubalus bubalis), one of the most important economic species raised in the world, especially in the East Countries. Cytogenetics covers several aspects of the genetic improvement. In the present paper, an update on the evolutionary, clinical and molecular cytogenetics of the water buffalo is reported.

Evolutionary cytogenetics: Two main types of buffaloes are present in the world: the African buffalo (Syncerus caffer) and the Asiatic buffalo (Bubalus bubalis). The latter has two subspecies: the river type (2n=50) and the swamp type (2n=48). These two species diverged by a tandem fusion translocation involving river buffalo chromosomes 4 and 9 (telomere of 4p and centromere of 9). This fusion was accompanied by loss of the nucleolus organizer regions (NORs) present in river buffalo chromosome 4p, and large portions of constitutive heterochromatin (HC) present in river buffalo chromosome 9. River and swamp buffaloes are normally crossbred, especially to increase milk production in swamp buffaloes. The hybrid (2n=49) could have a lower fertility due to unbalanced chromosome embryos. Further informations differentiating tribe bovinae (cattle and water buffalo) from the remaining ones belonging to bovidae family will be given.

Clinical cytogenetics: The study of water buffalo karyotype is very important because several studies have demonstrated that about 20% of females with reproductive problems (lack of oestrus in fertility age or large delay in the return in oestrus) show sex chromosome abnormalities and almost of them have been found to be sterile for serious damages occurring in internal sex adducts.

In Italy, in collaboration with the National Buffalo Breeder Association (ANASB), cytogenetic controls of bulls and males addressed to the reproduction, as well as on some females with reproductive problems, have been performed to eliminate the carriers of chromosome abnormalities, adding economic value to river buffalo breeding. These studies are routinely performed by using both CBA- and RBA-banding techniques. Specific cases are also studied by fluorescence in situ hybridization (FISH) technique and specific molecular markers (generally bovine BAC-clones).

Molecular cytogenetics: The availability of specific molecular markers containing coding sequences (generally bovine or ovine BAC-clones) and the use of the FISH-technique have opened the door to the molecular cytogenetics also for water buffalo. Indeed, cytogenetic maps with specific and detailed location of loci

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containing expressed coding (and no-coding) sequences along the chromosomes, have been performed for this species, although these maps are not particularly dense. However, they are useful: (a) to increase our knowledge on water buffalo genome by detailed physical description of mapped loci per single chromosome and chromosome band; (b) to study such chromosome abnormalities; (c) to anchor genetic maps (linkage, RH-maps) to specific chromosome regions; (d) to delineate the karyotype evolution of river buffalo versus cattle, sheep and related bovids, as well as between bovids and humans, especially to transfer useful information from human to animal genomes.

**Keywords**: chromosome abnormality, evolution, gene mapping, karyotype, water buffalo

**INTRODUCTION**

Water buffalo is one of the most important economic domestic species in the world, especially in the east countries. In addition, it is the only domestic species which is numerically growing (+2% per year as world average) with higher mean values in Italy (+8%), India (+4%) and Brazil (+5%) (Borghese and Mazzi, 2005). Water buffalo banded karyotypes have been performed by Di Berardino et al (1981) and Di Berardino and Iannuzzi (1981). Then a series of contributions have been published on the cytogenetics of water buffalo, noticeably increasing our knowledge on this species. Cytogenetics covers several aspects of the genetic improvement. In the present paper, an update on the evolutionary, clinical and molecular cytogenetics of the water buffalo is reported.

**EVOLUTIONARY CYTOGENETICS**

Buffaloes belong to the tribe Bovini, family Bovidae, sub-order Ruminantia, order Cetartiodactyla. Two main species of buffalo are known in the world: the African buffalo (*Syncerus caffer*) and the Asiatic buffalo (*Bubalus bubalis*).

The *Syncerus caffer* has two subspecies: the *Syncerus caffer caffer* (2n=52) and a fundamental number (FN) equal to 60, and the *Syncerus caffer nanus* (2n=54 and FN=60). These two subspecies interbreed, although the F1 has 2n=53 and may have reduced fertility due to the presence of unbalanced gametes giving rise to unbalanced zygotes (and embryos) which can die in early embryonic life, as occurring in cattle heterozygous carriers of rob (1;29) (Gustavsson, 1980). The main cytogenetic difference between these two species is the presence of four (*Syncerus caffer caffer*) and three (*Syncerus caffer nanus*) biarmed autosome pairs, while the other chromosomes are acrocentric, including X (the largest acrocentric) and Y (a small acrocentric) chromosomes. According to the chromosome banding techniques, the biarmed pairs in *Syncerus caffer caffer* correspond to centric fusion translocations of cattle (ancestral bovid) chromosomes 1;13, 2;3, 5;20 and 11;29, respectively (Gallagher and Womack, 1992).

The Asiatic (water) buffalo (*Bubalus bubalis*) has also two subspecies: the river buffalo with a karyotype 2n=50 and FN=60 (Figure 1) and the swamp buffalo (2n=48, FN=58). These two subspecies differ by one chromosome pair (and FN), because of a tandem fusion translocation between river buffalo (BBU) chromosomes.
4 and 9 (telomere of BBU4p and centromere of BBU9) originating the largest swamp buffalo chromosome 1 (Di Berardino and Iannuzzi, 1981). Thus, all chromosome arms and biarmed pairs are conserved between the two species. Crosses between the two species are fertile, although the hybrid has 49 chromosomes and may have a lower reproductive value.

The river buffalo karyotype (2n=50) (Figure 1) is formed by five biarmed autosomes and 20 acrocentric chromosomes, including both X (the largest acrocentric) and Y (small acrocentric) chromosomes. The five biarmed river buffalo chromosomes (from BBU1 to BBU5) correspond to five centric fusion translocations of homoeologous cattle chromosomes and bovine syntenic groups (U) according to CSKBB (1994) and ISCNDB (2001): BBU1 (1;27-U10/U25), BBU2 (2;23-U17/U20), BBU3 (8;19-U18/U21), BBU4 (5;28-U3/U29), and BBU5 (16;29-U1/U7). The fusion of these biarmed pairs was accompanied by a substantial loss of constitutive heterochromatin (HC), with very small C-bands found in the centromeres of the biarmed pairs, compared to centromeres of all acrocentric chromosomes, including X, which shows the largest heterochromatin block (Figure 2). The Y chromosome shows variable C-banding patterns depending on the degree of chromosome denaturation. Indeed, the Y chromosome appears completely heterochromatic or with a strong C-band that is distally located (Figure 2). Thus, the C-banding technique (especially CBA-banding) is the simplest cytogenetic technique to distinguish river buffalo sex chromosomes (especially the Y-chromosome) from the autosomes and used for studying river buffalo sex chromosome abnormalities (Iannuzzi et al., 2000a, 2001a, 2004, 2005; Di Meo et al., 2008a).

Because river buffalo BBU4 originated from centric fusion of cattle chromosomes 5 and 28 (CSKBB, 1994), and river buffalo BBU9 is homoeologous to cattle chromosome 7 (CSKBB, 1994),

The following cattle homologous chromosomes (and bovine syntenic groups) are present in swamp buffalo chromosome 1: BTA5 (U3), BTA28 (U29) and BTA7 (U22). During the tandem fusion event, the centromere of BBU9 was apparently deleted or inactivated while the nucleolus organizer regions (NORs) present at the telomeres of BBU4p (BTA28) (Iannuzzi et al., 1996) were lost (Di Berardino and Iannuzzi, 1981). Indeed, there are six nucleolus organizer (NO) chromosomes in river buffalo, located at the telomeres of 3p, 4p, 6, 21, 23, and 24 (Iannuzzi et al., 1996) while the swamp buffalo has five NO-chromosomes (Di Berardino and Iannuzzi, 1981). However, FISH-mapping with two river buffalo probes containing satellite DNA similar to bovine SAT I and SAT II (79% and 81% similarity, respectively) revealed signals in the proximal region of swamp buffalo chromosome 1q (at the presumed region of tandem fusion) with only the SAT I probe, although the signal intensity was stronger in the acrocentric chromosomes than in the biarmed pairs (Tanaka et al., 1999). This result was also confirmed with C-banding in river buffalo (Di Meo et al., 1995) (Figure 2). The SAT II probe produced a signal on all centromeric chromosome regions but not on the proximal regions of swamp buffalo chromosome 1q (Tanaka et al., 1999). Thus, large portions of HC and satellite DNA present in river buffalo BBU9 and the NORs present at the BBU4p telomere were
lost during the tandem fusion translocation originating the swamp buffalo chromosome 1.

In contrast to the autosomal chromosomes which were highly conserved among bovids, sex chromosomes diverged through more complex chromosome rearrangements. X-chromosome of bovids can be mainly reduced to three types: the submetacentric cattle type, the acrocentric river buffalo type, and the acrocentric sheep (or goat) type with small and visible p-arms. Chromosome banding comparisons among these species demonstrated that large portions of these chromosomes are conserved (reviewed in Iannuzzi and Di Meo, 1995), with the presence of large blocks of constitutive heterochromatin (HC) in BBU-X (Di Meo et al., 1995) (Figure 2) and their absence in both BTA-X and OAR/CHI-X (Iannuzzi and Di Meo, 1995). Further details were done when comparative FISH-mapping techniques were used and gene order analysed. Considering X-chromosome of water buffalo as that very similar to that of the ancestral bovid, a centromere transposition or centromere repositioning differentiated cattle X-chromosome from that of river buffalo (with losses of constitutive heterochromatin) (Iannuzzi et al., 2001b, 2009). Goat and sheep X-chromosomes differentiated from that of river buffalo by at least four chromosome segment transpositions, including that of centromere region (reviewed by Iannuzzi et al., 2009).

Similar chromosome events occurred during the karyotype evolution of the Y-chromosome. Indeed, comparative FISH-mapping analyses performed among the Y-chromosomes of cattle (Bos taurus), zebu (Bos indicus, BIN), river buffalo and sheep/goat revealed complex chromosome rearrangements. BTA-Y (submetacentric) and BIN-Y (acrocentric with small and visible p-arms) differ in a centromere transposition (or repositioning) or pericentric inversion since they show the same gene order along the distal regions (Di Meo et al., 2005). BTA-Y and BBU-Y differ in a pericentric inversion with loss (from BBU-Y to BTA-Y) or gain (from BTA-Y to BBU-Y) of HC, BBU-Y being larger than BTA-Y. OAR-Y/CHI-Y (very small metacentrics) differ from BBU-Y in a pericentric inversion and greater loss of HC and from BTA-Y and BIN-Y in a centromere transposition with loss of HC (Di Meo et al., 2005).

Because no biarmed chromosome pairs are shared between African (Syncerus) and Asiatic (Bubalus) buffaloes (CSKBB 1994; Gallagher and Womack, 1992), crosses between them are not possible because the hybrid would have an unbalanced chromosome set. Thus, the designation of two separate genera is also supported by chromosomal morphology.

Another Asiatic buffalo (and endangered) species is Bubalus depressicornis (2n=48), which is found on the Sulawesi (Indonesia) island. It is fitted in two subspecies: Bubalus depressicornis depressicornis (Lowland Anoa) and Bubalus depressicornis quarlesi (Mountain Anoa). This species is the smallest buffalo in the world, with a mature size between 70 (Mountain Anoa) and 100 cm (Lowland Anoa) tall with a shape and size similar to that of a goat. This species is very similar to the river buffalo from a cytogenetic point of view. Indeed, four of the six biarmed chromosome pairs in Anoa are centric fusion translocations of cattle homoeologues similar to those occurred in river buffalo (1;27, 2;23, 8;19, 5;28) whereas the other
Anoa biarmed chromosomes are different combinations of cattle chromosomes (11;20 and 17;15) (Gallagher et al., 1999).

A standard karyotype using six banding techniques and G and R banded ideograms has been performed for river buffalo (CSKBB, 1994). A comparative genetic comparison between river buffalo and cattle, goat and sheep standard karyotypes (ISCNDB, 2001) has been established by Iannuzzi et al., (2001c) using the same molecular markers assigned to G/Q and R-banded cattle chromosomes to construct cattle G- and R-banded ideograms, as well as definitive chromosome homologies between river buffalo and related bovids (ISCNDB, 2001).

**CLINICAL CYTOGENETICS**

Relationships between chromosome abnormalities and reproductive problems have been found in several domestic animal species, including the river buffalo (Yadav and Balakrishnan, 1982; Yadav et al., 1990; Prakash et al., 1992, 1994; Iannuzzi et al., 2000a, 2001a, 2004, 2005; Patel et al., 2006; Di Meo et al., 2008a, 2011).

Two types of chromosome abnormalities are present in the animals: numerical and structural. **Numerical autosomal chromosome abnormalities** are generally very rare in domestic animals. Indeed, the animal phenotype (morphological conformation) is abnormal in the carriers and thus, these abnormalities are directly eliminated by the breeders.

On the contrary, **numerical sex chromosome abnormalities** are generally tolerated by the species. This is essentially due to the nature of sex chromosome: chromosome Y is a small chromosome and one of the X-chromosome is randomly and genetically inactivated. These abnormalities are often responsible of sterility or low fertility for serious damages occurring in the internal sex adducts, especially in the females. Since the carriers of these sex chromosome abnormalities show normal body conformation, including external genitalia, these chromosome abnormalities escape the genetic selection of the breeders and, without a cytogenetic control, these animals (in particular the females), are kept in the farm for long time (also 4-5 years) without production of calves (and productions) with great economic damages for the breeders.

In Italy, in collaboration with veterinary practioners (and Veterinary Faculty of Naples), 171 females with reproductive problems (**females which have reached the reproductive age but do not remain pregnant also in presence of the bull**) were cytogenetically investigated during the last years. About 20% of these females were found carriers of sex chromosome abnormalities and all of them were sterile for the presence of serious damages to the internal gonadal systems, varying from atrophy of Muller ducts (Figure 3) to complete absence of internal sex adduct (closed vagina).

The more common sex chromosome abnormality found in river buffalo has been the mosaicism XX/XY (freemartin) (Figure 2) found in the 90% of river buffalo females examined so far for reproductive problems. All these female carriers were sterile for serious damages of the Muller’s ducts. Only in two different cases the breeders knew that the female was twin with a male, thus potentially alarmed for a possible freemartin (in cattle the 92% of XX/XY mosaicism are freemartin). In all
remaining cases the female freemartin were from single bird. This means that the male co-twin died in early embryonic life (*and adsorbed by the mother*) but the presence of male cells (*even for a brief time*) was sufficient to originate serious damages in the internal sex adducts in the female co-twin. Indeed, in cattle while the placental anastomosis occurs around 20 days of embryonic life, sex differentiation underwent later (about at 40 days) (Ruvinsky and Spicer, 1999). So, there is enough time (two weeks) to originate these damages for the presence in the two twins of both male (XY) and female (XX) cells. Considering the high genetic similarity between cattle and river buffalo, it is normal to think that in female river buffaloes occurs the same phenomenon.

Other type of sex chromosome abnormality found in river buffalo have been: (a) the X-monosomy (Prakash et al., 1992; Iannuzzi et al., 2000a, Di Meo et al., 2008); (b) the X-trisomy (Prakash et al., 1994; Iannuzzi et al., 2004); and (c) the sex reversal syndrome which has been found only in two female Italian river buffaloes so far (Iannuzzi et al., 2001a, 2004). In the latter syndrome, the females have normal female morphology (including external genitalia) but a male genotype (XY).

A particular case of XXY has been found in a male river buffalo calf raised in India (Patel et al., 2006). This animal had two Xs joined by a centric fusion translocation (originating a large biarmed chromosome) and a Y-chromosome.

**Structural chromosome abnormalities** are generally genetically balanced, although in humans about 50% of reciprocal translocations are unbalanced for losses of genetic material. In river buffalo only two cases of autosomal chromosome abnormalities have been found so far. Both of them were complex abnormalities which required the “fission” (*a submetacentric chromosome is divided along the centromere originating two acrocentric chromosomes*) of chromosome 1 and subsequent fusion of the short arms (1p) with an acrocentric chromosome which was chromosome 23 in a female cow (Di Meo et al., 2011), and with chromosome 18 in a bull (Albarella et al., 2013). This latter case has been very interesting because the bull (named “Magnifico”) was very famous and largely used in the A.I. Cytogenetic analysed were also performed in its offspring (36 females and 14 males): 15 animals (30%) were carriers of the same chromosome abnormality of the bull, thus confirming that the chromosome abnormality was transmitted to the progeny. After this discovery all semen samples of the bull were not used more for the AI.

Under the request of the Italian “National Buffalo Species Association” (ANASB), systematic cytogenetic analyses of bulls present in various farms or addressed to AI have been performing in our lab. Until now, in about 60 examined bulls, no one chromosome abnormality has been found, thus supporting that Italian river buffalo bulls have a normal karyotype. However the study of “banded karyotype” should be “routinely applied and required” for all bulls before their use for the reproduction, as well as for all females with reproductive problems. In particular all bulls should be “cytogenetically certified” by accredited cytogenetic laboratories before their use for reproduction.

**Cytogenetic test** as those of chromosome abnormalities (CA, *chromosome breaks, chromatid breaks, fragments*) and SCE (*Sister Chromatid Exchange*) (Figure 4) have been applied to calves born with limb defects (Peretti et al., 2008) or naturally exposed to mutagens, like animals raised in polluted areas (Ahmed et al.,
1998), or to dioxins (Genualdo et al., 2012). These test revealed pronounced chromosome fragility in both calves affected by limb malformations as well as in buffaloes raised in polluted areas or naturally exposed to dioxins, compared to control animals. Since similar results have been found also in other domestic species naturally exposed to mutagens (reviewed in Genualdo et al., 2012), cytogenetic test have been revealing a powerful tool to test the animal genomes under the environmental condition and, indirectly, to check the entire food chain simply monitoring domestic species which are before humans in the food chain.

Molecular cytogenetics Assignments of both type I (known genes) and type II loci (SSRs/microsatellite marker/STSs) have been performed in river buffalo using a somatic cell hybrid panel (El Nahas et al., 1996) and FISH-mapping techniques (Iannuzzi, 1998; Iannuzzi et al., 2003). In the most recent cytogenetic map (Di Meo et al., 2008b), 388 loci, mostly containing coding sequences, have been assigned to river buffalo by FISH-mapping techniques (mostly), noticeably extending the river buffalo cytogenetic map. In Figure 5 is reported an example of river buffalo cytogenetic map in chromosomes 10 and 2p and their comparison with human chromosome 6 to show the chromosome regions conserved between the two species. These maps are very useful for comparative mapping studies among boids and between boids and humans to transfer useful genetic information from richer genomes (humans) to those of domestic animals, as well as to better anchor linkage and radiation hybrid maps to specific river buffalo chromosome regions. Furthermore, the availability of molecular markers and of the FISH-technique allows to follow the chromosome abnormalities also in the germinal lines, in particular in the sperms. These markers can be found also useful application in the production of in vitro embryos. Indeed, since about 50% of these embryos are polyploid (and abnormal), the cytogenetic control using FISH-mapping techniques can be very useful to check abnormal embryos so to improve the technique producing embryos reducing the percentages of abnormal ones.

CONCLUSIONS

Although several genetic studies have been performed in water buffaloes, more work is necessary to genetically improve this species so to add economic value to all buffalo productions. Cytogenetic approaches are very useful to explain the reproductive problems often found by the breeders during the animal breeding. Cytogenetic screening with banded karyotypes (and relative certificates) should be performed for all bulls, especially those used in AI. These certificates must be given by accredited cytogenetic laboratories working on river buffalo. Cytogenetic test are also very useful to test the genome stability of animals and, indirectly, the animal welfare and food chain. Indeed, when animals are exposed to mutagenic compounds, cytogenetic test reveal higher chromosome fragility, compared to that of animal not exposed (control). The use of molecular markers and the FISH-technique not only can improve our knowledge on buffalo genome, but they can be used to better study the chromosome abnormalities and to check both sperms and embryos before their use. However, a better collaboration among breeders, veterinary practioners, cytogeneticists and breeder associations is necessary if we want to bring up the genetic improvement of buffaloes.
ACKNOWLEDGEMENTS

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REFERENCES


Figure 1. Reverse RBA-banded normal river buffalo karyotype (2n=50, XY) in an Italian bull. Numbering between parenthesis are the homoeologous chromosomes of cattle.

Figure 2. Reverse CBA-banding in a male (left) and female (right) river buffalo metaphase plates. The two types of cells were found in a female affected by mosaicism XX/XY (freemartin). Sex chromosomes are indicated. Note the large and centromeric HC-block in the X-chromosome (with a proximal and additional C-band) and the Y-chromosome (left image) which is C-band negative at the centromere (differently from all acrocentric chromosomes) but C-band positive at the telomeric regions. C-banding technique is the simplest one to distinguish sex chromosomes from autosomes and, for this reason, very useful to detect sex chromosome abnormalities in this species.
Figure 3. Internal sex adducts in a female river buffalo found carrier of a XX/XY mosaicism (freemartin). Note the pronounced atrophy of the Muller’s ducts.

Figure 4. Reverse river buffalo metaphase plate treated for sister chromatid exchange (SCE) test. Seven SCEs (arrows) were found in this cells.
Figure 5. Cytogenetic map of river buffalo chromosomes 10 (homoeologous to cattle chromosome 9) and 2p (homoeologous to cattle chromosome 23) and its comparison with human chromosome 6 (HSA6). Note the inversions occurred in both river buffalo chromosomes, when compared with HSA6. (From Di Meo et al., 2008).
Invited Papers
Buffalo Nutrition and Feeding Symposium
Enhancing Buffalo Production Efficiency through Rumen Manipulation and Nutrition

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ABSTRACT

Swamp buffaloes are important ruminants which have been contributing to the integrated livestock-crop production system and the livelihood of the farmers. The rumen of buffalo is fermentation vat capable of producing volatile fatty acids and synthesizing microbial protein for the hosts’ use. In addition, rumen methane is also produced and eructated out to the environment. Conducted researches have revealed significant information pertaining to the uniqueness of the buffalo rumen hosting a higher population of predominant species of cellulolytic bacteria and fungal zoospores, but with a lower protozoal population. Moreover, they produce a high concentration of rumen ammonia nitrogen and ability for rumen nitrogen recycling. Currently, the use of bio-molecular techniques can reveal additional interesting information concerning rumen microorganisms and their potential role in improving rumen fermentation and the detoxification processes in the rumen, as well as for swamp buffalo productivity. Using nested PCR, the results were found that the strain of Synnergistes jonesii was present in swamp buffaloes consuming high level of Leucaena. Local feed resources availability in various seasons can contribute as essential sources of carbohydrate and protein and can significantly impact to the rumen fermentation and the subsequent productivity of the buffalo. Development of the food-feed-system (FFS) can increase food for human and feed for the buffalo as well as enrich the nitrogen for the soil. Regarding digestibility, they are capable of digesting low-quality roughages and crop-residues with a digestibility of up to about five percent units higher than in cattle. Relating to rumen fermentation, the use of plant secondary compounds such as condensed tannins, saponins, dietary rich in minerals, as well as garlic and vegetable oils, have subsequently resulted in decreasing rumen protozoa, methanogens and methane mitigation. Rumen manipulation in buffaloes using dietary strategies should be recommended.

Keywords: fermentation, methane, food-feed-system, local feed resources, NPN, rumen ecology, swamp buffalo

IMPORTANT OF BUFFALO

Swamp buffaloes (Bubalus bubalis) have been important domesticated livestock for farmers engaged in integrated crop-livestock farming in many countries including China, Vietnam, Laos, the Philippines, Malaysia, Indonesia, Thailand, as well as in some countries of Africa and America etc. Their multiple functional roles are vital; serving as draft, transportation means, manure, meat, by-products as well

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as livelihood of the rural communities (Chantalakhana & Suntraraporn, 1979; Devendra, 1993). Recent research has been conducted investigating the uniqueness of their abilities in utilizing fibrous low-quality feeds including crop-residues producing fermentation end-products (volatile fatty acids, VFAs) and microbial protein for synthesis of useful products such as meat and milk. Furthermore, the use of molecular techniques to study existing rumen microbes namely bacteria, protozoa and fungi forming the rumen consortium and fermentation characteristics have been providing interesting and useful data pertaining to their abilities of digestion as well as potential applications in the food-feed-system to support sustainable livestock production (Wanapat, 2010; Wanapat & Rowlinson, 2007; Wanapat et al., 2011).

Livestock production, in particularly buffalo and cattle, are an integral part of the food production systems, making important contributions to the quality and diversity of human food supply as well as providing other valuable services such as work and nutrient recycling. Large increases in per capita and total demand for meat, milk and eggs are forecast for most developing countries for the next few decades. In developed countries, per capita intakes are forecast to change slightly, but the increases in developing countries, with larger populations and more rapid population growth rates, will generate a very large increase in global demand. Most importantly, the human-inedible materials such as roughages, tree fodders, crop residues and by products into human food by ruminant animals will continue as a very important function of animal agriculture. However, since much of the projected increase is expected to come from pork, poultry and aquaculture production, i.e. from species consuming diets high in forage carbohydrate, meeting future demand will depend substantially on achievable increases in cereal yields (Delgado et al., 1999). Therefore, there are opportunities and challenges for researchers to increase in animal productivity through the application of appropriate technologies, particularly in production systems, nutrition and feeding.

Buffaloes produce meat, milk, saving bank, draft power, transportation, and other purpose for human and on-farm manure to crop farming. Therefore, feed utilization of buffaloes is more effective than cattle when cattle and buffaloes were kept under similar conditions, particularly well-adapted to harsh environment and are capable of utilizing low quality roughages especially the agricultural crop-residues and by-products, hence their potential are therefore remarkable in terms of meat production using locally available feed resources. However, a decrease in the number of buffaloes has been occurring in some countries in the world due to influences associated with three factors: holsteinization which mean the substitution of low production buffaloes with high production of other ruminants; mechanization, which mean the substitution of draught animals with tractors and the poor market demand for buffalo products (Borghese, 2010). According to some countries, buffalo numbers have increased due to the demand for particular products obtained from buffalo milk and meat to both on the national and international market.

**RUMEN ECOLOGY AND FERMENTATION IN BUFFALOES**

According to Wanapat & Rowlinson (2007), Ruminal ammonia nitrogen (NH$_3$-N) has been reported to be an important nutrient in supporting efficient rumen fermentation. It was earlier reported that 5 mg% ruminal NH$_3$-N was optimum for
microbial fermentation in mixed culture in a closed system while a higher level would be required to achieve a maximum rate of fermentation in vivo, depending on the potential fermentability of feeds. In cattle fed low quality roughage, it was found a higher level of ruminal NH$_3$-N (15 to 20 mg%) increased digestibility and intake. Although a number of researchers showed that swamp buffaloes were more efficient than cattle in many aspects, namely N-recycling and fiber digestion, ruminal NH$_3$-N level in relation to efficient fermentation and intake. It was reported that when ruminal NH$_3$-N increased, from 1.7 to 5.6 mg%, total bacterial count, digestibilities of DM, NDF and ADF were increased. Wanapat & Pimpa (1999) reported that increasing level of ruminal NH$_3$-N to 17.6 mg% resulted in increased DM intake, protozoal population and highest concentration of urinary allantoin. Therefore, level of ruminal NH$_3$-N of 14 mg% was recommended as optimal in swamp buffaloes.

A comparative study on rumen bacterial and protozoal population and fungal zoospores in cattle (Brahman x Native) and swamp buffalo (Bubalus bubalis) was conducted. Forty animals, twenty of each, with same sex and similar age which were raised under similar condition in the Northeast of Thailand, were used. Rumen digesta were sampled bacterial population were higher in swamp buffalo than those in cattle (1.6 vs 1.36 x 10$^8$ cells/ml) having more population of cocci, rods and ovals. Lower rumen protozoal population in swamp buffaloes with lower numbers of Holotrichs and Entodiniomorphs were found as compared to those in cattle. Significant higher fungal zoospore counts were in swamp buffalo than those in cattle being 7.30 and 3.78 x 10$^6$, respectively. Study under electron microscope, revealed Anaeromyces sp. with acuminate apex were more predominant in the rumen of swamp buffalo. With these findings, cattle and swamp buffaloes showing differences in rumen bacterial, protozoal population and fungal zoospore counts, offer new additional information as why swamp buffaloes exhibit conditionally body weight better than cattle especially during long dry season without green grass. Studies on diurnal patterns of rumen fermentation and the effect of rumen digesta transfer from buffalo to cattle were conducted.

Based on these studies, diurnal fermentation patterns in both cattle and buffaloes were revealed. It was found that rumen NH$_3$-N was a major limiting factor. Rumen digesta transfer from buffalo to cattle was achievable. Monitoring rumen digesta for 14 days after transferred showed improved rumen ecology in cattle as compared to that of original cattle and buffalo. It is probable that buffalo rumen digesta could be transferred to others. However, further research should be undertaken in these regards in order to improve rumen ecology especially for buffalo-based rumen. With regards to urinary excretion of purine derivatives (PD) and tissue xanthine oxidase in swamp buffalo and cattle, Chen et al. (1996) conducted a comprehensive experiment for such species comparison. It was reported that activities of xanthine oxidases were found in plasma, liver and intestinal tissues, respectively and patterns of PD excreted were similar between buffalo and cattle having allantoin and uric acid. Based on Kang & Wanapat (2013, unpublished), swamp buffaloes could efficiency utilize NPN (urea) for increasing microbial protein synthesizes as compared to soybean meal.

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of PD excreted were similar between buffalo and cattle having allantoin and uric
acid.

**RESEARCH ON RUMEN MICROBES IN SWAMP BUFFALOES**

Livestock are already well-known to contribute to greenhouse gas (GHG)
emissions (Steinfeld et al., 2006; Goodland & Anhang, 2009). Livestock’s long
shadow, reported by Steinfeld et al. (2006) estimated that 7,516 million tons per year
of CO$_2$ equivalent or 18 percent of annual worldwide emissions are attributable to
cattle, buffalo, sheep, goats, and other animals. However, recent analysis with
different methodology and assumptions has shown at least 32,564 million tons of
CO$_2$ equivalent per year or 51 percent of annual worldwide GHG are produced
(Goodland & Anhang, 2009).

It has been reported that when cattle and buffaloes were kept under similar
conditions, buffaloes utilize feed more efficiently with the digestibility of feeds
being typically 3-5 percentage units higher (Wanapat & Wachirapakorn, 1990).
Ruminal ammonia nitrogen (NH$_3$-N) has been reported to be an important nutrient in
supporting efficient rumen fermentation. Earlier, it was reported by Satter & Slyter
(1974) that 5 mg% ruminal NH$_3$-N was optimum for microbial fermentation in
mixed culture in a closed system. However, in cattle fed low quality roughage
especially rice straw, higher levels of ruminal NH$_3$-N (15 to 20 mg%) were found to
increase digestibility and intake (Boniface et al., 1986; Perdok & Leng, 1990;
Wanapat & Pimpa, 1999). A number of researchers (Wanapat et al., 2000;
Devendra, 2002) have shown that swamp buffaloes are more efficient than cattle in
many aspects, namely N-recycling and fiber digestion, ruminal NH$_3$-N level in
relation to efficient fermentation and intake.

A comparative study on rumen bacterial and protozoal population and fungal
zoospores in cattle (Brahman x Native) and swamp buffalo (*Bubalus bubalis*) was
conducted. It was found that rumen bacterial and fungal zoospores population were
higher in swamp buffalo than those in cattle (1.6 vs 1.36 x 10$^8$ cells/ml) having a
higher population of cocci, rods and ovals. There was a lower rumen protozoal
population in swamp buffaloes with lower numbers of *Holotrichs* and
*Entodiniomorphs* as compared to those in cattle. Significantly higher fungal
zoospore counts were found in swamp buffalo than cattle (7.30 and 3.78 x 10$^6$,
respectively). Study under electron microscope, revealed *Anaeromyces* sp. with
acuminate apex were more predominant in the rumen of swamp buffalo. With these
findings, cattle and swamp buffaloes showing differences in rumen bacterial, protozoal population and fungal zoospore counts, offer new additional information as why swamp buffaloes maintain body weight and body condition score better than cattle especially during the long dry season without fresh grass and other forages (Wanapat, 2000; Wanapat et al., 2009; Wanapat et al., 2008).

Recent studies were conducted to investigate the effect of urea level with a variety of energy sources and varying roughage to concentrate ratio, in swamp buffaloes using PCR-DGGE and real-time PCR technique (Hart and Wanapat, 1992; Wanapat et al., 2009; Chanthakhoun et al., 2011; Khejornsart et al., 2011). Under this study, methanogenic bacterial diversity was investigated and the predominant populations of cellulolytic bacteria were found to be *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* in both rumen digesta and fluid.

Studies by (Wanapat & Cherdthong, 2009; Khejornsart et al., 2011) used a real-time polymerase chain reaction approach to determine the population of cellulolytic bacteria (*Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*) in digesta and rumen fluid of swamp buffalo (*Bubalus bubalis*) and beef cattle. It was found that the applicability of real-time PCR techniques for the quantification of cellulolytic bacterial numbers (*R. albus*, and *R. flavefaciens*) in the digesta of swamp were higher than those in cattle. However, at 4 h *R.albus* were significantly higher in buffalo than in cattle rumen fluid, but *R. flavefaciens* and *F.succinogenes* tended to be higher in cattle than those in buffalo, in rumen fluid. However, the digesta sample had higher cellulolytic bacteria than those found in the rumen fluid. This finding indicates higher ability of buffalo in digesting low-quality roughages.

However, bacteria are the most numerous of these microorganisms and play a major role in the biological degradation of dietary fiber. *F. succinogenes*, *R. albus*, and *R. flavefaciens* are presently recognized as the major cellulolytic bacterial species found in the rumen (Wanapat et al., 2008; Wanapat & Cherdthong, 2009; Khejornsart et al., 2011). Recent works on advances in molecular biology techniques allow the analysis of such bacteria without cultivation, thereby identifying many functional, but uncultured, bacteria as new targets for basic and applied research (Chanthakhoun et al., 2011).

Considerable research work concerning rumen microorganisms and their role in rumen fermentation have been conducted and shed more light in understanding the rumen efficiency of swamp buffaloes as compared to cattle in utilizing low-quality roughages. It is notable that the rumen of swamp buffaloes host a diversity of rumen microorganisms especially those of cellulolytic bacteria and with higher diurnal concentration of ammonia nitrogen (Hart & Wanapat, 1992; Wanapat, 2001; Wanapat, 2010; Wanapat & Pimpa, 1999). A number of trials have been conducted using different diets to study rumen microorganisms and their fermentation end-products. The results have revealed that *Ruminococcus flavefaciens* was significantly higher than *R. albus* and *Fibrobacter succinogenes*. Furthermore, improvement of rice straw by urea-treatment (Chanjula et al., 2004; Cherdthong et al., 2010) and the use of cassava hay could change rumen microorganisms as well as volatile fatty acids (VFAs). The use of the wild legume, *Phaseolus calcaratus* (Chanthakhoun
et al., 2011a,b), in the form of hay which contained higher protein and plant secondary compound like condensed tannins, and the use of various plant proteins including mulberry, luecaena and cassava hay could improve rumen ecology in buffalo fed on rice straw. In addition, a widely grown in the Tropics, tree fodder legume; Leucaena (*Leucaena leucocephala*) has been used as a protein supplement for ruminants. However, its use has been limited by the presence of mimosine, which is degraded in the rumen to a toxic compounds ((2,3-dihydroxy pyridone (2,3-DHP) and 3,4-dihydroxy pyridone (3,4-DHP)). Currently, Phesatcha et al. (2013) have reported the finding of *Synnergistes jonesii*, the rumen bacteria capable of degrading 2,3-DHP and 3,4-DHP in swamp buffaloes fed on Leucaena. This result indicates the presence of *Synnergistes jonesii* in swamp buffalo and the potential use of high level of *Leucaena*. The result was confirmed by relatively low concentration of urinary 2,3-DHP and 3,4-DHP by using HPLC.

**Rumen Manipulation in Swamp Buffaloes**

Plant secondary compounds (condensed tannins and saponins) are more important as ruminant feed additives, particularly on CH₄ mitigation strategy because of their natural origin in opposition to chemical additives. Tannins containing plants exhibited the anti-methanogenic activity mainly due to condensed tannins. There are two modes of action of tannins on methanogenesis: a direct effect on ruminal methanogens and an indirect effect on hydrogen production due to lower feed degradation. Also, there is evidence that some CT can reduce CH₄ emissions as well as reducing bloat and increasing amino acid absorption in small intestine. Methane emissions are also commonly lower with higher proportions of forage legumes in the diet, partly due to lower fibre contact, faster rate of passage and in some case the presence of condensed tannins (Beauchemin et al., 2008). Legumes containing condensed tannin (e.g., Lotuses) are able to lower methane (g kg⁻¹ DM intake) by 12-15% (Beauchemin et al., 2008; Rowlinson et al., 2008). Condensed tannins could reduce CH₄ production by 13 to 16% (DMI basis) (Woodward et al., 2004), mainly through a direct toxic effect on methanogens. McAllister & Newbold (2008) reported that extracts from plants such as rhubarb and garlic could decrease CH₄ emissions. In addition Sirohi et al. (2009) have shown that plant secondary compounds at lower concentrations could manipulate rumen fermentation favorably. At appropriate dose, saponins or saponins containing plants have been shown to suppress protozoal population, increase bacteria and fungi population, propionate production, partitioning factor, yield and efficiency of microbial protein synthesis and decrease methanogenesis, hence performance of ruminants were improved.

Manipulation of rumen fermentation using lemongrass, soapberry fruit, mangosteen peel power, garlic powder, and/or vegetable oil have resulted in maintenance of rumen pH, lowering protozoa and methanogens, increasing bacteria and zoospores as well as increasing propionic acid production (Wora-anu et al., 2007; Poungchompu et al., 2009; Kongmun et al., 2010). Mode of action of oils and plant secondary compounds has been presented in details by (Kobayashi et al., 2000; McIntosh et al., 2003; Calsamiglia et al., 2007; Patra and Saxena, 2009; Wanapat, 2010; Wanapat et al., 2010). Among various research papers dealing with factors contributing to rumen methane production (Shibata & Terada, 2010) have
recommended on estimation equation for rumen methane production based on dry matter intake, which has a high correlation coefficient. Functional roles of rumen microorganisms and their fermentation process have been comprehensively presented in which much research has been referred to (Figure 4) (Nocek & Russell, 1988).

Table 5, presents the data from both in vitro and in vivo trials using mangosteen peel powder (MP) and/or with other sources on rumen fermentation. Based on these results, MP supplementation both in in vitro and in vivo trials revealed significant increase in total volatile fatty acid production, as well as propionate production, while acetate, butyrate production and acetate to propionate ratio were significantly decreased. Condensed tannins and saponins contained in MP could attribute to the above effects. Similar effects especially the acetate and propionate ratio was found by Beauchemin & McGinn (2006) while total VFA were decreased. In addition, Poungchompu et al. (2009), however, used a combination of MP, soapberry fruit powder and garlic and found significant increase of propionate production in the rumen.

Supplementation of MP on DM intake, digestibility and rumen methane production are reported on Table 5. The findings showed that MP supplementation did not effect on DM intakes, while digestibility and rumen methane production (by estimation using VFAs) were significantly decreased. Effects of MP supplementation on ruminal microorganism population are shown in Table 6. MP supplementation had remarkably reduced rumen protozoa production, while predominant cellulolytic bacteria were increased. In addition, methanogens tended to be decreased. However, it was found that mangosteen peel powder significantly increased cellulolytic bacteria population (Kongmun et al., 2009). The condensed tannins and saponins present in the MP influence such changes in the rumen.

FOOD-FEED-SYSTEM (FFS)

Food-feed-system has been shown to produce both foods for human and feeds for animal production. Moreover, intercropping with legumes can enrich nitrogen in the soil. Some examples of the interventions are as follows. Wanapat et al. (2007) found that yield of cassava foliage, when intercropped with legume, cowpea produced 5.96 ton/ha of green cowpea pod. It was found that productivities of intercrops were improved with a biomass of 6.83 tonDM/ha of cassava foliage, and 0.89 ton DM/ha of cowpea residues (initial cutting at 4 months and thereafter 4 cuttings at 2 months interval. In addition, a legume, Stylosanthes, was also intercropped in the cassava plot, and it produced 3.51 ton DM/ha. The practice of cassava-legumes intercropping also improves farm productivity. However, some farmers encountered problems with making (drying) hay in the rainy season, therefore, the alternative strategies such as constructing solar-drying houses using simple materials such as plastic sheets and bamboo was recommended to farmers. As a result of food-feed-system, green cowpea pods were used for household consumption, as a gift to neighbors and sold for generating higher incomes, while cowpea residues and Stylosanthes fodder were used as animal feeds (Wanapat et al., 2001).
CONCLUSIONS

Swamp buffaloes will importantly continue to be an important ruminant serving in many aspects for the world population engaging the integrated farming systems. Their ability in utilizing high fibrous feeds especially those of agricultural crop-residues and by-products, through the presence of diverse microorganisms in the unique rumen ecology, providing energy and protein for their productive functions have been significantly discovered. Manipulation of the rumen ecology by dietary factors such as the use of plant secondary compounds could have an impact on rumen microorganisms and fermentation end-products. Furthermore, with advancement of molecular techniques, their applications in rumen buffalo research, would offer additionally useful data with regards to rumen ecology, particularly pertaining to microorganisms, methane production and mitigation, feed degradation and utilization, enzyme production, as well as meat quantity and quality.

ACKNOWLEDGEMENTS

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Table 1. Rumen ecology characteristics of swamp buffaloes under various feeding.

<table>
<thead>
<tr>
<th>Item</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruminal pH</td>
<td>6.5-6.8</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>38-39</td>
</tr>
<tr>
<td>NH₃-N, mg%</td>
<td>7.1-17.7</td>
</tr>
<tr>
<td>Blood-urea N, mg%</td>
<td>13.0-21.3</td>
</tr>
<tr>
<td>Ruminal volatile fatty acids (VFA), %</td>
<td></td>
</tr>
<tr>
<td>Acetate (C₂)</td>
<td>66.9-73.8</td>
</tr>
<tr>
<td>Propionate (C₃)</td>
<td>16.2-28.8</td>
</tr>
<tr>
<td>Butyrate (C₄)</td>
<td>4.7-6.6</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>96.7-115.3</td>
</tr>
<tr>
<td>Ruminal microbial population</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>Total viable count, x10¹² cells/ml</td>
<td>1.82-2.40</td>
</tr>
<tr>
<td>Cellulolytic, x10¹⁰ cells/ml</td>
<td>4.06-5.62</td>
</tr>
<tr>
<td>Proteolytic, x10⁶ cells/ml</td>
<td>3.84-5.33</td>
</tr>
<tr>
<td>Amylolytic, x10⁷ cells/ml</td>
<td>3.51-4.12</td>
</tr>
<tr>
<td>Protozoa, x10⁵ cells/ml</td>
<td>2.30-5.20</td>
</tr>
<tr>
<td>Holotrich</td>
<td>1.80-2.52</td>
</tr>
<tr>
<td>Entodiniomorph</td>
<td>0.35-1.30</td>
</tr>
<tr>
<td>Fungal zoospores, x10⁶ cells/ml</td>
<td>1.02-7.30</td>
</tr>
<tr>
<td>Urinary purine derivative, mM/d</td>
<td></td>
</tr>
<tr>
<td>Allantoin</td>
<td>22.4-37.4</td>
</tr>
<tr>
<td>Uric acid</td>
<td>4.9-9.1</td>
</tr>
<tr>
<td>Creatinine</td>
<td>660-722</td>
</tr>
</tbody>
</table>


Table 2. Numbers of bacteria, protozoa and fungal zoospores in the rumen of cattle and buffaloes raised under traditional system in the NE Thailand.

<table>
<thead>
<tr>
<th>Item</th>
<th>Cattle</th>
<th>Buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen pH</td>
<td>6.5±0.12</td>
<td>6.60±0.07</td>
</tr>
<tr>
<td>Microbial population,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria, x 10⁸ cells/ml</td>
<td>1.36±0.14</td>
<td>1.61±0.12</td>
</tr>
<tr>
<td>Coccus, x 10⁵ cells/ml</td>
<td>1.07±0.70</td>
<td>1.28±0.23</td>
</tr>
<tr>
<td>Oval*</td>
<td>&lt;</td>
<td>&gt;</td>
</tr>
<tr>
<td>Rod*</td>
<td>&lt;</td>
<td>&gt;</td>
</tr>
<tr>
<td>Protozoa, x 10⁵ cells/ml</td>
<td>3.82±0.88</td>
<td>2.15±0.41</td>
</tr>
<tr>
<td>Holotrich</td>
<td>2.52±0.70</td>
<td>1.80±0.36</td>
</tr>
<tr>
<td>Entodiniomorph</td>
<td>1.30±0.34ᵃ</td>
<td>0.35±0.13ᵇ</td>
</tr>
<tr>
<td>Fungal zoospore, x 10⁶ cell/ml</td>
<td>3.78±0.78ᵃ</td>
<td>7.30±0.93ᵇ</td>
</tr>
</tbody>
</table>

ᵃᵇ: in the same row with different superscripts differ (P<0.05)

*More or less as compare between cattle and swamp buffaloes

Source: Wanapat et al. (2000)
Table 3. Rumen pH and population of bacteria, protozoa, and fungal zoospores in rumen of swamp buffaloes and cattle.

<table>
<thead>
<tr>
<th>Item</th>
<th>Buffaloes</th>
<th>Cattle</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen pH</td>
<td>6.78±0.55</td>
<td>6.51±0.81</td>
<td>1.06</td>
</tr>
<tr>
<td>Microbial population, h- post feeding*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria, x10^8 cells/ml</td>
<td>3.3±1.3</td>
<td>2.2±0.6</td>
<td>0.25</td>
</tr>
<tr>
<td>Protozoa, x10^6 cells/ml</td>
<td>3.6±1.4</td>
<td>5.0±1.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Fungal zoospore, x10^5 cells/ml</td>
<td>7.1±1.4</td>
<td>3.8±1.7</td>
<td>0.06</td>
</tr>
<tr>
<td>NH₃-N, mg% (h- post feeding)</td>
<td>14.7±2.5</td>
<td>12.7±0.5</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*measured at 0, 2, 4, 6, 8 h, post-feedings.
Source: Wanapat et al. (2008)

Table 4. Quantitative measurement, R. albus and R. favefaciens population in rumen digesta and fluid between swamp buffalo and beef cattle using real-time PCR.

<table>
<thead>
<tr>
<th>Item</th>
<th>Buffaloes</th>
<th>Cattle</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen digesta, copies/ g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. albus (x 10^8)</td>
<td>6.04±0.84</td>
<td>2.92±1.96</td>
<td>0.026</td>
</tr>
<tr>
<td>R. favefaciens (x 10^8)</td>
<td>8.31±5.43</td>
<td>5.57±4.99</td>
<td>0.484</td>
</tr>
<tr>
<td>Rumen fluid, copies/ g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. albus (x 10^7)</td>
<td>3.02±1.67</td>
<td>1.58±1.13</td>
<td>0.112</td>
</tr>
<tr>
<td>R. favefaciens (x 10^7)</td>
<td>2.09±2.64</td>
<td>2.95±1.29</td>
<td>0.638</td>
</tr>
</tbody>
</table>

Source: Wanapat et al. (2009)
Table 5. Effect of mangosteen peel supplementation on rumen volatile fatty acid production in ruminants using *in vitro* and *in vivo* studies.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Level</th>
<th>TVFA</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C2/C3</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP</td>
<td>5 mg</td>
<td>─</td>
<td>─</td>
<td>+</td>
<td>+</td>
<td>─</td>
<td>Norrapoke et al (2012)</td>
</tr>
<tr>
<td><em>In vivo</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP</td>
<td>30g/kg</td>
<td>+</td>
<td>─</td>
<td>+</td>
<td>─</td>
<td>─</td>
<td>Pilajun and Wanapat (2011)</td>
</tr>
<tr>
<td>Combination</td>
<td>CO + MP</td>
<td>50 + 30 g/kg</td>
<td>─</td>
<td>─</td>
<td>+</td>
<td>─</td>
<td>Pilajun and Wanapat (2011)</td>
</tr>
<tr>
<td></td>
<td>MP + CAP</td>
<td>5 + 5 mg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>─</td>
<td>─</td>
<td>+</td>
<td>─</td>
<td>Norrapoke et al (2012)</td>
</tr>
</tbody>
</table>

GP = Garlic powder, MP = Mangosteen peel powder, MP = Mangosteen peel pellet, CO = Coconut oil, CAP = *Centella asiatica* powder, + = increased, ─ = decreased

<sup>a</sup>Supplementation in *in vitro* experiment diet.

Table 6. Effect of mangosteen peel supplementation on intake, digestibility and methane production in ruminants using *in vitro* and *in vivo* studies.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Level</th>
<th>DMI</th>
<th>Digestibility</th>
<th>Methane</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP</td>
<td>5 mg</td>
<td>+</td>
<td>─</td>
<td></td>
<td>Norrapoke et al (2012)</td>
</tr>
<tr>
<td><em>In vivo</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP</td>
<td>30 g/kg</td>
<td>nc</td>
<td>─</td>
<td>─</td>
<td>Pilajun and Wanapat (2011)</td>
</tr>
<tr>
<td>Combination</td>
<td>CO + MP</td>
<td>50 + 30 g/kg</td>
<td>nc</td>
<td>+</td>
<td>─</td>
</tr>
<tr>
<td></td>
<td>MP + CAP</td>
<td>5 + 5 mg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nc</td>
<td>+</td>
<td>─</td>
</tr>
</tbody>
</table>

GP = Garlic powder, MP = Mangosteen peel powder, MP = Mangosteen peel pellet, CO = Coconut oil, CAP = *Centella asiatica* powder, nc = not changed.

<sup>a</sup>Supplementation in *in vitro* experiment diet
Table 7. Effect of coconut oil and mangosteen peel supplementation on microbial abundance (Log copies/ml).

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>CO5</th>
<th>MP3</th>
<th>COM</th>
<th>SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacterial</td>
<td>9.78b</td>
<td>10.09a</td>
<td>9.84ab</td>
<td>9.92ab</td>
<td>0.08</td>
<td>0.042</td>
</tr>
<tr>
<td>Protozoa</td>
<td>6.40a</td>
<td>6.20b</td>
<td>6.33ab</td>
<td>6.14b</td>
<td>0.06</td>
<td>0.048</td>
</tr>
<tr>
<td>Methanogen</td>
<td>6.07</td>
<td>5.92</td>
<td>6.12</td>
<td>5.98</td>
<td>0.08</td>
<td>0.089</td>
</tr>
<tr>
<td><em>Fibrobacter succinogenes</em></td>
<td>9.04a</td>
<td>7.14c</td>
<td>8.88a</td>
<td>7.44bc</td>
<td>0.12</td>
<td>0.034</td>
</tr>
<tr>
<td><em>Ruminococcus flavefaciens</em></td>
<td>6.28</td>
<td>6.35</td>
<td>6.27</td>
<td>6.30</td>
<td>0.05</td>
<td>0.210</td>
</tr>
<tr>
<td><em>Ruminococcus albus</em></td>
<td>6.43</td>
<td>6.49</td>
<td>6.44</td>
<td>6.44</td>
<td>0.05</td>
<td>0.419</td>
</tr>
</tbody>
</table>

CO5, coconut oil 5% DMI; MP3, mangosteen peel 3% DMI; COM, combination of CO5 and MP3
Source: Pilajun & Wanapat (2012)

Figure 1. Diurnal rumen NH₃-N concentration of cattle and swamp buffalo fed on rice straw. Source: Wanapat et al. (2008)
Figure 2. Illustration of rumen swamp buffalo with ruminal bacteria, protozoa, fungal zoospores, fermentation process and fermentation end-products. Source: Wanapat (2012)

Figure 3. Cellulolytic bacteria population in the rumen swamp buffaloes determined by qPCR technique. Source: Wanapat & Pilajun (2008)
**Figure 4.** Role of plant secondary compounds (condensed tannins & saponins) on rumen fermentation process.

**Figure 5.** Energy and protein metabolism in the rumen microbial protein synthesis and methane production.
Figure 6. Food-feed-system pattern intercropped cassava and legume.
Invited Papers
Buffalo Production and Management Symposium
Nutritional and Other Management Practices for Optimum Semen Production in Buffalo Bulls

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ABSTRACT

To support the genetic improvement programme there is a need to produce quality semen from genetically superior bulls. This would entail maximum utilization of males through frozen semen technology with high fertility. Proper feeding management of the young bulls becomes more important for production of quality semen. However, bull’s feeding generally is not given due importance as a result its age at puberty and first semen collection get delayed. One of the major constraints in the exploitation of the potential of buffalo bull is a sharp seasonal trend in semen quality. It exerts its effect on reproductive performance through macro and micro climatic factors like temperature, humidity, rainfall, photo-period. Therefore suitable nutritional and management strategies are being searched for and incorporated so as to maximize quality semen production without discarding too many poor quality ejaculates.

Keywords: buffalo bulls, nutrition, puberty age, season effect, semen quality,

INTRODUCTION

A breeding bull is said to be half of the herd, as it sires the whole herd. Therefore, the importance of genetically superior bull as a producer of large quantities of normal fertile spermatozoa in any programme of natural and/or artificial breeding/insemination is obvious. The bull that has a high economical value attached with it; have to be maintained on proper nutrition and management to obtain optimum performance in terms of growth and semen production. However, bull’s feeding generally is not given due importance as a result its age at puberty and first semen collection get delayed. Obtaining semen at the earliest possible age from bulls is not only economical but also may increase productive life span and genetic testing of bulls at an early age. The onset of puberty is more closely related to body weight than the age and nutritional level modulates age at puberty. Thus nutrition ranks high among factors that control generation and output of sperms and accessory fluids in the male. But when we talk of nutrition, the environment and management goes with it and only one aspect cannot be taken in isolation. Weak and erratic libido is characteristically an inherent trait which the buffalo bull possesses. It has been found that semen collected during November (winter) produced significantly higher conception rate (40.9\%) than semen collected in June (summer) (34.0\%). It was attributed 40\% of the seasonal variation of buffalo fertility to the male (Heuer et al., 1987).

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OPTIMUM SEMEN PRODUCTION

The bulls to be used as semen donors must be selected at the age of 16-18 months. Breed, age, size, feed intake, play an important role in bringing on puberty in buffalo males. The following points must be taken into account to decide if a buffalo male will be a semen donor. The first step of bull selection is to know his ancestor’s history concerning milk or meat production. Once selected as semen donor a bull must be subjected to general clinical examination to find out any reproductive, pathological condition, chromosomal defects or any hereditary disease. Also the bull must be tested for some infectious contagious disease such as brucelosis, vibriosis, bovine viral diarrhoea (BVD) etc. It is important that young bulls selected for semen collection must be maintained alone in individual barns however, it is recommended to put them together once or twice a day with other young bulls in order to acquire homosexual behaviour an aspect very important for semen collection. Vale, 1994a has enumerated characteristics of normal buffalo ejaculate viz. Colour, white, milky with light blue tinge; Volume, 3 ml (2-8); Wave or swirl motion, > 3; Motility, > 70%; Vigour (individual motility), > 3; Concentration, 6 x 10^5 to 12 x 10^5; Live sperm, > 70%; Abnormal sperm, < 30% and pH, 6.5-7.5.

FACTORS AFFECTING OPTIMUM SEMEN PRODUCTION

Sexual Maturity

Contradictory reports are available in literature about the age at maturity and first ejaculation. The first signs of sexual interest and meiotic divisions of spermatogonial cells were found to occur as early as 9 month and age of puberty and first ejaculation in Egyptian buffalo bulls (14.2 month) is reasonably early (Ali et al, 1981). In another observation at NDRI, Karnal, a cloned buffalo bull, which was kept on intensive care with extra ordinary management, had donated the first ejaculate at the age of 18 months with a body weight of 450 kg which comes around 830 gm/d growth rate and 30 cm scrotal circumference. However, in spite of early puberty, bulls in Egypt are put to service at about 3 - 3.5 years of age and so are the bulls in India. Semen of best quality, with regard to sperm morphology, was observed in 3- to 5-year-old Murrah (Kumar et al., 1993; Chinnaiya and Ganguli, 1990; Singh et al., 2004) and Nili–Ravi buffalo bulls (Saeed et al., 1990). There were significant differences in the quality of semen of individual bulls (Mohan and Sahni, 1990; Galli et al., 1993; Kumar et al., 1993).

Nutrition

The feeding of the buffalo bulls has an influence on the production and quality of the semen (Chinnaiya and Ganguli, 1990, Dahiya et al., 2006). The level of feeding at various stages of growth seems to have an important influence on male and female reproductive performance. Severe under-nutrition or over-feeding and deficiency of specific nutrients are the most common causes of impaired reproductive capability of the bull in terms of semen production and quality. Moderate to severe nutritional deficiency, especially in rural buffaloes, due to feed shortage and/or poor quality feed leads to delayed puberty in buffalo bulls associated with loss of libido, depressed spermatogenesis and poor semen quality (Pant, 2002).
Season

The effect of season is both direct and indirect. It affects the animal directly through macro and micro climatic factors, which are the temperature, humidity, rainfall and photo-period. High heat stress during summer is known to depress the libido, semen quality and fertility of breeding buffalo bulls (Pant, 2000). Photoperiod is an important environmental factor influencing reproduction and sexual activity of buffalo bulls (Vale, 1997). In the temperate regions of the world, it has been found that the semen is of better quality during the winter and spring than in summer and autumn (Galli et al., 1993; Mohan and Sahni, 1990). In the tropical regions, the quality of semen was observed to be satisfactory during the rainy season. In the warm and humid tropical Amazon region, the best time to obtain semen is between January and June (Vale, 1994b). Winter and spring are the favorable season for the semen production and hot and hot-humid are the unfavorable season in buffaloes (Maink, 1984; Tuli, 1984).

Management

The effect of age, body size, body weight, scrotal circumference on the ability of buffalo male to produce semen has long been recognized. (Pant et al., 2003). In addition to nutrition, housing conditions also influence the quality and quantity of semen. Safety, ease, protection from environment and provision for exercise are the key point while planning accommodation for a bull. No hard and fast rule can be fixed for frequency of collection however, for getting quality semen, judicious use of breeding bulls in terms of number of ejaculates per collection and frequency of collection is essential. It will certainly prolong the reproductive life of a breeding bull. Semen collection once a week is an ideal frequency for a young bull between the age of 2-3 years. Thereafter two successive ejaculates with a time interval of 30 minutes, twice a week can be collected for harvesting good quality semen. As a regular prophylactic measure, breeding bulls are vaccinated against various contagious diseases. semen quality is affected by vaccination (Venkatareddy et al., 1991) as it is one of the major stress factors which cause rise in body temperature because of the febrile reaction occurring during post-vaccination period.

REMEDIAL MEASURES

Realistic remedial measures for optimum semen production includes:

1. Modifying nutritional environment
2. Ameliorating heat stress
3. Improving management practices

1. Modifying nutritional environment

Appropriate feeding and management of prepubertal buffalo bulls is thought to be of value, if bulls are to be prepared for breeding at right age. The feeding standards for maintenance and growth requirements of energy and protein are generally based on observation on female; it is possible that they underestimate the requirements of male. The feeding management as being followed for growing buffalo calves is followed until they attain a body weight of 350-400 kg. For the growing bulls the diet should contain about 12 (10 to 14) per cent CP and 60 per cent TDN on DM basis up to 15 months of age and attaining 300 to 350 kg weight

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DM intake should remain around 3.0 kg per 100 kg body weight as overfeeding may lead to fat deposition and obesity in bulls which reduces libido and vigor. Increase of fat thickness in buffalo males has also been associated with accumulation of fat in the scrotal region which causes an imbalance of temperatures exchange and reduction in the semen quality mainly sperm motility and normal morphology in the ejaculates (Vale, 1994a, 1997). The feeding standards (Kearl, 1982; Mandal et al., 2003) and the most recent feeding standard (Paul and Lall, 2010) are based on NRC feeding standards and can be referred for computation of the rations for growing animals.

**Supplemental feeding**

Although there are few studies establishing the minimum nutritional requirements for reproducing buffalo male, nutritional deficiency in vitamins, macro and micro minerals have also been associated with reduction in semen quality. Vitamin A and E are directly involved with the quality of semen in all domestic species. Deficiency of selenium a micro mineral has been associated with an increase in sperm tail abnormalities in several species. Supplementation diets with Vit. E or selenium and combination of two resulted in improvement of semen characteristics and acrosomal morphology (Gokcen et al., 1990). The role of minerals and vitamins in reproduction is very important for seminal aspects. Many authors have related Cu, Fe, I, Mn, Mo, Se and Zn as seven gold minerals of reproduction. Acute under nutrition and specific nutrient deficiencies i.e. vitamin A, manganese, copper, protein or change in Ca++/P+ ratio adversely affect the fertility of both young and mature bulls. However, proper maintenance under optimum nutritional condition rarely produced these symptoms. Zn is involved in more than 300 enzymatic reactions and most of them are related to reproduction functions as much in male as in female. In buffalo Zn function is related to the sperm motility and may affect the sperm metabolism (Ahmed and Tohamy, 1997). Significant improvement in semen quality was noticed in mature breeding buffalo bulls given vitmin AD₃ and E injection and water splashing at the hotter part of the day in summer season (Singh et al., 2000). Attempts have been made to find out the effect of feeding some protected nutrients on growth and semen quality of the bulls. It has been suggested that protection of critical amino acids such as methionine helps to improve the production performance. Bines et al. (1980) suggested methionine cause the changes in the contents of various hormones (e.g. insulin, thyroxin) in the blood, shifts in the balance of anabolic and catabolic metabolism. Dietary protein sources that are considered to be good sources of “bypass” or rumen undegradable intake protein (UIP) have also been used in the diet of growing buffalo bulls. (Dahiya et al., 2001). A study conducted by Singh et al. (2000) found that the effect of supplementation of methionine and lysine in the feed of buffalo bulls had brought about significant (P<0.01) improvement in quality (ejaculate volume, mass activity, live sperm, total sperm per ejaculate etc.) and freezability (pre and post freezing sperm motility and morphology) of buffalo bull semen. Studies have been conducted in our laboratory to find out the effect of feeding of cotton seed cake (CSC), mustard cake and fish meal (MCFM) as sources of rumen undegradable protein and ground nut cake (GNC) to young buffalo bulls on body growth, sexual maturity and semen production. Perusal of data presented in Table-2 showed the supremacy of cotton
seed cake over the ground nut cake as protein source in the ration of the breeding bulls. It was also observed that bulls fed CSC and MCFM started exhibiting sexual behavior by mounting each other around at the age of 27-28 months, which also corresponded to the testosterone level in bulls of these groups.

2. Ameliorating heat stress

The adverse effect of heat stress can be reduced either by modifying the environment or by enhancing the adaptive capacity of animals. Efforts should be made to create better micro environment around the animal and standardize the shelters. The will include the provision of environmental control devices (misters/foggers or sprinklers during peak period of heat stress), increased roof height and provision of shade in open paddocks to protect from radiation effect. The heat stress effect can be reduced by providing good quality green forage during summer and the diet must be made more energy dense to provide sufficient energy. Minimize high fibre diets like straws and stovers by decreasing dry forages in the diet. The feeding of antioxidants (Zn, Se, Vit. E) during summer can also improve health and fertility of the bull. The increase in feeding frequency and that too in cool hours improves feed intake and production under heat stress. Water intake is closely related to dry matter intake by reducing body temperature through absorbed heat energy.

3. Improved Management practices

The maintenance of bulls in good condition is an essential requirement for successful breeding programme. A bull should be kept in an individual pen of rough concrete floor of about 40-50 sq. m with adequate loafing area of about 80 sq. m area with katcha floor, manger and water trough with access to continuous water supply. Provision of trees and land scaping around bull shed should be given priority to protect the bull from exposure to direct sun. Adequate light and ventilation arrangements are desirable for a bull shed. Semen quality (Venkatareddy et al., 1991) is adversely affected by vaccinations against FMD, HS and BQ. To certain extent this adverse effects can also be reduced by immuno-modulators. Therefore, it is advised that vaccination schedule may be followed when the season is unfavorable as during that time the semen production is usually of poor quality especially for purpose of freezing. The following measures are also necessary for proper management of breeding bulls.

- The breeding bull must be selected with true to the breed characters and should come from parents with high index of production.
- A bull should be free from communicable diseases.
- A bull should not be allowed to put on fat and should be subjected to regular exercise to maintain vigor and libido.
- Always handle a bull in firm manner and never trust him as bulls have an unpredictable viciousness
- The preputial washing prior to the semen collection is an important aspect as most of the microbial contaminations in the semen come from the preputial area.

CONCLUSIONS

An ever increasing amount of knowledge on the basic physiological mechanisms underlying the reproductive function of buffalo bulls is piling up. This
will lead to an improvement and a better refinement of protocols aimed at the control of semen production efficiency and its quality. There is a big gap on the information on the effect of minerals and vitamins supplements in the male buffalo reproduction. In the near future new approaches for molecular understanding the role of the vitamins and some minerals in the processes of spermatogenesis and sperm preservation will be very necessary. For maximizing quality germplasm production the critical control points need to be focused are: avoidance of overfeeding as it leads to excessive fattening which lowers the libido, good management and exercise of bull be regularized, provision of good quality of energy and protein sources, provision of Vitamin A and E along with minerals especially Calcium, Phosphorus and Manganese during all ages of bull.

REFERENCES


Kearl, L.C. (1982) Nutrient Requirement of Animals in Developing Countries. International Feed Stuff Institute, Utah Agriculture Experimental Station, Utah State University, Logon, Utah, USA.


Table 1. Age, body weight at first ejaculation, growth and seminal attributes of different groups.

<table>
<thead>
<tr>
<th></th>
<th>G-I (CSC)</th>
<th>G-II (MCFM)</th>
<th>Control (GNC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Rate (g/day)</td>
<td>480.00</td>
<td>444.00</td>
<td>400.00</td>
</tr>
<tr>
<td><strong>Age and body weight at first ejaculation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.wt. (kg)</td>
<td>436.00</td>
<td>421.00</td>
<td>401.75</td>
</tr>
<tr>
<td>Age (months)</td>
<td>31.60</td>
<td>33.60</td>
<td>32.75</td>
</tr>
<tr>
<td><strong>Testosterone levels (mg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 month</td>
<td>0.394</td>
<td>0.416</td>
<td>0.315</td>
</tr>
<tr>
<td>24 month</td>
<td>0.597</td>
<td>0.780</td>
<td>0.410</td>
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<tr>
<td>28 month</td>
<td>0.822</td>
<td>0.770</td>
<td>0.512</td>
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<tr>
<td><strong>Seminal Attributes</strong></td>
<td></td>
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<tr>
<td>Volume/ ejaculate (ml)</td>
<td>2.13</td>
<td>0.75</td>
<td>1.07</td>
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<tr>
<td>Mass activity</td>
<td>1.01</td>
<td>0.22</td>
<td>0.15</td>
</tr>
<tr>
<td>Sperm concentration (million/ml)</td>
<td>1181.75</td>
<td>352.00</td>
<td>324.00</td>
</tr>
</tbody>
</table>
Privatization of Artificial Insemination Services under Smallholder Production System

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ABSTRACT
Efforts to privatize AI services for water buffaloes in the villages was initiated in 2006 through training of selected and interested individuals to conduct “pay per service” in their respective communities, the VBAITs. Government covered the training, equipping and nurturing until the VBAITs are fully functional. From 2006-2012, there were 636 VBAITs trained and represents 61.5% of the all trained AI technicians and is an increase of about 11 folds from the 2005 level. The share of VBAIT AI service in 2005 was only 5.92% while government AI technicians accounted for 94.0%. After 6 years of sustained trainings of VBAIT, the overall AI services rose to 52.5%, whereas the government AI technicians share went down to only 47.2%. Among the trained VBAITs, 38.1% were inactive, a bit higher than 29.3% inactivity among trained government AI technicians. However, among the active AI technicians, the average AI services/year/technician for VBAIT is 97.1 and 66.8 for government AI technicians. Also, the number of technicians with services of 200 or more per year was higher among VBAITs. Nurturing of VBAITs is very essential for their establishment in their service areas, and for those who have been totally capacitated, VBAITs formed their association. There are indications that VBAIT when fully capacitated and empowered can run and manage the AI system in a privatized mode.

Keywords: artificial insemination, privatization, village-based artificial insemination technician

INTRODUCTION
The urgency of creating opportunities for generating additional income for rural families is brought about by the increasing population, and thus growing number of available rural labor against the background of declining size of land holdings. Use of existing resources among rural family, such as the swamp buffalo, has been demonstrated to significantly contribute to increase income through the introduction of dairy genetics. This approach has been of value in improving production capacities among the resulting crossbreds, a magnitude as much as two times for growth, and 3-4 folds for milk production. Consideration has to be taken of the fact that there are about 3.3 million of carabaos in the custody of some 2.5 million farming families.

In view of the limited number dairy of buffaloes in the country that may be used for natural mating, introduction of dairy genetics through artificial insemination becomes the most practical approach to achieve the desired improvement in milk production. 2013.

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and meat productivity. For this purpose AI on swamp buffalo was initiated in the Philippines as early as 1984 and was done by the limited number of AI technicians of Local Government Units (LGUs). The added major challenge is the widely but thinly scattered animals in smallholder mixed farming system as access to animals for AI service is not easy. Thus, due to the limited number of AI technicians, the diffusion rate of AI services rendered by the government technicians remained not more than 3.0% of all breedable females in the country.

Budgetary constraints among LGUs is another issue and is a major cause why many trained AI technicians are inactive. Other than issue for gasoline and travel expenses, the supply of liquid nitrogen on sustained basis for efficient and effective AI service is also affected. Due to these limitations of the existing system it becomes clear that the more practical approach to increasing AI service coverage without necessarily increasing cost on the national government is through privatization of AI services.

Therefore, the proposed undertaking seeks to privatize and also expand the coverage of the AI program in support of ongoing efforts aimed at increasing the dairy buffalo herd population in the Philippines. This in turn will create new jobs, and increase income of target rural farming families.

MATERIALS AND METHODS

The project has four important components, each complementary to each other and all are needed to achieve the project objectives. These components are:

Training of Private Village-Based AI Technicians (VBAIT) - This activity is carried out nationwide and utilized the existing training facilities of the Philippine Carabao Center (PCC) network. The target is to train 2,000 private village-based artificial insemination technicians (VBAITs) over the project period at the account of the government. Prospective VBAITs are selected from their supposedly target communities through a process that involved local village officials, the provincial livestock officer, and PCC officers.

For this purpose, PCC capacitated five centers, each equipped with adequate training animals, suitable training facilities, retrained trainors, and adequate fund to carry out the desired trainings.

Utilization and harnessing the potentials of trained VBAIT- The program made available LN₂ tanks, AI guns and other AI paraphernalia, and each VBAIT received a set upon completion of AI training. All trained VBAIT also received nurturing support up to a point where each has been fully integrated in the service community by way of having developed adequate competencies, confidence as well as acceptability among target clientele. The scheme is development of privatized AI service on a “pay per service”. It is also part of the nurturing process to organize the VBAIT into functional organization with the aim of allowing the group to run all the AI related activities, such as LN₂ and semen distribution, etc. as part of the privatization process.

Strengthening of the semen processing laboratory- This involved improvement in the capacity of the existing semen laboratory to meet the national requirements for frozen semen of the expanded AI initiatives. More proven sires for semen production were assigned at the semen processing facility.
Improvement of liquid nitrogen distribution- One of the major requirements for a successful AI program is the availability of LN$_2$ down at the field level in order to preserve the quality of frozen semen. For this purpose, the program established LN$_2$ depot at PCC Headquarters, Muñoz, Nueva Ecija and served LN$_2$ requirements for Regions I, II and III. This depot has the capacity to fill up the requirements of all existing and future AI technicians in these regions. Thus, the commercial LN$_2$ manufacturing company delivers LN$_2$ in bulk truck, at least once/month, or as frequent as the case may need. This replaced the common practice of buying LN$_2$ by the 35 liter tanks at frequent times, coupled with frequent travels to and from the main depot of the LN$_2$ manufacturing company, located some 100km away. To meet LN$_2$ requirements for Regions 4 and 5, arrangement for bulk purchase from private company, CIGI (now Linden) was made. Similar arrangement was made to cover other regions in Central and Southern islands of the country.

For the first two years, VBAIT received free of charge LN$_2$ and frozen semen from the program. Thereafter, these items are paid by the VBAIT at cost, taking into consideration the two years establishment of VBAIT costumer relation, allowing the VBAIT to have adequate clientele in their respective community, and thus is able to generate enough income from their services.

RESULTS AND DISCUSSIONS
Training of VBAIT

The number of Village-based AI Technicians (VBAIT) trained by the five training centers from 2006 to 2012 is shown in Table 1. The first two years of VBAIT training implementation (2006-2007) was important learning period, giving PCC the chance and time to assess both the strategies and targets. This is because during the second year of full efforts of selection and training, the absorptive capacities of the training centers were assessed to be only to a maximum 6 trainings/year instead of the projected 8 sessions/year. So instead of the estimated full capacity of being able to train 480 VBAIT/year, it was only 360 VBAIT/year. Yet despite this estimated capacities, the output of the 5 training centers was only 241 (CY 2007).

Towards the end of 2007, the program monitoring team gathered field data on the activities of the trained VBAIT. It was reported that only 24% of those trained in 2006 and 39% of those trained in 2007 were indeed active doing AI in the field. The result of the 2007 evaluation team was the single reason for the program implementing unit to reassess strategies and targets, and caused the temporary suspension of training until a revised, more appropriate approach, particularly on selection and identification of would-be technicians was finalized. In the subsequent year (2008), the training was resumed but with limited target due to very rigid selection criteria set for prospective trainee. In fact, the few number of qualified trainee becomes the limitation starting 2008, not the absorptive capacity of the training centers anymore.

Prior to 2006, the number of private AI technicians was only 56 and represented only 12.0% of the all AI technicians (442) servicing water buffaloes. By 2012, the number of trained VBAIT (2006-2012) reached 636 and represent 61.56%
of all trained AI technicians, both government and VBAIT. Training process from 2006 to 2012 resulted in increase of government AI technicians by only 2 folds, whereas the number in trained VBAIT increased by 11 folds.

**Utilization of VBAIT for water buffalo upgrading in the villages**

The efficiency of AI technician training, measured in terms of % active in service after training is presented in Table 1. Prior to 2006, the efficiency of AI tech training was 50.0% and 44.6% for government AI technician and private AI technician, respectively. For the period 2006-2012, the cumulative AI technician training efficiency as of 2012 was much higher, 71.7% for government AI technician and 61.9% for VBAIT.

The surge in the number of trained VBAIT in 2006 and 2007 was followed by high % of inactivity, and it was found to be associated with poor system of selection of trainees. After 2007, the selection process became rigid, and thus the number of private AI technicians recommended for training was dramatically reduced in subsequent years. The overall inactivity among trained government technicians reached to 28.9% and was lower than those noted among trained private AI technicians recorded to be 38.1%. The inactivity among trained VBAITs has been associated 70% of the cases to three major reasons, namely, a) no time to do AI, b) decided to get employment abroad, and c) transferred to another job (Palacpac et al., 2013; Pol, 2012).

There was dramatic improvement in the AI training efficiency from 2006 to 2012, both for government and private AI technician with the rate of improvement higher in private AI technician (from 33.9% to 61.9%) than the government AI technician (50.0% to 71.7%)

On the other hand, about 44.7% of those classified as inactive VBAIT are those located in island provinces and in far plung areas of Southern Philippines. In view of long distance from the concerned PCC Center, nurturing of these technicians until they gain full confidence and competence becomes the major concern, added to the issue of difficulty in their access to supply of LN2 and frozen semen being out of route of the LN2 delivery van.

**Insemination Services of VBAIT:** The performance of trained AI technicians from 2003- to 2012 are presented in Table 2. Between 2003-2005, the contributions of private AI technicians to overall AI services was only 5.92% while government AI technicians contributed 94.0%. Dramatic change in the share of private AI technicians in the overall services were noted starting 2006 to 2012. From 5.92% share in prior year, it went up to 20.5% in 2006 and continued to grow to as much as 52.5% in 2012, an increase by 8.8 folds. This would indicate that by 2012, the private technicians share is greater than the contribution of government technicians in the overall AI services. The fact that active private AI tech constitutes only 43.2% of the total AI force servicing water buffalo in the country, it only suggests that private AI technicians have higher annual AI services/technician.

One very interesting development to date is the ability of PCC at CLSU and PCC at MMSU to organize the VBAIT under their watch. The association of VBAIT at PCC at MMSU has established their own by-laws, elected their officers and conducts meetings regularly. In fact, the VBAIT association has been planning on
taking over the responsibility of semen and LN$_2$ distribution to all its members, an activity that used to be done by PCC.

**Efficiency of AI Services:** The efficiency in AI services by AI technicians expressed as number of AI services/year is summarized in Table 3. Both types of AI technicians have very similar efficiency, with government and VBAIT having 82.6% and 75.5% of the technicians rendering 100 and below services/year, respectively. On the other hand, there is indication that there is higher percentage of private AI technician performing 200 and above AI services/year that of the government AI technician. In a more detailed study of VBAIT, confined within the national Impact Zone, Pol (2012) reported that there are considerable indicators that farmers are willing to call VBAIT service for a fee. In fact, in the same Impact Zone, Palacpac et al. (2013) reported that 68.0% of farmer-clients prefer VBAIT services owing to easy and immediate access.

**Development of LN$_2$ Distribution System**

The LN$_2$ depot established at PCC Gene Pool and Headquarters proved to be very useful and efficient in meeting the LN$_2$ requirements of Regions I, II, and III as originally planned, plus the cryogenic requirements of the national gene pool and the central semen laboratory.

The program has also procured two units light truck and 5 units of Van, all have been assigned for LN$_2$ distribution. The bulk LN$_2$ tank (1,000 liter) each mounted into the delivery vans were found to incur large LN$_2$ evaporation during long distance transport. Thereafter, its usage was only limited as LN$_2$ storage not needing frequent and long distance travel. As a replacement for long distance LN$_2$ transport, several mother tanks (35 liters) are now in use instead of bulk LN$_2$ tanks in order to minimize LN$_2$ losses.

The provision of L300 van dedicated to LN$_2$ distribution also proved very helpful in taking several 35 liter tanks at one time to the CIGI LN$_2$ depot in Sta. Rosa, Laguna for Region IV and V and from the Legacy LN$_2$ Company in Cebu for Regions VI to XII and Southern island.

Even at the early period of program implementation, the LN$_2$ distribution system in place has been capitalized by other existing AI program such as those rendered by Provincial and Municipal governments, accessing LN$_2$ at cost from the system, as this resulted in reduced LN$_2$ cost on their part and an easy accessibility to many LGU AI technicians.

**Other Relevant Observations**

Other relevant observations from the implementation of the program are herein summarized.

a. There are considerable numbers of successful VBAIT able to perform more than 200 services/year and earn an estimated added income of Php 100,000.00/year. A VBAIT only spend, at the most, two hours to do one AI service per day to be able to carry out the 200 AI services/year. These VBAITs are also actively engaged in their regular farming activities, and thus continuously generate income from their original farming.

b. Those successful VBAIT have already established credibility among the large animal raisers in their communities, and thus, these animals raisers are willing
to pay for the VBAIT services. The number of paying clients continue to increase in the VBAIT’s customers list.

c. Nurturing VBAITs until they reach a stage of full confidence and competence and is able to totally integrate with their service community is very essential. This lesson is shown in a PC Center-led nurturing model initiated by PCC at CLSU, and has been perfected by PCC at MMSU. In the case of PCC at MMSU, almost 100% of VBAIT are in active mode.

d. In areas geographically far from the reach of concerned PC Centers, it is important that another nurturing model be developed, such as the LGU-led system. In this case, PCC should also ensure that concerned LGUs are capacitated to be able to carry out the needed nurturing efforts for the VBAIT.

e. The successful organization of VBAIT at Region I, organized by PCC at MMSU is a very good development. It exemplifies the empowerment of the technicians by joining forces together towards a common goal. In the future, a national association of VBAIT be formed and empowered to run the national AI program.

REFERENCES
Table 1. Number of technicians trained and number of active in AI service, 2003-2012.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Government</th>
<th></th>
<th></th>
<th>Private</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trained</td>
<td>No.</td>
<td>%</td>
<td>Trained</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>2003-2005</td>
<td>386</td>
<td>194</td>
<td>50.2</td>
<td>56</td>
<td>25</td>
<td>44.6</td>
</tr>
<tr>
<td>2006</td>
<td>61</td>
<td>447</td>
<td>55.9</td>
<td>109</td>
<td>165</td>
<td>33.9</td>
</tr>
<tr>
<td>2007</td>
<td>86</td>
<td>533</td>
<td>50.0</td>
<td>241</td>
<td>406</td>
<td>46.0</td>
</tr>
<tr>
<td>2008</td>
<td>18</td>
<td>551</td>
<td>49.5</td>
<td>34</td>
<td>440</td>
<td>49.5</td>
</tr>
<tr>
<td>2009</td>
<td>33</td>
<td>584</td>
<td>52.3</td>
<td>48</td>
<td>488</td>
<td>46.9</td>
</tr>
<tr>
<td>2010</td>
<td>30</td>
<td>614</td>
<td>58.6</td>
<td>45</td>
<td>533</td>
<td>47.0</td>
</tr>
<tr>
<td>2011</td>
<td>79</td>
<td>693</td>
<td>69.2</td>
<td>58</td>
<td>591</td>
<td>58.5</td>
</tr>
<tr>
<td>2012</td>
<td>90</td>
<td>783</td>
<td>71.7</td>
<td>101</td>
<td>692</td>
<td>61.9</td>
</tr>
</tbody>
</table>

Note: Deliberate privatization effort was initiated in 2006.
Table 2. AI services in water buffaloes, by type of technician, 2003-2012.

<table>
<thead>
<tr>
<th>Year</th>
<th>TOTAL Services</th>
<th>GOVERNMENT</th>
<th></th>
<th></th>
<th>PRIVATE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>% of Total</td>
<td>Active Tech</td>
<td>AI served/tech</td>
<td>Number</td>
<td>% of Total</td>
<td>Active Tech</td>
</tr>
<tr>
<td>2003-2005</td>
<td>48,966</td>
<td>46,065</td>
<td>94.0</td>
<td>194</td>
<td>2,901</td>
<td>5.92</td>
<td>25</td>
</tr>
<tr>
<td>2006</td>
<td>12,333</td>
<td>9,799</td>
<td>79.4</td>
<td>250</td>
<td>3,573</td>
<td>20.5</td>
<td>56</td>
</tr>
<tr>
<td>2007</td>
<td>14,746</td>
<td>8,725</td>
<td>59.1</td>
<td>267</td>
<td>6,021</td>
<td>4.8</td>
<td>187</td>
</tr>
<tr>
<td>2008</td>
<td>16,737</td>
<td>8,419</td>
<td>50.3</td>
<td>273</td>
<td>8,318</td>
<td>49.6</td>
<td>218</td>
</tr>
<tr>
<td>2009</td>
<td>21,569</td>
<td>11,347</td>
<td>52.6</td>
<td>306</td>
<td>10,222</td>
<td>47.3</td>
<td>229</td>
</tr>
<tr>
<td>2010</td>
<td>24,814</td>
<td>12,413</td>
<td>50.0</td>
<td>360</td>
<td>12,401</td>
<td>49.9</td>
<td>251</td>
</tr>
<tr>
<td>2011</td>
<td>58,908</td>
<td>32,368</td>
<td>54.9</td>
<td>480</td>
<td>26,540</td>
<td>45.0</td>
<td>346</td>
</tr>
<tr>
<td>2012</td>
<td>79,248</td>
<td>37,565</td>
<td>47.4</td>
<td>562</td>
<td>41,683</td>
<td>52.5</td>
<td>429</td>
</tr>
</tbody>
</table>


Table 3. Number of insemination in water buffaloes in the villages/technician/year, by type of AI technician, 2010-2012.

<table>
<thead>
<tr>
<th>No. of AI Services/AI Technician</th>
<th>ATECHNICIAN TYPE</th>
<th></th>
<th></th>
<th>TOTAL AI SERVICES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Government</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>450</td>
<td>981</td>
<td>69.0</td>
<td>601</td>
<td>58.6</td>
</tr>
<tr>
<td>51-100</td>
<td>194</td>
<td>13.6</td>
<td>204</td>
<td>19.9</td>
</tr>
<tr>
<td>101-150</td>
<td>90</td>
<td>6.3</td>
<td>72</td>
<td>7.0</td>
</tr>
<tr>
<td>151-200</td>
<td>65</td>
<td>4.5</td>
<td>41</td>
<td>4.0</td>
</tr>
<tr>
<td>201-250</td>
<td>26</td>
<td>1.8</td>
<td>35</td>
<td>3.4</td>
</tr>
<tr>
<td>251-300</td>
<td>21</td>
<td>1.4</td>
<td>17</td>
<td>1.6</td>
</tr>
<tr>
<td>&gt;300</td>
<td>44</td>
<td>3.0</td>
<td>54</td>
<td>5.2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1,421</td>
<td>100.0</td>
<td>1,024</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Anatolian Water Buffaloes Husbandry in Turkey

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ABSTRACT

Anatolian water buffalo is found mostly in the half northwestern of Türkiye including north part of the middle Anatolia. It is more common along the coast of Black sea. It is also found in Eastern Anatolia. From a taxonomical point of view it is classified as ‘Mediterranean’ type. In the past, buffalo farming has been an important production source for Türkiye (1,117,000 heads assessed in 1971), while currently the population size is of only 110,000 heads. In Turkey most farmers keep 1-2 buffaloes for family consumption and this system is very widespread in villages while farms with around 100 heads are located near to the big cities. In despite of the popular indifference, farming of this species has survived in order to promote productive systems in agreement with sustainable rural development and trend to revalue autochthonous genetic types. There are in situ and ex situ conservation programme and incentive premiums in order to stop the reducing tendency of population. Nation-wide water buffaloes improvement program has also initiated called community based herd improvement program.

Anatolian water buffalo is reared for triple aptitude: meat, milk and draught. Most meat is especially used for making sausage, which is a very popular typical product in Anatolia, prepared with buffalo meat, beef and mutton spiced especially with garlic. Concerning milk production, research is evidencing that controlled farming and feeding conditions may significantly improve performances. Milk is also employed to make a very popular traditional product, known as ‘lüle kaymagi’.

The Anatolian water buffalo has dark brown, dark grey to black coat, with muzzle, hoofs and horns hairs usually black to black grey. Horns are relatively long, resembling the Mediterranean type, and narrow. They have relatively longer body and face smaller girth and longer thicker legs. The ears are wide and hairy inside. The neck is long and not unduly thick. The withers height tends to be the same as height at sacrum. The body is coarse and sometimes angular. White marks sometimes occur on head, on the lower part of legs and at the end of tail. The horns of female are longer and thinner than those of the male.

INTRODUCTION

Generally small structured with easy temperament and having generally long horn. Colour of horn and nail of legs are black and coat colour wearied from dark black to light block of Brown. Body coat cowered with long hair. Young animals have black hairs till 1-1.5 years and came reddish black colour often weaning. Usually has beard under chin. Average carcass weight estimates 110 kg. It is estimates 100,000 head of buffalo populations exist in Türkiye. Adult body weight of water Buffalo raised in Turkey is approximately 400 kg. Average lactation yield and length of Buffalo of Türkiye are 1 ton and 250 day respectively. Average age at first insemination is 22-24 month. Average open day period is three month. First heat cycles are observed generally after 3 month of calving. There are raised in coastal area of North Anatolia especially Samsun and North part of central Anatolia relatively close to coastal area such as Tokat, Trakya region (Istanbul) has the biggest number of buffalo population of Türkiye. Additionally east part of Türkiye such as Muş, Kars, Sivas has also water buffalo populations. Afyon in western Anatolia and Diyarbakır in South Eastern Anatolia has also buffalo population. Average rate of live weight gaining is 400 gram in young period up to 1 years of age. Buffaloes are raised as the form of family operation 3-5 head of farm size. They are used in forest area also for their pulling power. When the buffaloes used as draft animals are castrated in 12 years of age. They can be used up to 12 years of age as for pulling

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power. Most important reason for raising buffaloes are their milk meat and especially cream or (fat) characteristics which is favourable for consumer buffalo for meat is favourable for garlic flavoured Sausage. Buffalo cream is favourable as for additives to the famous Turkish deserts. They are also raised as meat production with average size of 50 head of herd in the region close to big cities. Average withers height of adults of male is 140 cm. Average fat content and total solid content of milk in first lactation is 8% and 16% respectively.

According to FAO (2000) data, there are about 166 million domesticated buffaloes raised in the five world continents. However, there are about 158 million buffaloes left in the world (FAO statistics, 2003). Roughly 97 percent of them or 153 million heads are water buffaloes essentially found in the Asian region.

Also, in Turkey the buffaloes population have declined dramatically over the last decades. The total population according to FAO statistics is 164,000 heads (2003 http://faostat.fao.org/). Formerly buffaloes have been used as draft animals for centuries. Also they have been used for source of meat and milk products. Than buffalo number was decreased, because of increasing demand for cattle breeding and increasing technology in agriculture.

In despite of the popular indifference, farming of this species has survived in order to promote productive systems in agreement with sustainable rural development and trend to revalue autochthonous genetic types. In this context, beginning from 1963 some provincial, regional and national research institutes have established programmes aimed to revalue and improve reproductive and productive potentiality of Anatolian water buffalo. In 1989 improvement plans has been established by crossing Anatolian buffalo with “Murrah” buffalo imported from Bulgaria.

Concerning milk production, research is evidencing that controlled farming and feeding conditions may significantly improve performances. Indeed, in comparison with buffaloes reared in village conditions, buffaloes under experimental conditions show more favourable values for mean lactation period (245 d vs 250 d), maximum and minimum milk yield (1.715 vs 1.603 litres and 442 vs 186 litres, respectively) and mean fat milk percentage (8% vs 7%). Milk is also employed to make a very popular traditional product, known as ‘lüle kaymağı’ whose preparation and chemical composition were described by Uraz (1970).

Buffalo for draught purpose is limited to tree stump hauling in forest area when mechanical equipments may not be used. In Türkiye most farmers keep 1-2 buffaloes for family consumption and this system is very widespread in villages while farms with around 100 heads are located near to the big cities.

Concerning housing system, most farmers own 1-2 buffaloes as source of animal protein for family consumption and this system, very widespread in villages, is based on pasture resource exploitation; on the contrary, big herds (around 100 heads) are located near to the big cities and buffaloes in this case receive concentrate supplemented feed (maize, wheat, barley, cottonseed and sugar beet by-products) when available. Although artificial insemination forms cornerstone of buffalo improvement program yet at present it represent only very rare occasion mostly research purposes.

Artificial insemination is not applied in villages while is widely used in experimental farms, such as Afyon and Bandırma district of Turkey previously and at Department of animal science of Mustafa Kemal University currently by (Sekerden, 2007). The programme is currently in progress at Zootechnics Institute of Bandırma district in the Turkish province of Balikesir employing artificial insemination and breeding programmes. The larger farms with 40-50 females maintain their own males and the villages usually share a common male.

Natural mating keeping sires within the herd continuously are practiced. Live weight is about 450-500 and 700-800 kg in the adult female and male respectively. The water buffalo population and the amount of production from water buffalos in Turkey are also decreasing. There is only one water buffalo breed called Anatolian water buffalo in Türkiye. The water buffalo in-situ conservation program was conducted in Balikesir province of western Türkiye. The number of water buffalo population were decreased 1,178,000 (1970) to 847,268 (2010) and increased 97,632 in 2011 again.
The latest attempt regarding Conservation of animal genetic resources was establishing water buffalo breeding organization of Turkiye started in 2008 and central water buffaloes breeder association were established in 2011. Nationwide water buffaloes improvement program were initiated under the name of community based improvement program. So far 14 provinces and 2,873 farm total of 16,082 adult individual water buffaloes were included in the nationwide improvement program.

Recently this kind support put on implementation that water buffalo breeding stock breeder who is member of breeder union and join the nationwide herd improvement program can receive 250 Euro per head as incentive premium. The aim of this project is to create superior breeding stock. Other water buffaloes breeders rather than included in improvement program also receive support as premium but lower (150 Euro) than included improvement program. Due to opening door for subsidizing the support to the farmer; this activity will help very much stopping the tendency for decreasing the number of water buffaloes in Turkiye.

The water buffalos in Turkey are named as Anatolian water buffalo and they are among Mediterranean water buffalos which are subgroup of river buffalos (Soysal et al., 2005). They are mostly bred in Samsun and Sinop in North Anatolia sea shore; in Çorum and Amasya in Middle Anatolia; in Afyon and Balikesir in Inner West Anatolia; in Sivas and Muş in East Anatolia and in Diyarbakır in Southeast Anatolia (Atasever and Erdem, 2008). In Turkey water buffaloes are particularly bred for milk production and they are slaughtered for meat production after they finish their productive ages (Şekerden, 2001). The cream produced from Anatolian water buffalo milk is a popular product which is consumed together with many local desserts (Soysal et al., 2005). In some regions, Anatolian water buffalo meat is also used for cheese production.

Anatolian water buffalo meat is consumed as fresh or in meat products like Turkish style fermented sausage, pastrami and salami. In the recent years there has been a rise in the production for only meat. Anatolian water buffalo meat is more commonly used in Turkish sausage as it decreases the fermentation duration and is believed to give taste. In some regions in Turkey, Anatolian water buffalo breeding is a traditional production model which has great importance in the economy and culture of its breeders. In recent years strong emphasize were placed on the management of farm animal genetic researches in general and also water buffaloes husbandry in particular. In general effort related conservation and sustainable utilization of the farm animal genetic resources are coordinated and supported financially and technically by the general directorate of agricultural researches and policy of ministry of food agriculture and livestock.

According to the Yılmaz et al. (2011) certain carcass and meat quality characteristics of Anatolian water buffalos were given on following tables.

There is only one water buffalo breed called Anatolian water buffalo in Turkiye. The water buffalo in- situ conservation program was conducted in Balıkesir province of western Turkiye. There is also ex situ conservation program for Anatolian water buffalo breed in Turkiye at Bandırma Animal research Institute in Turkiye. The latest attempt regarding Conservation of animal genetic resources was establishing water buffalo breeding organization of Turkiye started in 2008. In situ and ex situ conservation and incentive premium support programs are carried out to stop the declaring the population number.

Due to opening door for subsidizing the support to the farmer; this activity will help very much stopping the tendency for declaring the number of water buffalo in Turkiye.

Recently (2009) this kind support put on implementation that water buffalo breeding stock breeder who is member of breeder union can receive 150 Euro per head as incentive premium.

GENETIC CONSTITUTION OF POPULATION

The Anatolian water buffalo are involved in a protection program of gene resources with the declaration of guidelines for subsidizing animal farming by the Ministry of Agriculture and Rural Affairs in Turkey. So it is highly important to characterize the molecular structure of Anatolian buffalo in Turkey. In the last decade, many molecular studies have been conducted using
microsatellites (Gargani et al., 2009; Soysal et al., 2007), mtDNA (TURKHAYGEN I Project, 2012), ISSR markers (Aytekin et al., 2011) and PRNP gene promoter (Oztaban et al., 2009).

In order to reveal the genetic constitution of Anatolian water buffalo several molecular genetic diversity studies has been carried on.

The genetic variation and relationship among six Turkish water buffalo populations typical of different regions was assessed using a set of twenty-six heterologous (bovine) microsatellite markers. Between 7 and 17 different alleles were identified per microsatellite in a total of 254 alleles. The average number of alleles across all loci in all the analyzed populations was found to be 12.57. The expected mean heterozygosity (H_e) per population was between 0.5 and 0.58. The overall polymorphic information (PIC) value was between 0.33 and 0.86. Significant departures from Hardy-Weinberg equilibrium were observed for 44 locus-population combinations. Population differentiation was analyzed by estimation of the $F_{ST}$ index (values ranging from 0.053 to 0.123) among populations. The PCA analysis identified three clusters: the Merzifon and Danamandira populations represented one cluster each, and the Afyon, Coskun, Pazar and Turhal formed a single cluster. The assignment of individuals to their source populations performed using the Bayesian clustering approach implemented in STRUCTURE 2.2 software evidentiate a high differentiation of Merzifon and Danamandira populations as well. The results of this study could be useful for the conservation strategies of the Turkish buffalo (Soysal et al., 2008).

Another molecular genetic study for indigenous water buffalo population to Anatolia were characterised with 11 cattle autosomal microsatellite loci. A set of 4 cattle microsatellite loci was found to be polymorphic in the Anatolian buffalo genome. Genotyping of these polymorphic microsatellite loci revealed alleles ranging from 3 to 9. The observed heterozygosity ranged from 0.550 to 0.775 and the expected heterozygosity ranged from 0.494 to 0.815. The $F_{IS}$ value changed from –0.101 to 0.205. This result shown that, Anatolian water buffalo population samples seemed to be in Hardy-Weinberg expectation (Soysal et al., 2007).

**MORPHOMETRIC CHARACTERISTICS OF WATER BUFFALOE POPULATION OF TURKIYE**

An investigation done by Soysal et al. (2007); 76 males and 127 females of the Istanbul district and 32 males and 70 females raised in Danamandra village of Silivri district were measured. On each buffalo, withers height, rump height, body length, chest depth and chest width were determined. The results showed a significant difference between males and females starting from 12 months in buffaloes of Danamandra village and from 3 years of age in animals of Istanbul district.

There is only one water buffalo breed called Anatolian water buffalo in Turkiye. The water buffalo in- situ conservation program was conducted in Balıkesir province of western Turkey. The number of water buffaloes population were decreased 1,178,000 (1970) to 847,268 (2010) and increased 97,632 in 2011 again. The latest attempt regarding conservation of animal genetic resources was establishing water buffaloes breeding organization of Turkiye started in 2008 and central water buffaloes breeder association were established in 2011. Nationwide water buffaloes improvement program were initiated under the name of community based improvement program. So far 14 provinces and 2,873 farm total of 16,082 adult individual water buffaloes were included in the nationwide improvement program. Recently this kind support put on implementation that water buffalo breeding stock breeder who is member of breeder union and join the nationwide herd improvement program can receive 250 Euro per head as incentive premium. The aim of this project is to create superior breeding stock. Other water buffalo breeders rather than included in improvement program also receive support as premium but lower (150 Euro) than included improvement program. Due to opening door for subsidizing the support to the farmer; this activity will help very much stopping the tendency for decreasing the number of water buffaloes in Turkiye.

Buffalo improvement program for Anatolian water buffalo were designed as simple selection program in the beginning stage. Farmers were selected according to their willingness to take part of in the improvement program. Animals registered and ear tagged then selected on the
basis of conformation and milk yield related data. The young bulls were also selected on the basis of their conformation and milk yield of their dams.

There is only one water buffalo breed called Anatolian water buffalo in Turkiye. The water buffalo in-situ conservation program was conducted in Balikesir province of western Turkiye. The number of water buffalo population were decreased 1,178,000,819,709 to 847,268 (2010) and increased 97,632 in 2011 again. The latest attempt regarding Conservation of animal genetic resources was establishing water buffalo breeding organization of Turkiye started in 2008 and central water buffalo breeder association were established in 2011. Nationwide water buffalo improvement program were initiated under the name of community based improvement program. So far 14 provinces and 2,873 farm total of 16,082 adult individual water buffalo were included in the nationwide improvement program. Recently this kind support put on implementation that water buffalo breeding stock breeder who is member of breeder union and join the nationwide herd improvement program can receive 250 Euro per head as incentive premium. The aim of this project is to create superior breeding stock. Other water buffalo breeders rather than included in improvement program also receive support as premium but lower (150 Euro) than included improvement program. Due to opening door for subsidizing the support to the farmer; this activity will help very much stopping the tendency for decreasing the number of water buffalo in Turkiye.

REFERENCES
Table 1. Means and standard errors (SE) for certain carcass quality characteristics of male and female Anatolian water buffalos.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male</th>
<th>Female</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot carcass weight, kg</td>
<td>325.40 ±2.65</td>
<td>288.20 ±5.99</td>
<td>***</td>
</tr>
<tr>
<td>Carcass length, cm</td>
<td>127.07 ±1.00</td>
<td>132.16 ±1.93</td>
<td>*</td>
</tr>
<tr>
<td>Chest depth, cm</td>
<td>45.37 ±0.43</td>
<td>46.26 ±0.60</td>
<td>NS</td>
</tr>
<tr>
<td>Leg length, cm</td>
<td>72.62 ±1.30</td>
<td>67.87 ±0.84</td>
<td>**</td>
</tr>
<tr>
<td>Leg width, cm</td>
<td>29.96 ±0.65</td>
<td>28.99 ±0.61</td>
<td>NS</td>
</tr>
<tr>
<td>Conformation score</td>
<td>5.20 ±0.42</td>
<td>5.50 ±0.40</td>
<td>NS</td>
</tr>
<tr>
<td>Fatness score</td>
<td>7.70 ±0.47</td>
<td>7.30 ±0.68</td>
<td>NS</td>
</tr>
<tr>
<td>Backfat thickness, cm</td>
<td>19.84 ±1.69</td>
<td>19.77 ±2.06</td>
<td>NS</td>
</tr>
<tr>
<td>Fat colour parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lightness (L*)</td>
<td>64.00 ±1.04</td>
<td>60.03 ±0.96</td>
<td>*</td>
</tr>
<tr>
<td>Redness (a*)</td>
<td>6.14 ±0.49</td>
<td>7.01 ±0.89</td>
<td>NS</td>
</tr>
<tr>
<td>Yellowness (b*)</td>
<td>7.03 ±0.63</td>
<td>7.08 ±0.59</td>
<td>NS</td>
</tr>
<tr>
<td>pH_u</td>
<td>5.49 ±0.01</td>
<td>5.44 ±0.01</td>
<td>***</td>
</tr>
</tbody>
</table>

NS= Not significant (P>0.05). *=P<0.05; **=P<0.01; ***=P<0.001.
Table 2. Least-squares means for meat quality characteristics of Anatolian water buffalos due to gender and aging duration.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Gender (G)</th>
<th>Aging Duration (AD)</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>7-day</td>
<td>21-day</td>
</tr>
<tr>
<td>WHC*, %</td>
<td>9.81</td>
<td>9.98</td>
<td>10.57</td>
<td>9.23</td>
</tr>
<tr>
<td>Cooking loss, %</td>
<td>27.40</td>
<td>27.93</td>
<td>28.84</td>
<td>26.49</td>
</tr>
<tr>
<td>Shear force, kg</td>
<td>3.26</td>
<td>3.18</td>
<td>3.54</td>
<td>2.90</td>
</tr>
<tr>
<td>Colour parameters at 1 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lightness (L*)&lt;sub&gt;1h&lt;/sub&gt;</td>
<td>39.33</td>
<td>36.49</td>
<td>37.35</td>
<td>38.48</td>
</tr>
<tr>
<td>Redness (a*)&lt;sub&gt;1h&lt;/sub&gt;</td>
<td>21.78</td>
<td>22.98</td>
<td>21.29</td>
<td>23.47</td>
</tr>
<tr>
<td>Yellowness (b*)&lt;sub&gt;1h&lt;/sub&gt;</td>
<td>7.48</td>
<td>7.56</td>
<td>7.30</td>
<td>7.74</td>
</tr>
<tr>
<td>Colour parameters at 24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lightness (L*)&lt;sub&gt;24h&lt;/sub&gt;</td>
<td>40.87</td>
<td>38.23</td>
<td>38.82</td>
<td>40.29</td>
</tr>
<tr>
<td>Redness (a*)&lt;sub&gt;24h&lt;/sub&gt;</td>
<td>23.99</td>
<td>25.89</td>
<td>25.30</td>
<td>24.58</td>
</tr>
<tr>
<td>Yellowness (b*)&lt;sub&gt;24h&lt;/sub&gt;</td>
<td>7.84</td>
<td>7.91</td>
<td>8.90</td>
<td>6.85</td>
</tr>
</tbody>
</table>

*WHC=Water holding capacity
NS= Not significant (P>0.05).
*=P<0.05; **=P<0.01; ***=P<0.001.
Table 3. Means and standard errors (SE) for sensory characteristics of male and female Anatolian water buffalos.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male</th>
<th>SE</th>
<th>Female</th>
<th>SE</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour intensity</td>
<td>4.54</td>
<td>0.12</td>
<td>4.53</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Tenderness</td>
<td>4.67</td>
<td>0.12</td>
<td>4.68</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Juiciness</td>
<td>4.29</td>
<td>0.12</td>
<td>4.23</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Flavour intensity</td>
<td>4.87</td>
<td>0.12</td>
<td>4.96</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Flavour quality</td>
<td>4.79</td>
<td>0.12</td>
<td>4.79</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>4.66</td>
<td>0.12</td>
<td>4.69</td>
<td>0.12</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, Not significant (P>0.05).

Table 4. Several characteristics about Anatolian water buffalo raised in Türkiye.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactation Yield (kg)</td>
<td>1070.5±279.9</td>
<td>709.6±23.0</td>
<td>Şekerden et al (2000b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uslu, N.T. (1970b)</td>
</tr>
<tr>
<td>Lactation Length (day)</td>
<td>269.2±70.0</td>
<td>222.0±44.2</td>
<td>Şekerden et al (2000a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Şekerden et al (2000b)</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>8.1±0.205</td>
<td>6.6±0.68</td>
<td>Kök, S., (1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Şekerden et al (2000a)</td>
</tr>
<tr>
<td>Adult Body Weight</td>
<td>518.6±17.2</td>
<td>411.0±9.07</td>
<td>Şekerden et al (2000a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uslu N.T. (1970a)</td>
</tr>
<tr>
<td>Calving Interval</td>
<td>434.3±57.1</td>
<td>365.2±17.5</td>
<td>Şekerden et al (2000a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>İlarslan et al (1983)</td>
</tr>
<tr>
<td>Age at first Insemination (day)</td>
<td>679.7±210.9</td>
<td></td>
<td>Şekerden et al (2000a)</td>
</tr>
<tr>
<td>Age at first calving (day)</td>
<td>1313.2±234.8</td>
<td>964.1±3.94</td>
<td>Şekerden et al (2000b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>İlarslan et al (1983)</td>
</tr>
<tr>
<td>Birth Weight (Male)</td>
<td>34.3±1.20</td>
<td>26.7±0.52</td>
<td>Alaçam et al. (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uslu N.T., (1970b)</td>
</tr>
<tr>
<td>Birth Weight (Female)</td>
<td>31.6±0.90</td>
<td>22.1±0.48</td>
<td>Alaçam et al. (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uslu N.T., (1970b)</td>
</tr>
<tr>
<td>Service Period</td>
<td>112.45</td>
<td>70.8</td>
<td>İlarslan et al (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Şekerden et al (2000b)</td>
</tr>
<tr>
<td>Gestation Length (day)</td>
<td>326.5±5.8</td>
<td>317.0±51.5</td>
<td>İzgi and Asker, (1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>İzgi and Asker, (1989)</td>
</tr>
<tr>
<td>Daily Live Weight Gaining (gr)</td>
<td></td>
<td></td>
<td>Şekerden et al (2000c)</td>
</tr>
<tr>
<td>(0-3 Month) (Male)</td>
<td>0.483</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily Live Weight Gaining (gr)</td>
<td></td>
<td></td>
<td>Şekerden et al (2000c)</td>
</tr>
<tr>
<td>(3-6 Month) (Male)</td>
<td>0.456</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth Weight (Female)</td>
<td>31.6±0.90</td>
<td>22.1±0.48</td>
<td>Alaçam et al. (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uslu N.T., (1970b)</td>
</tr>
<tr>
<td>Service Period</td>
<td>112.45</td>
<td>70.8</td>
<td>İlarslan et al (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Şekerden et al (2000b)</td>
</tr>
</tbody>
</table>
Table 4. Several Characteristics about Anatolian water buffalo raised in Türkiye (Continued).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation Length (day)</td>
<td>326.5±5.8 (artificial insemination)</td>
<td>317.0±51.5 (natural insemination)</td>
<td>İzgi and Asker, (1989)</td>
</tr>
<tr>
<td>Daily Live Weight Gaining (gr) (0-3 Month)</td>
<td>(Male) 0.483</td>
<td>(Female) 0.456</td>
<td>Şekerden et al. (2000c)</td>
</tr>
<tr>
<td>Daily Live Weight Gaining (gr) (3-6 Month)</td>
<td>(Male) 0.305</td>
<td>(Female) 0.294</td>
<td>Şekerden et al. (2000c)</td>
</tr>
<tr>
<td>Daily Live Weight Gaining (gr) (6-9 Month)</td>
<td>(Female) 0.357</td>
<td>(Male) 0.314</td>
<td>Şekerden et al. (2000c)</td>
</tr>
<tr>
<td>Daily Live Weight Gaining (gr) (9-12 Month)</td>
<td>(Male) 0.504</td>
<td>(Female) 0.360</td>
<td></td>
</tr>
<tr>
<td>Total Solid Matter of Milk</td>
<td>17.7 (3. Lactation)</td>
<td>15.3 (1. Lactation)</td>
<td>Şekerden et al. (2000b)</td>
</tr>
<tr>
<td>Ash % of Milk</td>
<td>0.830</td>
<td>0.743</td>
<td>Şekerden et al. (2000a) Soysal and Kök (1997)</td>
</tr>
<tr>
<td>Water of Milk</td>
<td>82.3</td>
<td></td>
<td>Kök (1996)</td>
</tr>
<tr>
<td>Caseine % of Milk</td>
<td>3.4 (3. Lactation)</td>
<td>3.0 (1. Lactation)</td>
<td>Şekerden et al. (2000b)</td>
</tr>
</tbody>
</table>
Table 5. Mean, standard deviation and comparison between males and females*, distinctly for class of age, of somatic traits measured on buffaloes raised in Danamandra village, of Silivri district of Istanbul province of Turkiye.

<table>
<thead>
<tr>
<th>Class of Age</th>
<th>N</th>
<th>X ± S_{X}</th>
<th>N</th>
<th>X ± S_{X}</th>
<th>N</th>
<th>X ± S_{X}</th>
<th>N</th>
<th>X ± S_{X}</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 Year</td>
<td>28</td>
<td>87.46±12.01</td>
<td>3</td>
<td>131.33±5.02</td>
<td>11</td>
<td>126.54±9.59</td>
<td>1</td>
<td>141.30</td>
</tr>
<tr>
<td>1 ≥ Years &lt;2</td>
<td>22</td>
<td>90.95±10.74</td>
<td>5</td>
<td>105.00±13.64</td>
<td>0</td>
<td>-</td>
<td>32</td>
<td>134.15±5.32</td>
</tr>
<tr>
<td>2 ≥ Years &lt;4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 4 Years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Withers height
Male
Female

2. Rump height
Male
Female

3. Tail base height
Male
Female

4. Body length
Male
Female

5. Chest width
Male
Female

*Different letter means significant difference for P<0.05.
Table 5. Mean, standard deviation and comparison between males and females*, distinctly for class of age, of somatic traits measured on buffaloes raised in Danamandra village, of Silivri district of İstanbul province of Türkiye (continued).

<table>
<thead>
<tr>
<th></th>
<th>≤1 Year</th>
<th>1 &gt; Years ≤2</th>
<th>2 &gt; Years ≤3</th>
<th>3 &gt; Years ≤4</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>X ± S_x</td>
<td>N</td>
<td>X ± S_x</td>
<td>N</td>
</tr>
<tr>
<td>1. Withers height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>110.75±9.93</td>
<td>18</td>
<td>122.1±8.29</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>99.00±11.15</td>
<td>10</td>
<td>122.7±8.09</td>
<td>12</td>
</tr>
<tr>
<td>2. Rump height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>112.00±10.29</td>
<td>18</td>
<td>122.78±8.40</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>103.60±11.69</td>
<td>10</td>
<td>125.5±7.60</td>
<td>12</td>
</tr>
<tr>
<td>3. Body length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>118.25a±6.85</td>
<td>18</td>
<td>123.17±8.11</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>102.2b±10.47</td>
<td>10</td>
<td>121.9±8.07</td>
<td>12</td>
</tr>
<tr>
<td>4. Chest depth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>29.50a±3.00</td>
<td>18</td>
<td>59.67±5.19</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>45.20a±8.70</td>
<td>10</td>
<td>61.70±7.10</td>
<td>12</td>
</tr>
</tbody>
</table>

*Different letter means significant difference for P<0.05 (small letter) or P<0.01 (capital letter).
Table 6. Several body traits of Anatolian water buffalo of Türkiye; (Soysal and Kök, 1997).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Adult</th>
<th>4 Year</th>
<th>3 Year</th>
<th>2 Year</th>
<th>1 Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Withers Height</td>
<td>138.23</td>
<td>133.14</td>
<td>132.83</td>
<td>131.73</td>
<td>129.38</td>
</tr>
<tr>
<td>Rump Height</td>
<td>135.71</td>
<td>132.57</td>
<td>133.0</td>
<td>125.88</td>
<td>129.85</td>
</tr>
<tr>
<td>Shoulder Height</td>
<td>130.77</td>
<td>128.43</td>
<td>127.83</td>
<td>122.63</td>
<td>123.85</td>
</tr>
<tr>
<td>Tai Head Height</td>
<td>126.23</td>
<td>123.26</td>
<td>123.83</td>
<td>117.38</td>
<td>120.08</td>
</tr>
<tr>
<td>Height of Tuber coxa</td>
<td>122.31</td>
<td>118.46</td>
<td>118.5</td>
<td>112.5</td>
<td>116.23</td>
</tr>
<tr>
<td>Body Length</td>
<td>145.09</td>
<td>142.43</td>
<td>145.67</td>
<td>132.0</td>
<td>137.85</td>
</tr>
<tr>
<td>Chest Depth</td>
<td>77.20</td>
<td>71.10</td>
<td>74.67</td>
<td>67.38</td>
<td>69.46</td>
</tr>
<tr>
<td>Chest Girth</td>
<td>56.86</td>
<td>41.56</td>
<td>45.33</td>
<td>36.38</td>
<td>39.23</td>
</tr>
<tr>
<td>Width Between tiber coxa</td>
<td>31.11</td>
<td>27.78</td>
<td>24.17</td>
<td>29.375</td>
<td>25.85</td>
</tr>
<tr>
<td>Width Between Hipbone</td>
<td>60.94</td>
<td>56.52</td>
<td>52.0</td>
<td>52.63</td>
<td>51.38</td>
</tr>
<tr>
<td>Head Length</td>
<td>48.03</td>
<td>50.83</td>
<td>51.5</td>
<td>49.50</td>
<td>49.33</td>
</tr>
<tr>
<td>Width of Head</td>
<td>19.57</td>
<td>20.30</td>
<td>22.5</td>
<td>20.0</td>
<td>20.69</td>
</tr>
<tr>
<td>Front Shin Bone Circumference</td>
<td>23.80</td>
<td>22.814</td>
<td>24.58</td>
<td>22.25</td>
<td>19.92</td>
</tr>
<tr>
<td>Chest Circumferences</td>
<td>222.77</td>
<td>196.59</td>
<td>215.17</td>
<td>179.0</td>
<td>178.31</td>
</tr>
</tbody>
</table>

Table 7. Preliminary data on milk constituent of water buffalo population of İstanbul included in herd improvement program.

<table>
<thead>
<tr>
<th>variable</th>
<th>N</th>
<th>Mean</th>
<th>Standart error</th>
<th>Standart deviation</th>
<th>Coefficient of variation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>30</td>
<td>7.593</td>
<td>0.487</td>
<td>2.670</td>
<td>35.16</td>
<td>1.410</td>
<td>15.350</td>
</tr>
<tr>
<td>Protein</td>
<td>30</td>
<td>3.437</td>
<td>0.155</td>
<td>0.851</td>
<td>24.76</td>
<td>1.760</td>
<td>5.600</td>
</tr>
<tr>
<td>Non fat solid matter</td>
<td>30</td>
<td>9.291</td>
<td>0.420</td>
<td>2.300</td>
<td>24.75</td>
<td>4.750</td>
<td>15.150</td>
</tr>
<tr>
<td>Dansity</td>
<td>30</td>
<td>1.0187</td>
<td>0.00115</td>
<td>0.00629</td>
<td>0.62</td>
<td>1.0100</td>
<td>1.0300</td>
</tr>
<tr>
<td>Lactoze</td>
<td>30</td>
<td>5.203</td>
<td>0.235</td>
<td>1.288</td>
<td>24.75</td>
<td>2.660</td>
<td>8.480</td>
</tr>
<tr>
<td>Freezing degree</td>
<td>30</td>
<td>-0.6010</td>
<td>0.0277</td>
<td>0.1517</td>
<td>-25.25</td>
<td>-0.9900</td>
<td>-0.3000</td>
</tr>
</tbody>
</table>
Table 8. Preliminary data on length of lactation (days milked) of water buffalo population of İstanbul included in herd improvement program.

<table>
<thead>
<tr>
<th>N</th>
<th>Mean</th>
<th>Standart error</th>
<th>Standart deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>175</td>
<td>198.81</td>
<td>1.68</td>
<td>22.7</td>
<td>141.00</td>
<td>260.00</td>
</tr>
</tbody>
</table>

Table 9. Preliminary data yield of lactation of water buffalo population of İstanbul included in herd improvement program.

<table>
<thead>
<tr>
<th>N</th>
<th>Mean</th>
<th>Standart error</th>
<th>Standart deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>175</td>
<td>1072.6</td>
<td>26.5</td>
<td>350.2</td>
<td>339.00</td>
<td>2051</td>
</tr>
<tr>
<td>Group</td>
<td>Total</td>
<td>12.18 ± 2.7</td>
<td>120.8 ± 2.3</td>
<td>119.4 ± 2.4</td>
<td>112.7 ± 2.3</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>90.3 ± 5.3</td>
<td>94.4 ± 4.0</td>
<td>84.4 ± 4.3</td>
<td>82.4 ± 3.1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>109.7 ± 3.0</td>
<td>109.2 ± 4.3</td>
<td>106.5 ± 4.1</td>
<td>101.5 ± 3.9</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>129.8 ± 2.7</td>
<td>128.4 ± 2.7</td>
<td>118.2 ± 1.7</td>
<td>109.4 ± 2.3</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>131.3 ± 2.3</td>
<td>130.3 ± 2.9</td>
<td>128.7 ± 2.7</td>
<td>129.6 ± 1.2</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>79.1 ± 2.4</td>
<td>208.8 ± 2.3</td>
<td>194.4 ± 2.4</td>
<td>127.2 ± 2.3</td>
</tr>
</tbody>
</table>

Table 10. Distribution female water buffalo body measurement according to the age and provinces (mean and standard error).
N

3

13
4
6
2
25
15
4
18
4
41
28
4
11
4
47
13
12
13
38
31
17
18
66

Age
Group

3

1
2
3
4
Total
1
2
3
4
Total
1
2
3
4
Genel
1
2
3
Genel
1
3
4
Total

94.3 ± 2.0
107 ± 5.4
122.5 ± 4.5
130.7 ± 2.5
105.7 ± 3.1
94.4 ± 4.6
127.0 ± 3.4
133.5 ± 2.1
143.0 ± 3.2
120.5 ± 3.4
85.7 ± 1.5
102.5 ± 5.7
114.8 ± 1.4
136.0 ± 5.8
98.2 ± 2.7
83.6 ± 1.9
113.7 ± 3.2
127.0 ± 2.8
108 ± 3.3
91.1 ± 1.4
124.5 ± 2.7
133.7 ± 1.1
111.8 ± 2.5

125 ± 5.0

CY (cm)
X±Se

94.6 ± 2.1
106.7 ± 5.7
123 ± 3.9
129 ± 3.9
106 ± 3.1
94.5 ± 4.7
128.0 ± 3.5
131.5 ± 2.4
140.0 ± 2.9
119.9 ± 3.3
86.8 ± 1.5
102.5 ± 6.6
113.9 ± 1.0
130.5 ± 5.7
98.2 ± 2.4
85.6 ± 1.7
113.6 ± 3.1
124.0 ± 3.2
107.6 ± 3.1
93.8 ± 1.3
122.7 ± 2.8
132.4 ± 1.0
111.8 ± 2.3

124 ± 5.5

SAGY(cm)
X±Se

92.4 ± 2.0
105 ± 5.3
119.3 ± 4.8
126.3 ± 2.5
103.6 ± 3.0
92.5 ± 4.5
125.5 ± 3.1
128.7 ± 2.3
137.5 ± 3.0
116 ± 3.3
84.0 ± 1.5
99.2 ± 1.0
111 ± 1.3
126.7 ± 4.1
95.2 ± 2.4
83.1 ± 1.8
111.3 ± 3.2
121.9 ± 3.0
105.3 ± 3.1
91.5 ± 1.4
122.4 ± 2.7
131.0 ± 1.0
110.3 ± 2.4

119 ± 3.3

SY(cm)
X±Se

90 ± 2.2
103.5 ± 5.3
115.8 ± 4.4
120 ± 2.0
100 ± 2.99
90.8 ± 3.9
120.0 ± 3.7
125.2 ± 2.2
133.5 ± 2.7
113.8 ± 3.1
82.5 ± 1.7
98.7 ± 6.1
108.8 ± 1.2
120.7 ± 4.3
93.2 ± 2.3
81.1 ± 1.7
108.0 ± 2.6
117 ± 2.6
101.8 ± 2.8
89.5 ± 1.2
117.5 ± 2.2
124.8 ± 0.7
106.3 ± 2.1

122 ± 3.6

KSY(cm)
X±Se

87.5 ± 1.9
100.2 ± 5.08
110.6 ± 4.2
113.5 ± 0.5
97.2 ± 2.69
86.5 ± 3.5
115.0 ± 3.6
120.2 ± 2.3
129.0 ± 3.8
108.9 ± 2.8
79.0 ± 1.4
95 ± 6.1
103.3 ± 1.1
115.2 ± 4.5
89.1 ± 2.1
77.3 ± 1.7
103.4 ± 2.5
110.6 ± 2.3
96.9 ± 2.6
85.6 ± 1.3
113.2 ± 2.2
120.5 ± 0.7
102.2 ± 2.1

111 ± 1.0

OTYY(cm)
X±Se

84.4 ± 2.9
99 ± 4.3
109.3 ± 4.3
121 ± 2.0
95.6 ± 3.22
84.4 ± 5.3
120.7 ± 1.2
123.2 ± 2.75
133.5 ± 3.9
109.7 ± 3.2
78.1 ± 2.2
93.5 ± 7.7
106.1 ± 3.0
128.7 ± 6.4
90.3 ± 2.9
71.5 ± 1.6
103.5 ± 2.5
115.5 ± 3.4
96.7 ± 3.4
80.9 ± 1.7
111.5 ± 3.1
122 ± 1.2
100.0 ± 2.5

113.66 ± 2.6

VU(cm)
X±Se

42.1 ± 1.3
46 ± 2.1
52.5 ± 0.4
55.5 ± 0.5
46.3 ± 1.2
41.6 ± 2.3
51.0 ± 0.9
55.3 ± 1.0
65.7 ± 3.9
51.4 ± 1.5
37.5 ± 1.1
43.7 ± 4.6
51.7 ± 1.8
64.5 ± 3.6
43.6 ± 1.5
32.5 ± 1.6
50.4 ± 1.1
53.6 ± 1.0
45.3 ± 1.7
41.1 ± 0.6
52.4 ± 1.3
57.3 ± 0.5
48.4 ± 1.0

72 ± 2.5

GD(cm)
X±Se

109.6 ± 3.1
128.5 ± 4.6
152.8 ± 5.4
159.5 ± 0.5
144.6 ± 4.1
106.8 ± 7.0
152.0 ± 4.8
168.0 ± 4.6
192.0 ± 6.5
148.7 ± 5.7
100.7 ± 3.3
141.0 ± 7.7
145.5 ± 5.5
180.0 ± 8.8
120.7 ± 4.7
99.3 ± 3.7
135.1 ± 3.5
154.2 ± 4.4
129.4 ± 4.3
105.6 ± 2.6
162.5 ± 6.2
182.8 ± 4.6
141.3 ± 4.8

179 ± 10.4

GC(cm)
X±Se

10.9 ± 0.4
11.7 ± 1.0
14.5 ± 1.1
20.5 ± 1.5
12.6 ± 0.6
14.1 ± 0.7
18.0 ± 2.4
21.7 ± 1.0
25.7 ± 1.7
19.4 ± 0.8
12.9 ± 0.4
15.0 ± 0.9
16.7 ± 1.0
25.1 ± 3.7
14.8 ± 0.6
12.0 ± 0.6
13.2 ± 0.5
18.5 ± 1.4
14.6 ± 0.7
12.4 ± 0.3
16.1 ± 0.6
18.8 ± 0.5
15.2 ± 0.4

19.66 ± 1.3

OYAG(cm)
X±Se

24.7 ± 1.0
28.7 ± 2.8
36.5 ± 2.4
40.5 ± 1.5
29.4 ± 1.46
26.2 ± 1.9
35.5 ± 2.5
40.8 ± 1.2
48.0 ± 2.9
36.2 ± 1.5
24.0 ± 0.9
34.3 ± 2.3
37.7 ± 2.0
52.7 ± 5.3
30.0 ± 1.55
20.2 ± 0.6
32.0 ± 1.2
40.1 ± 1.6
30. ± 1.5
24.9 ± 0.7
37.4 ± 1.4
42.9 ± 0.5
33.0 ± 1.1

45.8 ± 6.5

KYAG(cm)
X±Se

20.7 ± 0.8
23.2 ± 2.2
30.6 ± 2.1
32.5 ± 0.5
24.4 ± 1.1
26.6 ± 1.4
34.2 ± 0.8
37.4 ± 1.2
42.2 ± 1.8
34.3 ± 1.1
25.4 ± 1.0
28.7 ± 1.6
30.0 ± 1.8
37.0 ± 1.9
27.7 ± 0.9
22.3 ± 0.6
30.4 ± 1.2
35.7 ± 0.8
29.5 ± 1.0
23.0 ± 0.7
30.6 ± 1.2
34.7 ± 0.9
28.2 ± 0.8

36.5 ± 2.0

GG(cm)
X±Se

Not: traits; CY = Withers Height, SAGY = Rump Height, SY = Seoulder (back) heiğht, KSY =Height Of Coxae,OTYY = Height At Tuber İchii, VU = Body Length, GD = Chest
Deepth, GÇ = Chest Girth, OYAG = Width Between Tuber İchii,KYAG = Width Between Tuber Ichii, GG = Chest Width and age group; 1 = 0 – 6 month; 2 = 6 month – 1 year; 3 =
1 year – 2 year; 4 = 2 year – 3 year; 5 = greater than 4 year

Tokat

307

Samsun

Balıkesir

Afyon

Çorum

Sakarya

Provinces

Table 10. Distribution male water buffalo body measurement according to the age and provinces (mean and standart error) (Continued).

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**Table 11.** Preliminary data concerning lactation length (days milked) and yield of the population of water buffalo herd improvement program of Turkiye.

<table>
<thead>
<tr>
<th>Provinces</th>
<th>N</th>
<th>Lactation length (days milked)</th>
<th>N</th>
<th>Lactation yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>İstanbul</td>
<td>175</td>
<td>198.81 ± 1.68</td>
<td>175</td>
<td>1072.6 ± 26.5</td>
</tr>
<tr>
<td>Düzce</td>
<td>39</td>
<td>150.41 ± 12.28</td>
<td>39</td>
<td>435.12 ± 44.13</td>
</tr>
<tr>
<td>Balıkesir</td>
<td>12</td>
<td>144.50 ± 13.12</td>
<td>12</td>
<td>695.87 ± 128.86</td>
</tr>
<tr>
<td>Afyon</td>
<td>274</td>
<td>243.87 ± 2.29</td>
<td>274</td>
<td>1063.38 ± 26.40</td>
</tr>
<tr>
<td>Bitlis</td>
<td>184</td>
<td>286.5</td>
<td>184</td>
<td>841.75</td>
</tr>
<tr>
<td>Tokat</td>
<td>486</td>
<td>147.9 ± 1.63</td>
<td>486</td>
<td>708.5 ± 15</td>
</tr>
<tr>
<td>Samsun</td>
<td>543</td>
<td>219.1 ± 1.65</td>
<td>543</td>
<td>624.8 ± 10.80</td>
</tr>
<tr>
<td>Diyarbakur</td>
<td>684</td>
<td>-</td>
<td>684</td>
<td>751.166 ± 19.53</td>
</tr>
</tbody>
</table>

**Table 12.** Number of water buffalo and name of provinces included in nationwide water buffalo improvement project in Turkiye.

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Number of farmer</th>
<th>Heifer</th>
<th>Bull</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFYON</td>
<td>135</td>
<td>1145</td>
<td>26</td>
<td>1171</td>
</tr>
<tr>
<td>BALKESİR</td>
<td>125</td>
<td>995</td>
<td>20</td>
<td>1016</td>
</tr>
<tr>
<td>BİTLİS</td>
<td>297</td>
<td>1652</td>
<td>77</td>
<td>1729</td>
</tr>
<tr>
<td>DİYARBAKIR</td>
<td>514</td>
<td>1927</td>
<td>87</td>
<td>2014</td>
</tr>
<tr>
<td>DÜZCE</td>
<td>105</td>
<td>1105</td>
<td>22</td>
<td>1127</td>
</tr>
<tr>
<td>İstanbul</td>
<td>45</td>
<td>1002</td>
<td>38</td>
<td>1040</td>
</tr>
<tr>
<td>SAMSUN</td>
<td>88</td>
<td>1974</td>
<td>37</td>
<td>2011</td>
</tr>
<tr>
<td>TOKAT</td>
<td>149</td>
<td>1020</td>
<td>50</td>
<td>1070</td>
</tr>
<tr>
<td>ÇORUM</td>
<td>163</td>
<td>911</td>
<td>36</td>
<td>947</td>
</tr>
<tr>
<td>GİRESUN</td>
<td>521</td>
<td>1095</td>
<td>31</td>
<td>1126</td>
</tr>
<tr>
<td>KAYSERİ</td>
<td>104</td>
<td>956</td>
<td>24</td>
<td>980</td>
</tr>
<tr>
<td>KÜTAHYA</td>
<td>383</td>
<td>911</td>
<td>7</td>
<td>918</td>
</tr>
<tr>
<td>SİVAS</td>
<td>243</td>
<td>879</td>
<td>35</td>
<td>914</td>
</tr>
<tr>
<td>MUŞ</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOPLAM</td>
<td>2873</td>
<td>15572</td>
<td>490</td>
<td>16062</td>
</tr>
</tbody>
</table>

**Table 13.** Preliminary data obtained regarding weight at several ages of water buffalo population of the community based water buffalo improvement program in Turkiye.

<table>
<thead>
<tr>
<th>Provinces</th>
<th>N</th>
<th>Birth weight (kg)</th>
<th>N</th>
<th>Weight at 6 month (kg)</th>
<th>N</th>
<th>Yearling weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>İstanbul</td>
<td>637</td>
<td>34.011 ± 0.270</td>
<td>371</td>
<td>107.87 ± 1.29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DÜZCE</td>
<td>393</td>
<td>32.785 ± 1.133</td>
<td>6</td>
<td>75.200 ± 8.70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BALKESİR</td>
<td>399</td>
<td>33.536 ± 0.336</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AFYON</td>
<td>756</td>
<td>29.160 ± 0.215</td>
<td>377</td>
<td>89.202 ± 0.501</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BİTLİS</td>
<td>1097</td>
<td>29.350 ± 0.150</td>
<td>791</td>
<td>85.100 ± 0.650</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOKAT</td>
<td>639</td>
<td>27.7 ± 0.182</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SAMSUN</td>
<td>504</td>
<td>28.96 ± 0.309</td>
<td>362</td>
<td>100.54 ± 0.893</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DİYARBAKIR</td>
<td>957</td>
<td>28.37 ± 0.22</td>
<td>684</td>
<td>83.27 ± 1.12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Population</td>
<td>n</td>
<td>$H_o$</td>
<td>$H_E$</td>
<td>$n_A$</td>
<td>$F_{IS}$</td>
<td>Microsatellite number</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>----</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>----------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Pazar</td>
<td>32</td>
<td>0.55</td>
<td>0.62</td>
<td>5.76</td>
<td>0.10</td>
<td>26</td>
</tr>
<tr>
<td>Danamandira</td>
<td>18</td>
<td>0.53</td>
<td>0.62</td>
<td>5.14</td>
<td>0.13</td>
<td></td>
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Invited Paper
Buffalo Socio-Economic and Sustainable Production Symposium
Changing Dynamics in Buffalo Production Systems in South Asian Region

Om Prakash DHANDA*

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ABSTRACT

Buffalo population in South-Asian countries is increasing more rapidly than the rest of the world due to their unique qualities and emerging role in economic development. This region possesses most of the well-known breeds of buffaloes which are reared by adopting extensive, semi-intensive and intensive production systems by the farmers. But, Bangladesh does not follow intensive production system as almost all the animals are swamp type and low producers. However, these systems are being replaced from extensive or semi-intensive to more intensive high input systems due to new socio-economic pressures and perceptions. These approaches are expected to provide enhanced food security and economic prosperity to the people of the region.

PREFERENCE FOR BUFFALO

The buffalo population in Indian sub-continent is growing annually at the rate of 1.5 percent. The buffaloes have spread over the traditionally non-buffalo regions as these animals possess certain unique qualities in comparison to cattle. In the present scenario where trend shows a decline in area under forage production and also decline in grazing community lands over the past 4 decades, milk producing animals will have to depend more and more on crop residues. The problem is likely to get further aggravated due to growing human population resulting in higher pressure on land for food crops.

Buffaloes are known to be better converters of poor-quality roughage into milk and meat. They are reported to have 5% higher digestibility of crude fiber than high-yielding cows; and 4-5% higher efficiency of utilization of metabolic energy for milk production (Mudgal, 1988). Buffaloes can gain as much as 1.0-kg daily body weights a day on good-quality roughage and concentrate.

The investigations carried out during the past three decades or more have amply confirmed that the buffaloes digest feed more efficiently than do cattle, particularly when feeds are of poor quality and are high in ligno-cellulose. It has also been found that buffaloes can digest crude protein, fat, calcium, phosphorus and non-protein nitrogen more efficiently than other ruminants. The ability of buffaloes to digest fiber efficiently is partly due to the presence of some typical micro-organisms in the rumen which convert feeds into energy more efficiently than those in cattle. Other reasons for the buffalo's being a better converter of feed might be the higher dry-matter intake, longer retention time of feed in the digestive tract, ruminal characteristics more favourable to ammonia nitrogen utilization, less depression of...
cellulose digestion by soluble carbohydrates, superior ability to handle the stress environment and a wide range of grazing preferences.

Hence, preference for buffaloes has continued to increase due to higher fat content of milk (7-8%), ability to thrive on harsh conditions, genetic potential for disease resistance and low quality rations as well as ever increasing export market for buffalo meat and milk products. It is expected that buffalo will ultimately emerge as the future animal of dairy-cum-meat industry in the region.

BUFFALO POPULATION DYNAMICS IN SOUTH ASIA

South Asian buffaloes dominate the world population (Table 1), representing about 75% of the world buffalo population (FAO, 2010). During the last ten years, world buffalo population has increased at the rate of 1.24 per cent per year, whereas, in South Asian countries the buffalo population increased at the rate of 1.49 per cent per year, largely contributed by India and Pakistan.

GLOBAL OVERVIEW OF BUFFALO MILK AND MEAT PRODUCTION

The world total milk production was 703.35 million tonnes in 2009 comprises cow milk, buffalo milk and other animals, which was a considerable jump from 697,573 million tonnes in 2008. The world cow milk output in 2009 was 587.44 million tonnes, of which USA was on top with 85.86 million tonnes and India was second largest producer with 45.14 million tonnes. On the other hand, regarding the world buffalo milk output, India ranks first with a production of 59.87 million tonnes in 2009, followed by Pakistan, China, Egypt and Iran, respectively. The world total output of buffalo milk in 2009 was 89.96 million tonnes. Out of the world milk output, cow milk represented 84 per cent in 2009 and 13 per cent taken up by buffalo milk. In recent years, cow milk production has been declining in many parts of the world like the European Union, USA, Australia, Japan and China. On the other hand, buffalo milk production has kept on increasing particularly in South Asia region. Of the total buffalo milk output, more than 90 per cent is produced in India and Pakistan and South Asian countries contribute about 93 per cent.

The annual growth rate in world buffalo meat production was 1.66 per cent, whereas, in South Asian countries the increased rate was 2.24 per cent during last ten years: 1998 – 2008 (FAO, 2010). Undoubtedly, majority of buffalo meat is from South Asia region, representing approximately 71 percent and with volume of 2,398,922 tons with the greater bulk contributed by India and Pakistan (Table 2).

GENETIC RESOURCES

Asia is famous for its riverine and swamp types of buffaloes. River buffaloes are generally large in size mostly with colour horned and are mainly available in India and Pakistan. These animals have a preference for water and basically raised the milk production. Swamp buffaloes are mostly found in Bangladesh and are mainly used as draught animals. A few animals are also found in North- Eastern State of India bordering Myanmar. India is a harbinger of some of the best riverine breeds of buffaloes. Murrah, Nili-Ravi, Surti, Mehsana, Jaffarabadi enjoy pre-eminent position among high producing germplasm. Some breeds like Bhadawari, Pandharpuri possess a very high fat (8.5%) and highly economical to regional areas.
of these habitats. Indian buffaloes have been categorized in five groups on the basis of well-defined characteristics and habitats.

Pakistan is also credited to possess high yielding Kundi and Nili-Ravi buffaloes in areas bordering India and contribute 71% of total milk to national pool.

BUFFALO PRODUCTION SYSTEMS

The buffalo production system in South Asian countries are categorised into extensive, semi-intensive and intensive production system.

_Extensive or Zero-input Production System:_ The typical extensive and traditional unique buffalo production system in India adopted by livestock breeders (Maldharis of Banni, Kachchh, Gujarat) and pastoralists predominantly depend on buffalo keeping under extensive production system. This system shows larger diversity in buffalo populations in remote areas, arid and semi-arid regions of the country like Banni area of Kachchh, Gujarat, Orissa and North-East region. The extensive production systems adopted by pastoralists in different parts of the country integrates well with the local feed/fodder resources, plants, soil and climate. The animals develop capacity to survive under shortage of feed/fodders, prevalent diseases, drought and other adverse climatic conditions.

The above examples for unique extensive buffalo production system in India based on indigenous knowledge adopted seasonal and spatial grazing system that are holistic, complementary to natural feed/fodder resources and symbiotic with agro-ecosystems. In Bangladesh, buffaloes rose under extensive production system in costal and hilly area, where, no housing provided and generally natural breeding practices. Animals are kept in a single herd in large number in open throughout the year. Animals maintained on natural pasture, no concentrate/mineral supplements and rice straw is fed.

_Semi-Intensive Buffalo Production System:_ In South Asia, small-scale semi-intensive systems have developed and intensification has led to combining grazing, stall-feeding of planted forage and crop residues. The semi-intensive or mixed farming system adopted in majority tropical highlands, arid, semi-arid, humid and sub-humid zones. The tropical highlands mix farming system in Himalayan region found in India, Nepal and Pakistan. The buffaloes are maintained in small numbers and have secondary importance in income generation, compared to the crops farming. In Uttaranchal Hills, dairy production by small scale farmers is widespread and buffalo kept in sedentary systems, grazing is done during day time, at night they are fed crop residues and tree leaves.

Under this system, buffaloes are allowed for grazing according to the season and supplemented with concentrate, mineral mixture, green and dry fodder as per the productivity and milk yield of animals. The supplements used for the feeding of buffaloes are highly variable and depends on stage of lactation, economic condition and requirement of buffalo keepers. In this system lactating and pregnant buffaloes are provided concentrates, green and dry fodders, whereas, dry, growing stock and male calves are least cared.

In Bangladesh, buffaloes are raised under a semi-intensive system with minimum inputs. They are allowed to graze on natural pasture on fallow land or road side in day time. No concentrate or mineral supplements are usually fed. For milch
buffaloes, the calves are usually separated from the dam in the evening or night and the milking only once in the morning. The staple food for buffaloes is rice straw, which is an inadequate source of energy and protein. Sugarcane leaves, micro silage of sugarcane leaves, cassava leaves, roadside grass, elephant grass, and maize with corn cob and pineapple bran are also used as feeding stuffs.

**Intensive Buffalo Production System:** Milk production system in the South Asian region changing very fast and market-oriented dairy farms are concentrated in out skirt or within urban / metros cities near fluid milk consumption centres. Less proximate production occurs only in those regions where there is an efficient market infrastructure. Therefore, the potential to increase dairy production depends largely on the unit costs of collection and transport. Those in urban peripheries are doubly advantaged, because with better access to markets, the unit costs of the support services such as input supply, animal health services and milk marketing decrease as production increases. In India, this system is predominantly found in and around large cities, such as Delhi, Mumbai, Calcutta, Bangalore, Ahmedabad, Surat. Usually these buffaloes are kept for milk production and are not for breeding purposes. After completing lactation majority dry buffaloes slaughter for meat production. This system in urban and peri-urban areas purchased selected buffaloes of high milk production potentials from the breeding tract and after extracting last drop of milk majority of them disposed in abattoir. For feeding the animals all feed items are purchased from local market due to which non-productive animals are immediately disposed of.

In Pakistan, intensive production system is growing rapidly in cities such as Karachi, Lahore, Rawalpindi and Islamabad. Pregnant buffaloes are purchased from rural areas, after calving, female calves usually sold or and a small number are kept as replacement for breeding while male calves are fattened for meat production. At the end of lactation, females are also slaughtered for meat purposes. Buffaloes under intensive production system are maintained under stall feeding and fed satisfactory diet comprising mixture of chaffed straw or stoves, concentrate mixture of locally available cakes, flours of grains, dal chunnies and bran etc are fed through mixing with chaffed green fodders during feeding time. Buffalo keepers adopted intensive production systems are also using compounded feeds and mineral mixtures for providing relatively more balance diet. However, intensive system for buffalo production is not practiced in Bangladesh.

**NEW INITIATIVES AND CHANGING PERCEPTIONS**

*Genetic Improvement:* Buffalo breeding programme in India was primarily based on culling and selection policy in Military Dairy Farms as these were only large herds available in pre-Independent India. However, All India Coordinated Research Project on Buffalo Breeding was launched in 1970 in order to improve production potential of buffaloes through breeding, feeding and management. This project could not make much headway in view of low intensity of selection of the sires. Therefore, a new initiative known as ‘Network Programme in Buffalo Breeding’ was started in 1993 by expanding the population base thereby increasing the intensity of selection. The females with the farmers were also included for progeny testing of sires. These steps resulted in significant improvement in average
milk production by achieving the production level of about 2100 litres in a lactation of 305 days.

Incentives for Buffalo Breeders: Some of the provincial Government like Haryana have introduced special monetary benefits schemes for Buffalo farmers for conserving and breeding pure elite females. These farmers are paid cash incentives by the Government for maintaining the first and second calves for a period of three years. The male calves from these superior females are purchased back by the State for rearing as future bulls.

Beef Production: The old and spent buffaloes are usually used as meat animals in South Asian countries. However, the recent data shows an annual increment of 2.25% in beef production mainly from India and Pakistan. This has been possible due to introduction of new scientific programmes of beef production in buffalo. For example, Hind Agro-industries Ltd, one of the biggest exporters of meat in the country has started project on male calves in the catchment area of the meat plant where the farmers are advised not to sell or dispose of male calves till they attain the live weight of 200 kg under buy back system. The expenditure incurred on feeding and health care of these calves during this period is borne by the company. The farmers are very happy with this arrangement as they receive supplementary income from the sale of the calves in addition to the money from sale of milk. Since the males are maintained and grown by the company under its own watch and care, the quality of meat from such males fetch high price in international market. So, it is a win-win situation, for all the stakeholders. Likewise, Federal Government is funding a national programme on “Rearing of males for Beef production” under self-employment scheme by the educated unemployment youth, these endeavours have started showing promising results and India has become the leader and occupies first position in export of buffalo Beef.

Specialised farming: The farmers have started realising the importance of dairy farming in raising their farm incomes. The mixed farming system consisting of one to two milk buffalo and some area under crops is not profitable and unable to meet growing demand of the household and a comfortable level of living. Hence, there is a shift in the system where a large number of farmers have started establishing big farms rather than the smaller ones. This change has been brought primarily due to the availability of markets and creation of infrastructure like roads and power. Secondly, the margin of profit in small production system is not as high as in industrial level.

Launch of National Dairy Plan: National Dairy Development Board, Government of India has launched a new initiative in the form of ‘National Dairy Plan’ for providing a further fillip to milk production in the country mainly from Buffaloes. The main emphasis the under this plan will be the use of superior sires through A.I. at farmers door-steps and balanced animal feed. In addition, setting up of more processing facilities and marketing of milk products will also be strengthened.

Biotechnological Interventions: Production of elite buffaloes by applying modern biotechnological tools and approaches like embryo production and transfer and Zona free cloning offers a great potential. Multiple ovulation and embryo transfer has been integrated with breeding strategy under Progeny testing
programme undertaken by the Central Institute of Research on Buffalos at Hisar, India. This is expected to reduce the time in selection of bulls of proven merit significantly thus increasing the availability of more semen doses for use in artificial insemination.

Application of newer techniques of cloning is also proving highly beneficial in enhancing reproductive efficiency of these animals. Recently a female calf named as ‘Mahima’ and male calf named as ‘Swarn’ were born to the recipients through a new technique known as “Hand Guided Cloning Technique” by using donor ear skin cell and seminal plasma cell, respectively, at National Dairy Research Institute, Karnal, India.

In conclusion, it may be mentioned that buffaloes have thrived and served the inhabitants of South Asian countries well and are further expected to play bigger role in increasing the income and food security of farmers due to their unique qualities explained earlier. Need to obtain more milk and meat from this species will be stronger in future as the number and quality of dual breeds of cattle capable of producing good quantity of milk and providing draft power is dwindling fast in view of fast mechanization of farm sector in these countries. All the stake holders, therefore, have to join hands in providing desired technical and financial support so as to turn the buffaloes into a highly efficient producing and profitable animal.

REFERENCES

Table 1. Buffalo population in world and South Asia.

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<th>Year</th>
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<th>South Asia</th>
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<td>1998</td>
<td>160,715,087</td>
<td>117,706,250</td>
<td>73.24</td>
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<td>2004</td>
<td>172,651,049</td>
<td>129,551,154</td>
<td>75.04</td>
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<td>2005</td>
<td>174,526,286</td>
<td>131,256,213</td>
<td>75.21</td>
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<td>2006</td>
<td>176,188,724</td>
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<td>75.16</td>
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<td>2007</td>
<td>177,376,972</td>
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<td>2008</td>
<td>180,702,923</td>
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<td>74.81</td>
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Table 2. Buffalo Milk and Meat Production in the World and South Asia (tons).

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<th>Milk Production (tons)</th>
<th>Meat Production (tons)</th>
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<td>South Asia</td>
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<td>1998</td>
<td>62,220,043</td>
<td>57,253,330</td>
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<td>2004</td>
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<td>70,553,042</td>
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<td>2008</td>
<td>89,277,195</td>
<td>83,182,205</td>
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Invited Papers
Buffalo Meat and Meat Products Symposium
Latest Concepts in Rearing Buffaloes for Meat Production

Surendra Kumar RANJHAN*

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*Corresponding email: sk_ranjhan19@yahoo.in, info@hind.in

ABSTRACT

India is world’s largest exporter of buffalo meat known as carabao in the international market. In 2011-12 alone, its exports stood at 1.28 million tonnes valued at Rs. 14,000 crores or US $ 2.5 billion. The Indian meat industry has seen a tremendous change during the last one decade wherein the modern state-of-art mechanized slaughter houses have changed the complete scenario of traditional meat industry. This change has come due to the stringent sanitary and phytosanitary (SPS) measures adopted by the exporting units as they are required by the importing countries. Now, about 32 meat processing plants are fully integrated right from slaughter to the production of frozen buffalo meat with Rendering and Effluent Treatment Plants to ensure pollution free and eco-friendly atmosphere around these Units. Additionally, six more state-of-art mechanized slaughter houses are coming. The importance of buffalo in India can be gauged by the fact that it is increasing faster than cattle, although in some East and Southeast Asian countries buffalo population has declined rapidly, which is a matter of concern. The primary importance of buffalo is for more milk in South Asia, and secondarily, for meat production. Therefore, its role for food security in India is well established. The price of buffalo meat is much cheaper than beef, chevon, mutton, pork and poultry and is, therefore, the cheapest source of protein to the weaker section of the society. Because of its competitive prices and better blending characteristics, it forms a major ingredient in corn beef, hotdogs and other value added meat products. In India, every year, about 10 million such male calves are removed from the buffalo production system due to intentional killing by the farmers to save dam's milk due to non-remunerative cost of raising male animals, thus incurring a loss of about Rs. 200 crores (US $ 18 million) per annum. These calves could otherwise be salvaged for meat production, which will not only improve the economic condition of the farmers but would also provide quality meat for domestic consumption at competitive prices and also for export market. Raising these male calves will generate additional employment.

INTRODUCTION

Meat Industry in India

The trends in livestock population, slaughter rate (number slaughtered as percentage of population), carcass weight and meat production in India in 2008 are shown in Table 1 (FAO, 2011). Meat production is estimated at 9.2 million tonnes, standing fourth in rank in world's meat production (DGFT – Table 2). It includes about 4.8 million tons of poultry meat and 4.49 million tons of red meat production. Buffalo in India contributes about 17% of total meat production. The contribution by

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cattle, sheep, goats, pigs and poultry is 16%, 3%, 6%, 6% and 51%, respectively. (Table 2)

Export of Meat

India's international trade in livestock and livestock products is mainly in live animals (17%), meat and meat products (82%), dairy products and eggs (1%). At the global level, India's export and import accounts for only 0.17% of each. Meat and meat products have dominated the exports from livestock.

The major export of meat is buffaloes, which is shown in Table 2. It may be seen that export of buffalo meat has increased significantly in the last five years with little decline in 2007-2008 due to worldwide recession. The export of buffalo meat in 2001-2002 was only 240,989 MT, which had increased to 972,863 MT (equivalent to 1.25 MT dressed carcass), valued at at Rs. 14,000 crores (US $ 2.5 billions) in 2011-2012. In the next one decade there is going to be another change in the Indian meat Industry catering to the international market. This change would be backward integration at the Primary Production level with individual identification and traceability. Globally, livestock markets are becoming redundant as contract farming models are becoming more popular. Contract farming allows the processor to get animal of his choice while assuring farmers of their veterinary support and better remuneration. In fact, until the livestock market concept is changed in India, especially for male buffaloes, the issue of identification and traceability will continue to dog the Indian meat sector which will get further complicated due to the forthcoming Codex Alimentarius Guidelines (WHO/FAO Joint Committee) for safe meat. The backward integration coupled with individual identification and traceability has to be introduced.

MATERIALS AND METHODS

Feedlot-Intensive Feeding of Male Buffalo Calves for Meat Production

In a Village Demonstration farm of Hind Agro Industries Limited (HAIL), in Aligarh in Western Uttar Pradesh in India, a commercial feedlot to house 5,000 male calves has been established. The facilities include environmentally controlled animal houses with slatted floors where urine and dung are collected in the Keller and are regularly pumped out for spraying in the forage field. The feedlot has its own feed compounding feed mill and about 100 acres agricultural land attached to the Feedlot to cultivate green fodder. The male calves at the age of 8-10 months are procured from the farmers and are quarantined for 15 days during which vaccinations and de-worming are provided. Thereafter, they are brought to the main farm and are fed on high protein / high-energy diet to put on an additional weight of 90-120 Kg in 3-4 months to produce quality meat. The composition of feed is as follows:

**Composition of the feed (pelleted)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
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<tr>
<td>Straw</td>
<td>20%</td>
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<tr>
<td>Maize</td>
<td>30%</td>
</tr>
<tr>
<td>Rape Seed cake / Mustard Cake</td>
<td>30%</td>
</tr>
<tr>
<td>Bran</td>
<td>5%</td>
</tr>
<tr>
<td>Urea</td>
<td>2%</td>
</tr>
<tr>
<td>Molasses</td>
<td>10%</td>
</tr>
<tr>
<td>Mineral Mix</td>
<td>1%</td>
</tr>
<tr>
<td>Salt</td>
<td>2%</td>
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</table>
The above mixture is pelleted and has been fed in the form either as complete feed and / or roughage fed separately and concentrate fed separately. The above mixture contains about 18% crude protein and 62% TDN. Each animal is fed at the rate of 2.5% of the body weight of pellets; 3 kg of spent brewer's grains, and 2 kg whole sugar cane / sorghum per day. This above ration provides 20% crude protein and 65% TDN with a dry matter intake of 4.0 to 5.0 kg per animal per day of 150 – 200 kg. The trials were conducted for two years (2010-2011 and 2011-2012) on 553 animals where individual records for daily feed intake and weekly body weights were taken to know the growth rate and feed efficiency.

**RESULTS**

The Murrah calves grew at the rate of 550 to 700 gm per head per day with feed conversion ratio of 5: 1. The cost of 1 kg live weight gain comes to around Rs. 69 to Rs. 100 (US$ 1.3–1.8) per kg live weight gain at a base cost price of year 2012.

At the commercial farm at Integrated Livestock Village Farm (ILVF) of Hind Agro Industries Limited in Aligarh, data for five trials is given in Table 6 which shows the cost of production for 1 kg live weight. In the five trials during 2011-2012 the cost of production varied from Rs. 69 – Rs. 100 per kg live weight gain. Taking 65% dressing percentage the cost of meat per kg comes to Rs. 106 to Rs. 153 per kg of dressed carcass which is much higher than the present price of buffalo carcass sold in the market (Rs. 110–120 per kg). However, the quality of meat is much better.

**DISCUSSIONS**

The Murrah and Murrah graded calves grew at the rate of 55–700 gm on a daily feed intake of 2.5 kg of concentrate mixture, 4 kg of green fodder and 1 kg of straw. The cost of rearing is on an average US $ 1.5 kg. With the present cost of buffalo meat ranging from US $ 2.5 per kg to US $ 3.8 per kg, it will be economical to produce animals for more sophisticated market where average price of deboned meat is about US $ 3.5 to 3.8 per kg.

**Implications**

In order to sustain the present meat exports from India “farm to fork” concept has to be followed which will involve individual identification and traceability of the animals at the primary production level. The Agricultural and Processed Food Products Export Development Authority (APEDA) of the Ministry of Commerce, Government of India, an apex meat export promoting authority, is likely to make it mandatory for all the meat exporting units to have corporate commercial farm to raise animals for meat. The present technology will be helpful in raising the animals.

**REFERENCES**


**PROCESS FLOW CHART**
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<th>Livestock Species</th>
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<th>Animals Slaughtered (Million)</th>
<th>Percent Slaughtered (%)</th>
<th>Carcass weight (kg)</th>
<th>Meat production (million Tonnes)</th>
<th>Share in total meat production (%)</th>
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<tr>
<td>Cattle</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>9.29</strong></td>
<td><strong>604.0</strong></td>
<td><strong>57.57</strong></td>
<td><strong>0.8</strong></td>
<td><strong>4.80 (</strong>)**</td>
<td><strong>51.0</strong></td>
</tr>
</tbody>
</table>

Table 2. Buffalo meat exports from India.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Meat Produced (Tonnes) *</th>
<th>Total Buffalo Meat Produced (Tonnes)</th>
<th>Total deboned Buffalo Meat Exports* (Tonnes)</th>
<th>Percentage* Export of Buffalo Meat Produced (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001-02</td>
<td>4,425,000</td>
<td>1,421,000</td>
<td>240,989</td>
<td>17.00</td>
</tr>
<tr>
<td>2002-03</td>
<td>5,622,000</td>
<td>1,428,000</td>
<td>295,456</td>
<td>21.00</td>
</tr>
<tr>
<td>2003-04</td>
<td>5,898,000</td>
<td>1,443,000</td>
<td>338,940</td>
<td>23.40</td>
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<tr>
<td>2004-05</td>
<td>5,922,000</td>
<td>1,471,000</td>
<td>302,280</td>
<td>20.00</td>
</tr>
<tr>
<td>2005-06</td>
<td>6,212,000</td>
<td>1,582,000</td>
<td>459,937</td>
<td>29.00</td>
</tr>
<tr>
<td>2006-07</td>
<td>6,251,250</td>
<td>1,621,210</td>
<td>494,111</td>
<td>30.00</td>
</tr>
<tr>
<td>2007-08</td>
<td>6,302,680</td>
<td>1,632,170</td>
<td>482,925</td>
<td>29.00</td>
</tr>
<tr>
<td>2008-09</td>
<td>6,322,450</td>
<td>1,633,500</td>
<td>483,737</td>
<td>29.1</td>
</tr>
<tr>
<td>2009-10</td>
<td>6,524,158</td>
<td>1,645,290</td>
<td>513,668</td>
<td>31.2</td>
</tr>
<tr>
<td>2010-11</td>
<td>8,285,500</td>
<td>1,825,450</td>
<td>724,273</td>
<td>38.8</td>
</tr>
<tr>
<td>2011-12</td>
<td>9,290,000</td>
<td>1,835,920</td>
<td>972,863</td>
<td>68.0</td>
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</tbody>
</table>

Table 3. Buffalo meat exports from India and top importing countries quantities and values.

<table>
<thead>
<tr>
<th>Country</th>
<th>Quantity (Tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2007-08</td>
</tr>
<tr>
<td>Vietnam</td>
<td>50,135</td>
</tr>
<tr>
<td>Malaysia</td>
<td>53,096</td>
</tr>
<tr>
<td>Egypt</td>
<td>28,730</td>
</tr>
<tr>
<td>Jordan</td>
<td>19,513</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>32,518</td>
</tr>
<tr>
<td>Philippines</td>
<td>55,625</td>
</tr>
<tr>
<td>Algeria</td>
<td>0</td>
</tr>
<tr>
<td>U.A.E.</td>
<td>26,212</td>
</tr>
<tr>
<td>Iran</td>
<td>10,075</td>
</tr>
<tr>
<td>Thailand</td>
<td>114</td>
</tr>
<tr>
<td>Iraq</td>
<td>6,564</td>
</tr>
<tr>
<td>Kuwait</td>
<td>37,477</td>
</tr>
<tr>
<td>Angola</td>
<td>43,348</td>
</tr>
<tr>
<td>Syria</td>
<td>114</td>
</tr>
<tr>
<td>Georgia</td>
<td>10,041</td>
</tr>
<tr>
<td>Oman</td>
<td>12,216</td>
</tr>
<tr>
<td>Congo</td>
<td>10,896</td>
</tr>
<tr>
<td>Gabon</td>
<td>7,997</td>
</tr>
<tr>
<td>Lebanon</td>
<td>5,441</td>
</tr>
<tr>
<td>Qatar</td>
<td>3,214</td>
</tr>
<tr>
<td>Myanmar</td>
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<tr>
<td>Azerbaijan</td>
<td>3,469</td>
</tr>
<tr>
<td>Armenia</td>
<td>5,926</td>
</tr>
<tr>
<td>Senegal</td>
<td>6,821</td>
</tr>
<tr>
<td>Ghana</td>
<td>9,603</td>
</tr>
<tr>
<td>Pakistan</td>
<td>9,948</td>
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<tr>
<td>Equatorial Guinea</td>
<td>1,517</td>
</tr>
<tr>
<td>Mauritius</td>
<td>3,594</td>
</tr>
<tr>
<td>Bahrain</td>
<td>2,317</td>
</tr>
<tr>
<td>Canada</td>
<td>0</td>
</tr>
<tr>
<td>Liberia</td>
<td>449</td>
</tr>
<tr>
<td>Other Countries</td>
<td>25,956</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>482,925</strong></td>
</tr>
</tbody>
</table>

Source: DGFT:
### Table 4. Buffalo meat exports from India and top importing countries quantities and values.

<table>
<thead>
<tr>
<th>Country</th>
<th>2007-08</th>
<th>2008-09</th>
<th>2009-10</th>
<th>2010-11</th>
<th>2011-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vietnam</td>
<td>4,317</td>
<td>12,118</td>
<td>14,192</td>
<td>12,563</td>
<td>40,083</td>
</tr>
<tr>
<td>Malaysia</td>
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<td>4,710</td>
<td>6,285</td>
<td>10,763</td>
<td>13,880</td>
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<tr>
<td>Egypt</td>
<td>2,272</td>
<td>4,496</td>
<td>3,849</td>
<td>10,554</td>
<td>11,432</td>
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<tr>
<td>Jordan</td>
<td>1,480</td>
<td>1,554</td>
<td>1,939</td>
<td>5,146</td>
<td>8,777</td>
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<tr>
<td>Saudi Arabia</td>
<td>2,794</td>
<td>2,686</td>
<td>3,294</td>
<td>6,647</td>
<td>9,466</td>
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<tr>
<td>Philippines</td>
<td>3,569</td>
<td>4,495</td>
<td>4,325</td>
<td>5,284</td>
<td>5,472</td>
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<tr>
<td>Algeria</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2,891</td>
<td>5,775</td>
</tr>
<tr>
<td>U.A.E.</td>
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<td>1,582</td>
<td>2,194</td>
<td>3,820</td>
<td>4,938</td>
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<tr>
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<td>1,112</td>
<td>849</td>
<td>2,278</td>
<td>4,364</td>
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<tr>
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<td>5</td>
<td>3</td>
<td>4</td>
<td>1,332</td>
<td>4,209</td>
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<td>Iraq</td>
<td>462</td>
<td>127</td>
<td>1,285</td>
<td>2,120</td>
<td>2,828</td>
</tr>
<tr>
<td>Kuwait</td>
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<td>3,213</td>
<td>3,543</td>
<td>3,090</td>
<td>2,831</td>
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<tr>
<td>Angola</td>
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<td>2,991</td>
<td>1,683</td>
<td>2,311</td>
<td>2,795</td>
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<tr>
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<td>919</td>
<td>1,967</td>
<td>2,283</td>
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<tr>
<td>Georgia</td>
<td>646</td>
<td>536</td>
<td>674</td>
<td>1,452</td>
<td>2,088</td>
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<td>1,075</td>
<td>1,066</td>
<td>1,296</td>
<td>1,930</td>
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<td>Congo</td>
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<td>1,218</td>
<td>1,307</td>
<td>1,575</td>
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<tr>
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<td>500</td>
<td>529</td>
<td>864</td>
<td>903</td>
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<td>698</td>
<td>671</td>
<td>1,138</td>
<td>1,110</td>
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<td>314</td>
<td>635</td>
<td>746</td>
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<td>474</td>
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<td>576</td>
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<td>190</td>
<td>205</td>
<td>245</td>
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<td>125</td>
<td>188</td>
<td>410</td>
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<td>Mauritius</td>
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<td>427</td>
<td>416</td>
<td>357</td>
<td>539</td>
</tr>
<tr>
<td>Bahrain</td>
<td>186</td>
<td>244</td>
<td>314</td>
<td>346</td>
<td>417</td>
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<tr>
<td>Canada</td>
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<td>0</td>
<td>17</td>
<td>0</td>
<td>268</td>
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<tr>
<td>Liberia</td>
<td>23</td>
<td>36</td>
<td>20</td>
<td>62</td>
<td>127</td>
</tr>
<tr>
<td>Other countries</td>
<td>1,680</td>
<td>4,364</td>
<td>6,418</td>
<td>2,930</td>
<td>2,918</td>
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<tr>
<td><strong>ALL COUNTRIES</strong></td>
<td><strong>35,474</strong></td>
<td><strong>50,859</strong></td>
<td><strong>58,307</strong></td>
<td><strong>86,510</strong></td>
<td><strong>136,178</strong></td>
</tr>
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</table>

Source: DGFT
Table 5. Buffalo meat export price over the years.

<table>
<thead>
<tr>
<th>Year</th>
<th>Rs / Kg</th>
<th>USD $ / kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000-2001</td>
<td>47.74</td>
<td>1.045</td>
</tr>
<tr>
<td>2001-2002</td>
<td>47.03</td>
<td>0.986</td>
</tr>
<tr>
<td>2002-2003</td>
<td>43.82</td>
<td>0.906</td>
</tr>
<tr>
<td>2003-2004</td>
<td>44.70</td>
<td>0.973</td>
</tr>
<tr>
<td>2004-2005</td>
<td>53.63</td>
<td>1.171</td>
</tr>
<tr>
<td>2005-2006</td>
<td>57.17</td>
<td>1.291</td>
</tr>
<tr>
<td>2006-2007</td>
<td>65.00</td>
<td>1.435</td>
</tr>
<tr>
<td>2007-2008</td>
<td>73.46</td>
<td>1.825</td>
</tr>
<tr>
<td>2008-2009</td>
<td>104.90</td>
<td>2.285</td>
</tr>
<tr>
<td>2009-2010</td>
<td>112.18</td>
<td>2.366</td>
</tr>
<tr>
<td>2010-2011</td>
<td>119.53</td>
<td>2.623</td>
</tr>
<tr>
<td>2011-2012</td>
<td>142.50</td>
<td>2.931</td>
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</tbody>
</table>

Source: DGFT 2012

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. of Animals</th>
<th>Initial Weight After 15 days of quarantine (Kg)</th>
<th>Final Weight At the end of 8 weeks (Kg)</th>
<th>Total Weight Gain Of all animals (Kg)</th>
<th>Weight Gain / head/Day Grams</th>
<th>Total Feed Cost Per animal for 8 weeks (Rs.)</th>
<th>Cost / Kg Live weight gain (Rs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>94</td>
<td>20529 &lt;br&gt; 218 ± 10</td>
<td>24567 &lt;br&gt; 261 ± 8</td>
<td>4038 &lt;br&gt; 40 ± 4</td>
<td>538</td>
<td>2967</td>
<td>69.00</td>
</tr>
<tr>
<td>Trial 2</td>
<td>60</td>
<td>12791 &lt;br&gt; 213 ± 18</td>
<td>14763 &lt;br&gt; 246 ± 5</td>
<td>1972 &lt;br&gt; 35 ± 6</td>
<td>598</td>
<td>3496</td>
<td>99.9</td>
</tr>
<tr>
<td>Trial 3</td>
<td>94</td>
<td>19460 &lt;br&gt; 202 ± 6</td>
<td>22952 &lt;br&gt; 244 ± 9</td>
<td>3492 &lt;br&gt; 39 ± 4</td>
<td>663</td>
<td>3060</td>
<td>82.72</td>
</tr>
<tr>
<td>Trial 4</td>
<td>160</td>
<td>30400 &lt;br&gt; 190 ± 7</td>
<td>34337 &lt;br&gt; 214 ± 4</td>
<td>6298 &lt;br&gt; 39.3</td>
<td>703</td>
<td>3570</td>
<td>95.42</td>
</tr>
<tr>
<td>Trial 5</td>
<td>145</td>
<td>25375 &lt;br&gt; 175 ± 8</td>
<td>31586 &lt;br&gt; 217 ± 6</td>
<td>6211 &lt;br&gt; 42.85</td>
<td>765</td>
<td>4230</td>
<td>98.72</td>
</tr>
</tbody>
</table>

Trial Period – 8 weeks of a few sample animals
Buffalo and Buffalo Meat in Thailand

Suthipong URIYAPONGSON*

Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand
*Corresponding email: suthipng@kku.ac.th

ABSTRACT

Buffalos are the important domestic animals in Thailand. There were 3.3 million buffalos in 1996, the highest in this region. Currently, the population has dropped to 1.3 million. Meat from buffalo is darker in color compared to beef because of more pigment and less intramuscular fat compared to beef. Panel test and tenderness measurement showed that water buffalo meat was less acceptable than beef. However, buffalo meat is an excellent source of special nutrients. It contains 18-24% protein and 1-3% fat. It has low cholesterol and Triglyceride. Buffalo meat also contains omega 6 and 3 which are important for human health. It is a good source of protein especially for those who are concerned about their health.

Keywords: meat production, Thailand, water buffalo

INTRODUCTION

Buffalos are the important domestic animal especially for small holders in the Northeast. The numbers of buffaloes have been rapidly decreasing every year. According to the Department of Livestock Development the number of buffalos has decreased 18% per year. Currently, Thailand has 1.3 million buffalos and approximately 40% are females (520,000 heads). The number of calf production each year is 145,500 heads, half of what we used for meat consumption (200,000 heads) per year. It was accounted for 26,279 tons of meat and the per capita consumption was 0.86 kg per head per year.

POPULATION OF BUFFALO IN THAILAND

The number of buffalos in Thailand rapidly decreased from 3.3 million in 1996 to 1.67 and 1.62 million in 2009 and 2010 respectively. Thailand used to have the highest population in Southeast Asia in 1996 but currently the number is one third compared to Myanmar and Vietnam (Table 1).

The population of buffalos in Thailand in 2011 was 1,234,179 heads. It divided in to 362,373 males (29%) and 871,806 females (71%). The females can be divided into 614,403 heads of heifer and 257,403 heads of buffalo cows as shown in Figure 1.

BUFFALO PRODUCTION

Buffalos are raised by small farmers and each farmer had only 2-4 heads for draft, manure, money saving or for family heritage. Few farmers use intensive system for calf production of buffaloes. Farmers have low income and low
education and have no plans to activate the younger generations to get involved in the buffalo production system.

BUFFALO MEAT - A MAJOR SOURCE OF MEAT PRODUCT

Buffalo has been used as a major meat in Thailand. Butchers usually sell both meats (cattle and buffalo) at the same price. Butcher shops make more money from buffalo than from cattle. This was due to price of buffalo being lower than cattle with the same weight. Most consumers don’t know or don’t care about the difference between both meats.

The characteristics of buffalo carcasses are similar to those of cattle. Despite heavier hide and head, the amount of useful meat (dressing percentage) from buffaloes is almost the same as cattle. Mediterranean type buffalo and Zebu cattle steer in Brazil yielded dressing percentages of 55.5% and 56.6% respectively. Swamp buffaloes dressing percentages have been measured in Australia at 53%. Generally buffalo have about 3% lower dressing percentages than cattle. According to the research water buffalo in Thailand had only 47-49% dressing percentages.

Most buffalo meat is derived from old animals slaughtered at the end of their productive life. As a result, much of the buffalo meat sold is of poor quality. But when buffaloes are properly reared and fed, their meat is tender and palatable.

The Asian buffalo meat production accounts to 91-92% of the world. India is the largest buffalo meat producer followed by Pakistan, China, Nepal and Thailand. Water buffaloes are exported for slaughter from India and Pakistan to the Middle East and from Thailand and Australia to Hong Kong. Demand for meat is so great that Thailand's buffalo population has dropped from 7 million to 5.7 million head in the last 20 years, a period in which the human population has more than doubled.

MEAT QUALITY

Buffalo meat and beef are basically similar. The muscle pH (5.4), shrinkage on chilling (2%), moisture (76.6%), protein (19%) and ash (1%) are all more or less the same in buffalo meat and beef. Buffalo fat, however is always white and buffalo meat is darker in color than beef because of more pigmentation or less intramuscular fat compared with beef. Taste panel tests and tenderness measurements showed that the meat of the water buffalo is as acceptable as that of cattle. Buffaloes may retain meat tenderness to a more advanced age than cattle because the connective tissue hardens at a later age, improving buffalo meat quality.

There are several ways to change or improve buffalo meat quality. Feed buffalo with different concentrate and roughage can change the chemical composition of meat. Rakiat (2008) reported that buffalo grazing in Guinea mixed Hamata pasture had better meat composition. It contained less triglyceride and lower ratio of Omega 6:3 which made better eating quality. Sompratana (2008) used concentrate diets at 1.5 and 2% of body weight and Guinee for roughage in 203 kg buffalos for 350 days. The results showed that meat from buffalos fed 2% concentrate had lower collagen, shear value and lower TBAR compared to buffalo fed 1.5% concentrate. Naveena et al. (2004) enzyme from curcumin ginger and papaya at 2.5 and 2% aging buffalo meat. The results showed that meat aged with
5% ginger had appearance tenderness and overall acceptability higher that control group.

CHEMICAL COMPOSITION OF BUFFALO MEAT

Buffalo meat is an excellent source of several nutrients. Sompratana (2008) and Rakiat (2008) reported that buffalo meat had 74-78% water, 18-24% protein, 1-3% fat and 1-2% ash. The water in buffalo meat decreases while protein and fat increase when the buffalo gets older. Buffalo meat has low cholesterol and Triglyceride (only 56.9 mg/100 g of cholesterol and 1.15 gm/100 gm of triglycerides, respectively). Buffalo meat also had Omega 6 and 3 which are important for human health. The Omega 6 and 3 in buffalo meat are 9.50 and 1.49% of total fat (Ziauddine et al., 1994). Chemical composition and the Omega 6 and 3 in buffalo meat are showed in Table 2 and 3.

HEALTHY DIET FROM BUFFALO

Buffalo meat has lower fat compared to chicken, beef and fish (46, 69, and 60%, respectively). Buffalo meat contains less cholesterol (56.9 mg/100g VS 106.6 mg/100 g) and triglyceride (1.38 g/100 g vs 1.77 g/100 g) compared to beef. Moreover, buffalo meat has more essential fatty acids such as omega 6 and omega 3 approximately 6.12 and 2.77% of total fatty acid. The number of Omega 6 is lower than pork and chicken while Omega 3 is higher. Eicopentaenoic acid (EPA) and Decahexaenoic acid (DHA) which are important for brain development is high in buffalo meat compared to beef and pork. Buffalo meat was the good source of CLA (1.83%) which were higher than in beef (1.47%) pork (0.6%) and chicken (0.32%), respectively.

REFERENCES

### Table 1. Water buffalo population in Thailand and some Asian countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>2010</th>
<th>2009</th>
<th>1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thailand</td>
<td>1,666,650</td>
<td>1,670,510</td>
<td>3,303,590</td>
</tr>
<tr>
<td>Myanmar</td>
<td>3,000,000</td>
<td>3,000,000</td>
<td>1,165,620</td>
</tr>
<tr>
<td>Vietnam</td>
<td>2,913,390</td>
<td>2,886,600</td>
<td>2,953,900</td>
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<td>Cambodia</td>
<td>702,074</td>
<td>739,946</td>
<td>743,928</td>
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</table>

Source: FAOSTAT (2012)

### Table 2. Chemical composition of buffalo meat.

<table>
<thead>
<tr>
<th>Compositions</th>
<th>(%)</th>
</tr>
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<tbody>
<tr>
<td>Moisture</td>
<td>74-78</td>
</tr>
<tr>
<td>Protein</td>
<td>18-24</td>
</tr>
<tr>
<td>Fat</td>
<td>1-3</td>
</tr>
<tr>
<td>Ash</td>
<td>1-2</td>
</tr>
</tbody>
</table>

Source: Sompratana (2008) and Rakiat (2008)

### Table 3. Chemical composition of buffalo meat.

<table>
<thead>
<tr>
<th>Compositions</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/gm of meat)</td>
<td>56.9</td>
</tr>
<tr>
<td>Triglyceride (gm/100 gm meat)</td>
<td>1.15</td>
</tr>
<tr>
<td>Omega 6 (% of total fat)</td>
<td>9.50</td>
</tr>
<tr>
<td>Omega 3 (% of total fat)</td>
<td>1.49</td>
</tr>
</tbody>
</table>

Source: Ziauddine et al. (1994)

### Figure 1. Classification of female buffalos in Thailand.

Sources (DLD, 2011)
Invited Papers

Buffalo Milk and Milk Products Symposium
Lactation Curve and Milk Flow

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ABSTRACT

The buffalo Mediterranean Italian breed was very much studied for lactation curve. Data on lactation curves after the first, the second and the third calving are reported. In all the 3 lactations we have similar curves, with the production peak on the 40th day, in the second and third lactations, while in the first lactation it is shown on the 50th day and with a longer duration. Lactation curves were different for the first and later parities with lactations of different duration also in Nili-Ravi buffaloes. Milk production and milk flow profiles are important parameters to be recorded and evaluated. In fact they give various advices on milking management. The milk flow curve is typical for each animal and must be recorded singly. Buffaloes are characterized by longer teat and teat canal, and particularly by stronger muscular resistance of teat wall, than in cow: it is necessary a high vacuum level for opening the teat canal and beginning milk ejection. A recent trial is reported, where the manual stimulation at different times provoked the increasing of released oxytocin and of the intra-mammary pressure and finally the increasing of cisternal teat largeness and the reduction of teat canal length. Buffalo is characterized by smaller cisternal fraction than in cattle. The cisternal milk fraction is immediately available while the alveolar fraction needs an oxytocin stimulation to release. In Mediterranean Italian buffaloes it has been observed a higher cisternal fraction of total milk than in Murrah breed. In buffalo management, the milking room and the milking machine are really a critical point and the characteristics of milking vacuum and milking pulsations are strictly connected with milk flow observations. Different curves obtained in recent trials during the milking with normal milking machine and with Automatic Milking System are reported and discussed. A recent study on Mediterranean Italian buffaloes from 104 different herds showed that 88.86% of milk is ejected in 4.24 min (main milking time), as 8.49% is ejected in 1.98 min (milk let-down) corresponding to the cisternal milk fraction. Only 2.65% is the milk ejected in the stripping phase. These results suggest to apply a premilking udder stimulation to reduce milk let-down time increasing the average milk flow and reducing total milking time. These results suggest to the detachment of milking cluster to reduce the blind phase with the following advantages: a) reducing the total milking time and consequently worker’s time, b) improving the farmer’s income and the milk quality thought the mastitis incidence decreasing.

Keywords: cisternal fraction, lactation, milk flow curve
1. LACTATION CURVE

Milk production during whole lactation is a continuous physiological function which describes the rate of milk secretion with advancement in lactation (Dongre et al., 2011). The lactation curve in dairy species can be seen as the graphical representation of milk yield during the time. There are many factors which affected the lactation curve in dairy species: breed, genetic basis, season and period of calving, age at calving, environmental factor and health status (especially on mammary gland). The typical shape of lactation curve has two characteristic parts: a rapid increase from calving to a peak period in early stage of lactation and a gradual decline from peak of yield to the end of lactation (Leon-Velarde et al., 1995).

The most important phase is the persistency of the lactation: persistency of milk production is the ability of animal to maintain milk production at high level after peak production, or usually refers to the rate of decline in daily yield after the peak of lactation (Togashi and Lin, 2004). Random regression model was applied for the analysis of test day record and to study the genetic persistency of the first lactation milk yield of Indian Murrah buffaloes (Geetha et al., 2006); these Authors found that heritability of test day milk yield varied from 0.33 to 0.58 in different test days. Chaudhry et al. (2000) calculated persistency of lactation in 2390 lactation records of Nili-Ravi buffaloes, with 87% persistent buffaloes; environmental, season of calving, parity and lactation length had a significant effect on the trait. Persistency was 9% heritable with a repeatability of 13%. Correlation with lactation length was positive: buffaloes with greater lactation length were more persistent.

The Mediterranean Italian breed was very much studied for lactation curve. In Figure 1 the lactation curves after the first, the second and the third calving are reported. The average production was 2160 kg in the first lactation, 2348 in the second lactation and 2356 in the third one. It clear that the buffalo potential production is observed in the second lactation (Coletta and Caso, 2008). In all the 3 lactations we have similar curves, with the production peak on the 40th day, in the second and third lactations, while in the first lactation it is shown on the 50th day and with a longer duration.

The effect of age and calving season on lactation curves in Mediterranean Italian buffaloes was demonstrated too by Catillo et al. (2002), as it is shown in Figure 2.

Lactation curves were different for the first and later parities with lactations of different duration also in Nili-Ravi buffaloes (Khan and Chaudry, 2000), who found a lactation length of about 289 days for an average milk yield of 1984 kg as about 59% of the lactations had length shorter than the standard lactation of a 10 months. The same Authors found different lactation curves according the parities (Figure 3, Khan and Chaudhry, 2001). These Authors found 10% of atypical lactations, with the first calves having the highest frequency.

According Penchev et al. (2011), the highest peak yield belongs to the buffaloes with the highest calving age, by 0.719 kg higher as compared with the earliest calves (Figure 4). In the latest age at calving the relative decline from second to third months is twice faster as compared with the earliest class. From month 3 to 4 the drop is even better pronounced (16% vs. 5.6%) while after that point the differences are negligible until the end of lactation.
2. MILK EJECTION AND MILK FLOW CURVE.

Milk production and milk flow profiles are important parameters to be recorded and evaluated. In fact they give various advices on milking management (Thomas et al., 2005). The milk flow curve is typical for each animal and must be recorded singly. The portable milkmeter Lactocorder® (WMB AG – Balgach, website) is the normally used instrument to relieve real time milk flow curves and their parameters, electrical conductivity of milk and it is used too to collect a sample for the qualitative analysis. Milk flow curves have been well studied in the cattle (Weis et al., 2004; Bruckmaier, 2005; Tancin et al., 2006), partially in sheep and goat (Dzidic et al., 2004; Bruckmaier et al., 1994; Boselli et al., 2009) and a relatively recent approach is the introduction of this method in buffalo (Boselli et al., 2004; Thomas et al., 2003; Bava et al., 2007; Di Palo et al., 2007; Borghese et al., 2007 a,b; Rasmussen et al., 2008; Boselli et al., 2005, 2008, 2010, 2011b,c) and in donkey (Boselli et al., 2011a).

The graphic representation of milk flow curve can be showed by different phases:
- the first is the increasing phase (milk letdown time and incline time), represented by the time elapsed between the attachment of the milking clusters and the time until constant milk flow;
- the second is the plateau with a constant milk flow (peak of milk flow is generally in this phase);
- the third is the decreasing phase and represents the time from the plateau phase until the detachment of the milking cluster at stop of milk flow;
- an eventual fourth phase may be the stripping yield, preceded by blind phase.

Graphical representation in milk ejection is visible through flow curves. They are typical and characteristic for each dairy species: cattle, buffalo, goat and donkey (see Figure 5, 6, 7, 8). Specifically, milk flow curves and other parameters are influenced by anatomical (Thomas et al., 2004; Ambord et al., 2009), physiological (Thomas et al., 2005), environmental factors (Pazzona, 1989; Dogra et al., 2000, Thomas et al., 2005; Caria et al., 2011; Caria et al., 2012) and health status (Boselli et al., 2004). Buffaloes are characterized by longer teat and teat canal, and particularly by stronger muscular resistance of teat wall, than in cow: it is necessary an high vacuum level for opening the teat canal and beginning milk ejection (Thomas et al., 2004; Ambord et al., 2009), even if the teat canal becomes shorter by an adequate stimulation (Ambord et al., 2010).

In a recent trial, we verified that the manual stimulation at different times provoked the increasing of released oxytocin and of the intra-mammary pressure and finally the increasing of cisternal teat largeness and the reduction of teat canal length (Borghese, 2012). We can obtain similar results, applying oxytocin injection, as oxiotocyn provokes immediately a progressive increasing of intra-mammary pressure and of cisternal teat largeness and a reduction of teat canal length (Figure 9-11).

In the Figure 12 a transversal section of the same teat is presented, where the great volume of cisternal teat, the increased teat diameter and the reduction of teat wall are very evident.

Species are characterized by smaller cisternal fraction than in cattle. The cisternal milk fraction is immediately available while the alveolar fraction needs an oxytocin stimulation to release. In Mediterranean Italian buffaloes it have been
observed a higher cisternal fraction of total milk than in Murrah breed (respectively 7.6%, as reported by Ambord et al., 2009, and 4.9%, as in Thomas et al., 2004, for different parity, number and stage of lactation).

In buffalo management, the milking room and the milking machine are really a critical point and the characteristics of milking vacuum and milking pulsations are strictly connected with milk flow observations (Pazzona, 1989; Dogra et al., 2000 Caria et al., 2011; Caria et al., 2012). Animal general health, with particular regard to mastitis, influences milk composition and milk flow parameters. In buffalo, Somatic Cell Count is a good indicator of subclinical mastitis (Pyorala, 2003; Thomas et al., 2004; Rosati et al., 2008). These differences characterize the milk flow curves in buffalo species and result in different relieved parameters as the total milking time and milk yield. Figures 13 to 23 give some examples of different milk flow curves. Figure 13 shows a normal milk flow curve, but from a farm where over-milking (about 3 minutes) was applied often. Visualisation of the long phase of over-milking must oblige the milkers to change the milking routine and to solve this problem, applying the automatic detachment.

Milking of the cisternal fraction is shown as the first curve in Figure 14 and known as a bimodal curve, which is not frequent in buffaloes, because of the small proportion of the cisternal fraction, that is normally the 6-7% of the total milk (cisternal+alveolar). Figure 15 is a curve from a buffalo with very low flow rate, a long plateau time, probably due to a tight and long teat canal, which is seen frequently in Italian buffalo herds. Most buffaloes need at minimum 8 minutes of milking time, but exceptions can be seen for buffaloes with a high peak flow rate and a short plateau time (Figure 16): in this case the maximum peak flow (about 3 kg/min) is similar to that one presented by dairy cattle, but the high over-milking is anyway a negative factor, for udder health and too for management economy. Therefore is a must to reduce over-milking and milking time: for this purpose could be convenient to introduce in the milking room a group of animals with similar milking time and anyway to apply the automatic detachment, to eliminate totally the over-milking.

Figure 17 shows a milk flow curve with a double profile, due to the partially milk emission disturbed or due to the repositioning of milking cluster. This problem can be avoided applying more attention by the milkers. Figure 18 is a milk flow curve of a buffalo not responding to manual stimulation during teat preparation until oxytocin was injected. This problem represents an economic loss, because of 6 minutes of wait and for the damage of the teat, extremely stimulated. The animals with oxytocin habit, anyway are a real problem for the livestock management, for the increasing cost and for more engagement during milking.

Figure 19, 20, 21, show different curves obtained during the milking with Automatic Milking System (AMS, Figure 24). The first is classified as failure (no attachment of cluster), the second as incomplete (partially attachment of cluster, only two quarters) and the third as complete one, perfectly attachment of cluster (Figure 21) (Boselli et al., 2011b). These data show that AMS could not be practised by all the buffalo dairy population, but AMS needs a very regular udder conformation with symmetric and perpendicular teats. In Figures 22 and 23, milk flow curves at quarter level are shown (Borghese, 2012). The fore quarters started
milk flow 30 after the rear ones because of the different anatomy. The rear quarters produce higher quantity of milk and show higher maximum flow and higher plateau.

In Mediterranean Italian breed, Borghese et al. (2007a) found a mean time from stimulation to milk let-down of 133±14 second(s), with no difference between morning and evening milking. Boselli et al. (2004) refer mean values of 105 s. versus 99.6 s. in the morning and in the evening respectively to reach the plateau phase. They calculated a peak flow of 1.32 kg/min in the morning versus 1.22 kg/min in the evening. Mean decline time of 110 s. in the morning versus 137 s. in the evening where calculated. Average flow rates were 0.86 kg/min in the morning and 0.77 kg/min in the evening.

In Murrah buffaloes, Thomas et al. (2004) found the time until milk let down of 69 s. versus 154 s. in two groups with a different concentrate feeding during milking. Time until a milk flow of 500 g was 195 versus 329 sec in the morning and 224 versus 383 s. in the evening, which highlights the importance of concentrate feeding during milking. In the same experiment peak milk flow was 1.18 and 1.13 kg/min in the morning versus 1.05 and 0.93 kg/min in the evening respectively for the groups. Dogra et al. (2000) found an average milk flow rate of 0.92 kg/min. Thomas et al. (2006) calculated mean flow rates of 0.49 kg/min, 0.18 kg/min and 0.10 kg/min among groups of dairy Murrah buffaloes submitted to different regimes of concentrate integration and manual pre-stimulation or not respectively.

Electrical conductivity can be measured simultaneously with the milk flow using the Lactocorder®. Conductivity is related to udder health, somatic cells count and mastitis onset. Boselli et al. (2005) found mean values of 4.11 mS/cm in milk of Mediterranean Italian buffalo. The correlated with somatic cell count was r = 0.37 (Pearson P<0.001) and indicates possibilities to use electrical conductivity in mastitis detection.

General udder health and particular teat pathologies may influence milk production in quantity, quality and milk ejection parameters. Other differences in the milking response are strictly connected with the quarters’ milk distribution (Weiss et al., 2004; Rodrigues Amaral and Corrêa Escrivão, 2005) as reported in other milking species (Bruckmaier et al., 1994).

The general health of animals and good livestock conditions are very important in milk production. Moreover, the milking machine is a very critical factor for the milk flow rates because of the characteristics of milking vacuum, milking pulsation and pulsation frequency, and other technical characteristics connected to the milking equipment (Pazzone, 1989; Dogra et al., 2000).

Caria et al. (2011) compared the effects of milking at low vacuum (36 kPa) and medium vacuum (42 kPa) on milk emission characteristics and milking system performances. The results showed that the low vacuum level did not influence both milk yield and milk ejection time.

While in another study Caria et al. (2012) verified the milkability at different vacuum levels (37, 40, 43, 46, 49 and 52 kPa): it is shown that the Mediterranean Italian buffalo is suitable to mechanical milking at different working vacuum levels. Milk yield was not influenced by the working vacuum level and resulted satisfactory also at lower levels; the vacuum levels of 37 and 40 kPa showed the best milkability
conditions, at which plateau phase was longer than decline phase and lag time was not affected by vacuum level.

3. INNOVATION IN BUFFALOES MILKING TECHNOLOGIES

A recent study analysed the principal milk flow traits and milk yield recorded in Mediterranean Italian buffaloes from 104 different herds, raised in the Latium Region (Centre of Italy) during 2005-2010 (Boselli et al., 2011c). This study showed that 88.86% of milk is ejected in 4.24 min (main milking time), as 8.49% is ejected in 1.98 min (milk let-down) corresponding to the cisternal milk fraction as observed in a previous work (Ambord et al., 2009). Only 2.65% is the milk ejected in the stripping phase.

These results suggest applying a premilking udder stimulation to reduce milk let-down time increasing the average milk flow and reducing total milking time. These results suggest to the detachment of milking cluster to reduce the blind phase with the following advantages: a) reducing the total milking time and consequently worker’s time, b) improving the farmer’s income and the milk quality thought the mastitis incidence decreasing.

About the introduction of AMS in buffalo, the preliminary results of recent study showed that there is a considerable variation in milk ejection and consequently in milk flow curves for the buffalo milked in AMS with forced system, in comparison with the conventional one.

The differences are the following: better pre-stimulation allowing a positive endogenous releasing of oxytocin, with reduced milk letdown phases; independent milk ejection for each teat, with optimal milking of all the quarters, with the reduction of over-milking; better milking hygiene during dairy routines, limiting the incidence of mastitis and with low value of somatic cell count; negative effects, because of failed or incomplete milking (17% of total), that limit the potential capability and efficiency of AMS; frequent air flow, which could cause alteration of milk composition (Boselli et al., 2011b).

REFERENCES


www.lactocorder.ch website
Figure 1. Effect of parity on lactation curve in Mediterranean Italian breed (Coletta and Caso, 2008).

Figure 2. Effect of parity on lactation curve in Mediterranean Italian breed (Catillo et al., 2002).

Figure 3. Effect of parity on lactation curve in Nili-Ravi breed (Khan and Chaudhry, 2001).
**Figure 4.** Different lactation curve in the cases of early and late calving for the Murrah Bulgarian buffalo cow (Penchev et al., 2011).

**Figures 5.** Typical milk flow curve of dairy cow (Holstein Friesian breed).

**Figures 6.** Typical milk flow curve of buffalo (Mediterranean Italian breed).
Figures 7. Typical milk flow curve of goat (Saanen breed).

Figures 8. Milk flow curve of donkey (Amiata breed, one milking for day).
Figure 9. (up-left), 10 (upright), 11 (down-left), 12 (down-right). Ultrasound cross section of fore teat at the time 0 (Fig. 9), after 3 minutes from oxytocin injection (Fig. 10), after more 20 seconds (Fig. 11).

Figure 13. Normal milk flow curve including over-milking.
Figure 14. Bimodal curve (with cisternal fraction) in a high producing buffalo (Borghese et al., 2007 b).

Figure 15. Slow milk ejection with a long plateau time caused by a tight teat canal.

Figure 16. Milking with a high peak flow but short plateau.
**Figure 18.** Buffalo with no milk ejection until oxytocin was injected.

**Figure 19.** Milk flow curve recorded with AMS: type 0 (failure milking).
Figure 20. Milk flow curve recorded with AMS: type 1 (incomplete milking).

Figure 21. Milk flow curve recorded with AMS: type 2 (complete milking).

Figure 22. Milk flow curve at quarter level (fore left) (Borghese, 2012).
**Figure 23.** Milk flow curve at quarter level (hind left) (Borghese, 2012).

**Figure 24.** AMS applied in Mediterranean Italian buffalo.
Influence of Fish Oil Supplementation on the Concentration of Conjugated Linoleic Acid (CLA) and Omega 6 and 3 in Buffalo Milk

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ABSTRACT
The aim of this research work was to investigate the influence of fish oil supplementation on the concentration of conjugated linoleic acid (CLA) and omega 6 and 3 in samples of buffalo milk. A total of 24 female buffaloes separated at random into three groups were fed for 49 days with: natural pasture (group I), supplemented with 70 ml of fish oil (group II) and 140ml of fish oil (group III). In the experiment the concentration of CLA showed differences (P<0.05) among the three groups, with a maximum of 7.14 mg/g fat in group II. No significant differences were found in omega-6 among the three groups. The highest value of 3.82 mg/g fat corresponded to group I, which had not been supplemented with fish oil. Significant differences were observed in omega 3 (P<0.05) in groups II and III with respect to group I. The highest average value of 2.42mg/g fat was obtained in group III. The closest relationship omega 6/3 (1.37:1) was observed in group III. As a result, the diets of groups II and III, which included fish oil, increased significantly the content of CLA and omega 3 with reductions in levels of omega 6.

Keywords: Bubalus bubalis, CLA, fish oil, milk, omega 6 and 3

INTRODUCTION
The CLA is the term used to describe one or more positional and geometric isomers of linoleic acid (cis-9, cis-12, octadecadienoic acid) that contains double conjugated bonds. Such bonds are usually found in positions 9 and 11, or 10 and 12. They can be cis or trans configuration. The CLA biologically active form would be represented by the isomer cis-9, trans-11 CLA (also called rumenic acid), representing 80 to 90% of total CLA in milk fat (Belury, 2002).

It has been shown that CLA inhibits the carcinogenesis onset in skin tumors, in tumors of the stomach and in mammary gland tumors, all of them induced experimentally with different carcinogens in mice (Ha et al., 1990, Ip et al., 1994). Hypocholesterolemic properties have been demonstrated in pilot studies with hamsters fed with a hypercholesterolemic diet and supplemented with CLA. This experiment showed a significant reduction in total cholesterol, LDL-cholesterol and triglycerides (Nicolosi et al., 1993). Hypocholesterolemic properties were also accepted April 10, 2013; online November 11, 2013.
demonstrated in rabbits supplemented with CLA (Lee et al., 1994). Linoleic and
linolenic acids are essential fatty acids, synthesized by plants but not by mammals.
These acids must be therefore supplied by food. They are the precursors for the
synthesis of polyunsaturated fatty acids (PUFA) in the series omega 3 and omega 6,
respectively (Gagliostro, 2004). CLA precursors are the PUFAs present in the forage
feed for ruminant as linoleic acid (cis-9,trans-12 C18: 2) and α-linolenic acid. The
former abounds in corn silage, cereals and various oilseeds such as sunflower and
soybean. α-linolenic acid is present in greater percentage in green pasture and flax.
(Gagliostro, 2004). The transformation of unsaturated fatty acids occurs in the
rumen by bacteria of the genus Butyrivibrio through ruminal biohydrogenation,
that can be improved by a strategic diet rich in unsaturated fatty acids. This improves
the salubrity of milk and meat from ruminants due to a higher CLA and omega 3
concentration (Chilliard et al., 2000).

Studies have shown that omega 3 fatty acids, eicosapentaenoic acid (EPA)
and docosahexaenoic (DHA) have hypcholesterolemic, antitrombic and
antiinflammatory properties in human beings (Williams, 2000). In human health the
concept of relationship omega 6 / omega 3 is convenient to be used (Gagliostro
2004). A lower ratio of omega-6/omega-3 fatty acids is more desirable in reducing
the risk of many of chronic diseases of high prevalence in Western societies, as well
as in developing countries (Simopoulos, 2002).

The main source of CLA in human diet are milk and dairy products, that
contain mainly cis-9, trans-11 C18:2 (rumenic acid) and trans-9, cis-11 C18:2.
(Gagliostro, 2004). This work aimed at investigating whether fish oil
supplementation in the diet of buffaloes bred in the Province of Corrientes and fed
on natural pastures modifies the CLA concentrations, the omega-3 and 6 fatty acids
and whether that supplementation improves the omega 6/3 relationship in the milk.
This paper is a summary of an earlier publication in a specialized journal.

MATERIALS AND METHODS
The animals used belong to a herd from a farm located in the town of San
Cosme, 35 km from the city of Corrientes, Province of Corrientes, Argentina. We
have worked with 24 multiparous buffaloes of Murrah breed and Murrah x
Mediterranean crossbred, identified with alphanumeric caravans, distributed in three
groups, consisting of eight animals each. All animals were fed with natural pastures
during the 49-day experience. Control group I, was fed only with natural pastures,
group II was daily fed with 70 ml of fish oil and group III with 140 ml of fish oil.
The animals supplemented with fish oil, made of 85% argentine haddock (Merluccis
hubbsi) and 15% anchovy (Anchoa marinii), received the ration at milking time in
an individual feeder to meet the assumption of independence.

The buffaloes were milked by hand in the morning. The samples (n = 24)
were obtained at the beginning and at the 49-day of the experience carried out
between May and July 2009 during the second stage of lactation of the experimental
herd. During the milking routine and after discarding the first streams, samples of
200 ml of milk were taken from each animal. The samples were packed in
disposable containers, frozen at -20°C and kept in polyurethane boxes until they
were taken to the laboratory.
Each sample was processed in duplicate to obtain the lipid profile. To obtain the total lipids, a mixture of chloroform and methanol according to Bligh and Dyer technique (1959) was used. The conversion of fatty acids in methyl esters was carried out with methanol NaOH and BF$_3$ at 14% boiling for 8 minutes. The methyl esters were obtained with hexane and analyzed with a gas chromatograph. Standards of methyl esters of 99% pure fatty acids (Lipid Standard 189-19 Sigma-Aldrich) were used.

The fatty acid composition was determined in an Agilent gas chromatograph equipped with a capillary column 60 mm long and 0.25 mm of internal diameter (Supelco 2340) and a flame ionization detector. The gas chromatography method used (GC-FID) met the ISO 15304 standard (2010).

Descriptive statistics was applied to assess the sample estimates for each treatment (mean, standard deviation, coefficient of variation and minimum and maximum ranges).

RESULTS AND DISCUSSIONS

In the experiment the concentration of CLA showed differences (P<0.05) among the three groups, with a maximum of 7.14 mg/g fat in group II. No significant differences were found in omega-6 among the three groups. The highest value of 3.82 mg/g fat corresponded to group I, which had not been supplemented with fish oil. Significant differences were observed in omega 3 (P<0.05) in groups II and III with respect to group I. The highest average value of 2.42 mg/g fat was obtained in group III.

The closest relationship omega 6/3 (1.37:1) was observed in group III. As a result, the diets of groups II and III, which included fish oil, increased significantly the content of CLA and omega 3 with reductions in levels of omega 6.

In our experience we have observed that the increase of the CLA fatty acids and omega 3 is related to the incorporation of the fish oil in the diet provided. However, a greater increase of these fatty acids was obtained when using low amounts of fish oil in the diet. This is due to the process of biohydrogenation in the rumen that causes a toxic effect when the amount of fish oil is exceeded. The amount of unsaturated fatty acids provided in the diet has a toxic effect on the microbial metabolic activity (CLA producer), mainly on the Butyrivibrio fibrisolvens microorganism with the consequent decrease in the production of CLA fatty acid (Maia et al., 2010).

CONCLUSIONS

Taking into account our work conditions, the fish oil used in the present experience increased significantly (p <0.05) the CLA and omega -3 content in milk of buffaloes and improved the omega -6 / -3 relationship. In our region where animals are fed with nutritionally deficient natural pastures, supplementation with fish oil is an excellent strategy to increase the values of fatty acids. Milk and its derivatives with higher CLA and omega -3 concentrations together with a close relationship between omega -6 and -3, give us a product that has nutritional, anticancer, hypocholesterolemic as well as antitrombotic properties having a big impact on human health. The possibility of increasing the concentration of these fatty acids.
acids through strategic supplementation, that is cost-effective, easily implemented and managed and does not alter the organoleptic properties of milk, is the challenge of research in the area of nutrition and technology of food.

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Buffalo Milk Cheese

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ABSTRACT

Buffalo milk cheeses are becoming increasingly popular throughout the world; buffalo’s milk is the preferred commodity in preparing many cheese varieties, namely soft, semi, hard cheeses and certain pickled cheeses. Buffalo milk cheeses display typical body and textural characteristics and unique in nature, the sensory qualities are far superior. Attempts have been made successfully to prepare other cheese varieties using buffalo’s milk. Pre-treatments are applied to buffalo’s milk for cheese making, with some cheese varieties simply made from raw milk, but most made from pasteurized milk of which the composition (e.g., fat: protein ratio) may have been standardized. However, there has been consistent interest in more novel and sophisticated strategies for pre-treatment of cheese-buffaloes milk. Approaches explored include the use of alternative processing technologies (e.g., membrane filtration, high-pressure treatment, homogenization, heat treatments more severe than pasteurization) or addition of either whey protein products, enzymes or emulsifying salts. Such additions to cheese milk seem to provide some definite advantages. New approaches have been attempted to accelerate the cheese ripening process. This article will review the key principles for pre-treatment of cheese-buffaloes milk. Aspects of using buffalo’s milk and its significance with respect to the quality and safety of the final products are discussed in this review.

Keywords: buffalo, challenges, cheese, opportunities, quality, safety

INTRODUCTION

Tradition and habits of buffalo milk consumption are quite diverse in different parts of the world. There is however an increasing demand for buffalo milk and buffalo milk products. More than 5 percent of the world's milk comes from water buffaloes. Buffalo’s milk is ranked second in the world after cow’s milk being more than 12% of the world, milk production (Ahmed et al., 2008). Buffalo milk is used in much the same way as cow's milk. It is high in fat and total solids, which gives it a rich flavor.

Cheeses are becoming increasingly popular throughout the world. Demand is rising at a rate that is among the highest for any food product. The preference of population, more so in Western Europe, to cheese varieties made from buffalo milk has given a new dimension to the growth and development of buffalo milk cheese industry. Cheese from buffalo is highly priced in the most of the world, it displays typical body and textural characteristics and unique in nature. Buffalo milk is the preferred commodity in preparing certain pickled cheeses of Middle East and traditional cheese varieties of India like Paneer. The yield of Cheddar, Swiss,
Cottage, Mozzarella and other types of cheeses is higher when prepared from buffalo milk and the sensory qualities of cheese (more so with cottage and Mozzarella cheese varieties) made from buffalo milk is far superior. World over, dairy technologists are tirelessly working to find ways to prepare other cheese varieties with buffalo milk. Another area where buffalo milk has made strong inroads is in the preparation of cheese spreads. They are popular for their mild flavor and the ease of use. Cheese spreads were successfully prepared from buffalo milk cheddar cheese.

Pre-treatments are applied to buffalo’s milk for cheese making, with some cheese varieties. However, there has been consistent interest in more novel and sophisticated strategies for pre-treatment of cheese-buffaloes milk. Approaches explored include the use of alternative processing technologies (e.g., membrane filtration, high-pressure treatment, homogenization, heat treatments more severe than pasteurization) or addition of sources of protein (e.g., whey protein products) or enzymes or emulsifying salts. Such additions to cheese milk seem to provide some definite advantages. New approaches have been attempted to accelerate the cheese ripening process. This article will review aspects of using buffalo’s milk and its significance with respect to the quality and safety of the final products.

BUFFALO’S MILK AND CHEESE QUALITY

Organoleptic quality

A sensory quality of cheese (more so with Domiati, Kariech, Feta, Cottage and Mozzarella cheese varieties) made from buffalo milk is far superior. It is known for its unique body and texture characteristics. The appearance of cheese has great value in its acceptability. Fresh and pickled buffalos milk white cheeses with high solids and protein had improved appearance and texture due to more stability resulted by protein fat interlacing and calcium links to casein. (Hofi 1984), while in case of low fat cheeses, the appearance was poor, rough and lacking lust (Sameen et al., 2008).

Nutritional Quality

Mozzarella cheese from buffalo’s milk had higher nutritional value (Sameen et al 2008). The same has been proved for other cheese types made from buffalo’s milk. Buffalo’s cheddar cheese was found superior in nutritional profile compared with cow cheddar (Murtaza et al 2008).

Yield

The richness of buffalo milk makes it highly suitable for processing. The yield of Domiati, Kariech, Feta, Cheddar, Swiss, Cottage, Mozzarella and other types of cheeses is higher when prepared from buffalo milk. Because of these high yields, processors appreciate the value of buffalo milk. One of the obvious advantages derived from the increased yield is the lower cost for the manufacture of the cheese. Obviously, the more cheese derived from the raw material, the less expensive it is to manufacture the cheese. Another advantage gained from increased yield relates to the disposal of the whey which is left after the cheese is formed. This byproduct is one for which few uses have been found and therefore is frequently disposed of through the sewage waste system. With increased yield from the milk, less whey is formed with a resulting reduction in pollution to our environment.
Advantage and disadvantage of using buffalo’s milk in cheese making
Advantage and disadvantage of using buffalo’s milk in selected cheese varieties are summarized in Table 1.

Approaches explored to enhance buffalo’s milk cheese quality
Several attempts have been tried to enhance cheese quality, when dealing with buffalo’s milk. A summary of such approaches attempts are listed in Table 2.

CONCLUSIONS
It is concluded that buffalo milk because of its chemical composition, offers excellent opportunities for the development of different dairy products. Substantial effort is still needed to the “conventional” methods for improvements hard cheese quality. An economical study should, however, be accomplished for a better evaluation of any new proposed approaches.

REFERENCES


Table 1. Advantage and disadvantage of using buffalo’s milk in selected cheese varieties.

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Reference</th>
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<tr>
<td>Mozzarella</td>
<td>white color, high yield more piquant &amp; aroma flavor</td>
<td>Harder body, less watery, lower metability, higher oiling off</td>
<td>Patel, 1986 Bikash et al., 1996 Mostafa et al., 1996 Sameen, 2008</td>
</tr>
<tr>
<td>Casskawal,</td>
<td>high yield</td>
<td>Hard body, sandy texture</td>
<td>El-Chabrawy, 1973</td>
</tr>
<tr>
<td>Pickled Domiat, Feta</td>
<td>white color, high yield, easy to handle</td>
<td>increase in cost, flavour development is slower</td>
<td>Hofi, 1984</td>
</tr>
<tr>
<td>Fresh Domiat</td>
<td>high yield, easy to handle &amp; cut, less whey drainage</td>
<td></td>
<td>Aly and Galal, 2002</td>
</tr>
<tr>
<td>Kariech</td>
<td>high yield, pure white shiny color, easy to handle, cut, preferred &amp; shape, less whey drainage</td>
<td></td>
<td>Hofi el al., 2004</td>
</tr>
<tr>
<td>Ras, Rommi</td>
<td>high yield</td>
<td>Hard body, sandy texture</td>
<td>Hofi, 1979</td>
</tr>
<tr>
<td>Paneer</td>
<td>high yield</td>
<td></td>
<td>Aneja et al., 2002</td>
</tr>
<tr>
<td>Cheddar</td>
<td>rich flavor, superior in nutritional profile</td>
<td></td>
<td>Murtaza et al., 2008</td>
</tr>
<tr>
<td>Gouda</td>
<td>40% of fat at the end of 90th day</td>
<td>Hard body</td>
<td>Jabbar, 2003</td>
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Table 2. Selected approaches explored to enhance cheese quality of cheeses made from buffalo’s milk.

<table>
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<th>Approaches</th>
<th>Cheese type</th>
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<tr>
<td>Addition of emulsifying salts</td>
<td>Casckawal</td>
<td>El-Chabrawy, 1973</td>
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<tr>
<td>Sodium citrates</td>
<td>Mozzarella</td>
<td>Teama et al., 1979</td>
</tr>
<tr>
<td>Sodium Polyphosphates</td>
<td></td>
<td>Mostafa et al., 1996</td>
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<tr>
<td></td>
<td></td>
<td>Mansour, 2005</td>
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<tr>
<td>Addition of sodium and potassium chlorides</td>
<td>Mozzarella</td>
<td>Paulson et al., 1998</td>
</tr>
<tr>
<td>Direct acidification</td>
<td>Mozzarella</td>
<td>Metzger et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Kariech</td>
<td>Ahmed, 2008</td>
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<td></td>
<td></td>
<td>Hofi el al., 2004</td>
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<tr>
<td>Ultrafiltration</td>
<td>Domiate</td>
<td>Hofi, 1984</td>
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<td></td>
<td></td>
<td>Hofi and Fayed, 1987</td>
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<tr>
<td></td>
<td></td>
<td>Hofi el al., 2001</td>
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<tr>
<td>Diafiltration</td>
<td>Domiate</td>
<td>Hofi, 1984</td>
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<tr>
<td>Addition of fermented whey protein concentrate</td>
<td>Iranian white</td>
<td>Madadlou et al., 2005</td>
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<tr>
<td>Addition whey protein concentrate</td>
<td>Domiate</td>
<td>El-Sheikh et al., 2001</td>
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<td></td>
<td>Mozzarella</td>
<td>Rudan et al., 1998</td>
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<tr>
<td>Adding adjunct cultures</td>
<td>Feta</td>
<td>Katsiari et al 2002</td>
</tr>
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<td>Casein /Fat ratio standardization</td>
<td>Casckawal</td>
<td>El-Chabrawy, 1973</td>
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<tr>
<td></td>
<td>Mozzarella</td>
<td>Teama et al.1979</td>
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<td></td>
<td></td>
<td>Sameen, 2008</td>
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<td>$\text{H}_2\text{O}_2$/ catalase treatment</td>
<td>Ras cheese</td>
<td>Hofi, 1975</td>
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<td></td>
<td>Casckawal</td>
<td>El-Chabrawy, 1973</td>
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<tr>
<td>Heat treatment</td>
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<td>Aly and Galal, 2002</td>
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<td>Casein micelles size</td>
<td>Mozzarella</td>
<td>Mansour, 2005</td>
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<td>Homogenization</td>
<td>Camembert</td>
<td>Ghosh, 1999</td>
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