BUFFALO BULLETIN

Editor: S. Sophon

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Aims

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Buffalo Bulletin is published quarterly in March, June, September and December. Contributions on any aspect of research or development, progress reports of projects and news on buffalo will be considered for publication in the bulletin. Manuscripts must be written in English and follow the instruction for authors which describe at inside of the back cover.

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A 10-year-old she buffalo was presented to the college clinics with the history of a 20 cm-in- diameter growth, which had appeared at the right shoulder region and gradually increase in size (Figure 1). The growth was round and soft. Haematological and biochemical parameters were within the normal physiological limits. It was diagnosed as a case of tumor involving the shoulder, and it was decided to perform surgery.

The animal was sedated with xylazine hydrochloride 0.01 mg/kg body weight intramuscularly. The animal was restrained on lateral recumbency, 2% lignocaine hydrochloride was infiltrated at the base of the tumor mass, and the mass was excised surgically. Post operatively animal was given enrofloxacin 5.0 mg/kg body weight for 7 days and meloxicam 0.50 mg/kg body weight intravenously for 5 days. Antiseptic dressing of the surgical wound was done with povidone- iodine solution once every two days. The animal made uneventful recovery within 15 days after surgery. The mass was subjected to histopathological examination. Sections showed lymphocytic, polymorphocytic, lymphoblastic, histocytic types, which were mixed cell types (Figure 2) (Madan Joshi et al., 1995) Neoplastic cells were lymphocytes with polyhedral shape, large nuclei placed eccentrically with mitotic figures. The animal was observed for one year after surgery, and there was no report of recurrence.

REFERENCE

INTRODUCTION

Fibromas are benign neoplasms of fibrocytes with abundant collagenous stroma. The occurrence of fibroma involving the skin is very rare in cattle as compared to papilloma (Theilen and Madewell, 1979). Depending on the amount of collagenous fibers, fibromas can be categorized as soft fibromas and hard fibromas. The authors place on record a case of cutaneous hard fibroma at the point of elbow in a she buffalo and its surgical management (Gopalakrishna Rao, 2004).

CASE HISTORY AND OBSERVATION

An 8-year-old she buffalo was presented to the college clinics with the history of a 25-cm-in. diameter growth, which had appeared at the left elbow and gradually increased in size (Figure 1). The growth was round and firm and interfered with the movement of the limb. Hematological and biochemical parameters were within the normal physiological limits. It was diagnosed as a case of tumor involving the elbow, and it was decided to perform surgery.

TREATMENTS AND DISCUSSION

The animal was sedated with xylazine hydrochloride 0.01 mg/kg body weight intramuscularly. The animal was restrained on lateral recumbency, and 2% lignocaine hydrochloride was infiltrated all around the site of operation. An elliptical incision was made around the tumor, and by blunt dissection the skin was released. The blood vessels were ligated using chromic catgut. Then, the growth was carefully dissected out. The wound was dressed with povidone iodine solution and closed with interrupted sutures by using silk. Post-operatively, 2.5 gms of strepto-pencillin was administered intra-muscularly for 5 days. The wound was dressed daily with povidone iodine solution, and the sutures were removed on the 10th post-operative day. No recurrence was observed in a period of one year. The mass (Figure 2) was subjected for histopathological examination. Histopathological features of hard fibroma showed whorls and interlocking bundles of collagen fibers with fibroblasts in all directions (Figure 3). Collagen fibers were dense and loose and showed various degrees of vascularisation. The epidermal overlying the fibroma was hyperplastic.
REFERENCES


Figure 1. Hard fibroma of the left elbow in a buffalo.

Figure 2. Excised tumourous mass.

Figure 3. Photomicrograph showing whorls and interlocking bundles of collagen fibers with fibroblasts in all directions H&E × 70.
TRICHOEPITHELIOMA IN A BUFFALO: A CASE REPORT

R.V. Suresh Kumar, P. Veena, P. Sankar, N. Dhanalakshmi, Ch. Srilatha and S. Kokila

INTRODUCTION

Tumors of the hair follicle account for approximately 5% of all skin tumors in dogs. They are extremely rare (Withrow and Vali, 2001) or not recognized in other species like bovines and are uncommon in cats. Trichoepitheliomas have a predilection for the back, neck, thorax and tail. The tumor is located within the dermis with extension into the subcutaneous tissue. Epidermal ulceration, alopecia of the skin overlying the mass and the secondary infection may present (Meuten, 2002). This paper reports a rare case of trichoepithelioma in a buffalo.

CASE HISTORY AND OBSERVATION

A 7-year-old she buffalo was presented to the college clinics with the history of a 12-cm-in-diameter growth, which had appeared at the mid tail region and gradually increased in size (Figure 1). The growth was round and hard. Haematological, and biochemical parameters were within the normal physiological limits. It was diagnosed as a case of tumor involving the mid tail, and it was decided to perform surgery.

TREATMENTS AND DISCUSSION

The animal was sedated with xylazine hydrochloride 0.01 mg/kg body weight intramuscularly. The animal was restrained on lateral recumbency, 2% lignocaine hydrochloride was infiltrated at the base of the tumor mass, and the mass was excised surgically. The mass was the subjected for histopathological examination. Section showed histopathological features of trichoepithelioma-like basal cells which were arranged in islands with keratinized centers (Figure 2). The presence of stratified squamous epithelium was also noticed along with basal cells and squamous cells. Few pleomorphisms or mitosis were noticed surrounding stroma was relatively acellular and consisted of fibroblasts and collagen which lacked organization. In general hair follicle tumors are benign and have an excellent prognosis after surgical excision. No local recurrences or metastasis was recorded even though many of the lesions had histological evidence of malignancy (Withrow and Vali, 2001).

REFERENCES


Department of Veterinary Surgery and Radiology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Figure 1. Trichoepithelioma of the tail in a buffalo.

Figure 2. Photomicrograph showing features of trichoepithelioma-like basal cells which was arranged in islands with keratinized centers H&E × 70.
ABSTRACT

The duplex-PCR technique, proposed to identify and differentiate cattle and water buffalo DNA using primers described by Rea et al. (2001) for identification of cattle and buffalo DNA in Italian cheese, was tested on mitochondrial DNA extracted from meat muscle samples.

The optimized PCR amplified 113 bp and 152 bp products for cattle and buffalo respectively. Meat processing technology (salting, drying, smoking, and cooking) affects the integrity of the extractable DNA. Also many times meat samples are brought to the laboratory for speciation one or two days after slaughter under unpreserved conditions. So different levels of autolysis were experimentally produced, and PCR successfully amplified *cyt b* gene from meat samples that were putrefied even after 48 h and cooked at various conditions.

The technique was successful in detecting up to 1 pg adulteration in a cattle-buffalo meat mixture. The test is a valuable tool for meat authentication and screening of cooked, putrefied and mixed samples of cattle and buffalo flesh.

Keywords: meat speciation, polymerase chain reaction, cattle, buffalo, *cyt b*

INTRODUCTION

The determination of food authenticity and the detection of adulteration are major issues in the food industry and are attracting an increasing amount of attention. Therefore, reliable techniques to identify the species of origin of components in a food product derived from animals are necessary for food authentication purposes. Identification of the species of origin of meat samples is relevant to consumers for the possible economic loss from fraudulent adulterations, medical requirements of individuals who might have specific allergies, and religious reasons (Miguel et al., 2004).

The conventional methodology used for the determination of species origin in meat products are predominantly based on immunochemical and electrophoretic analysis of protein. Additionally, through the acquisition of sequence data, DNA can potentially provide more information than protein, due to the degeneracy of the genetic code and the presence of many non-coding regions. Polymerase chain reaction (PCR) is the most widely used molecular biology technique. Following PCR, the amplified DNA fragments normally require further analysis to identify the species of origin. The methods used include Single Strand Conformation Polymorphism (SSCP), Restriction Fragment
Length Polymorphism (RFLP), Denaturing Gradient Gel Electrophoresis (DGGE), Thermal Gradient Gel Electrophoresis (TGGE) and Sequencing. A particular type of PCR, based on the multiplex-PCR principles described by Dieffenbach and Dveksler (1995) and Innis et al. (1990), was used for species identification in meat and meat products in two different ways by Fei et al. (1996) and Matsunaga et al. (1999). The advantage of this technique is that the species is directly identified by PCR, without requiring further analyses.

A number of studies have addressed meat species identification in the recent past and attempts were made to differentiate closely related meat species (Rajapaksha, 2002; Rodriguez, 2004; Maccabiani, 2005; Martina et al., 2006). Fewer studies have been published so far reporting the application of DNA-based techniques for differentiation of water buffalo meat from cattle meat, but with limited utility in the case of mixed and processed meat (Girish et al., 2005; Rastogi et al., 2004; Jain, et al., 2007). In this context, development of highly sensitive and specific methods for identification and differentiation of cattle and water buffalo meat is necessary for law enforcement.

In the present paper, a variation of multiplex-PCR, the duplex-PCR, is proposed to identify and differentiate cattle and water buffalo meat. A common primer is used along with two specific primers that allow two different DNA fragments to be amplified, one specific to cattle and the other to water buffalo. These are used to identify meat and meat products from the two species. This work presents a specific, sensitive, effective and inexpensive alternative to the existing methods.

**MATERIALS AND METHODS**

**DNA isolation from meat samples**

Mitochondrial DNA, along with genomic DNA, was extracted from meat samples of each species by using the method described by Ausubel et al. (1987) with some modifications. The quality and purity of DNA were checked on agarose gel electrophoresis and DNA was quantified using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies).

**Polymerase chain reaction**

A small fragment of the cyt b gene of mitochondrial DNA extracted from fresh, cooked and putrefied samples was amplified. For this purpose species specific primers described by Rea et al. (2001) for identification of cattle and buffalo DNA in Italian cheese were tested on DNA extracted from meat samples. A common forward primer (5'-CTT CTT ATT CGC ATA CGC AAT CTT ACG ATC - 3') and species specific reverse primers, cattle specific (5'-TGC TCT AAT CCC CCTA CTA CAC ACC TCC A- 3') and water buffalo specific (5'-TAT GAT GTT CCG GCC ATT CAG CCA ATG CC- 3') were used, as described by Rea et al. (2001).

Various combinations of primers and DNA of cattle and buffalo origin were tested in a final volume of 25 μl containing 1x PCR master mix (MBI Fermentas, Canada) 10 pmole of each primer and 90-100 ng of DNA template (cattle and/or buffalo). Amplification was performed in Master Cycler gradient thermocycler (Eppendorf, Germany) with the following cycling conditions: after an initial heat denaturation at 95°C for 5 minutes, 35 cycles were programmed as follows: 95°C for 30 seconds, 65°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 5
minutes. This optimized PCR amplified a 113 bp product for cattle and a 152 bp product for buffalo which were confirmed by using Genesnap and Genetool programmes (Syngene, UK) and running the products parallel to a 100 bp MW marker.

RESULTS AND DISCUSSION

Rea et al. (2001) successfully used an annealing temperature of 55°C for differentiation of cattle and buffalo DNA from cheese samples using the same set of primers, but in the present study mild cross reactions were observed when 55°C annealing was used (Figure 1) so the annealing temperature was increased by 2°C each time, until there were no cross reactions at 65°C (Figure 2 - lane 3 and 4). The specificity and sensitivity of the test greatly depends on the annealing temperature of the primers. These primers yielded PCR products only from the DNA extracted from species that they were designed for and showed no cross-reactivity with the DNA from sheep and goat. PCR products were not obtained for the samples of negative controls with any of the species-specific primer sets.

PCR profiles of cooked and putrefied meat samples

Species identification of cooked meat is often warranted. The processing technology (salting, drying, smoking, and cooking) applied during the manufacture of meat products affect the integrity of the extractable DNA, causing its degradation into small size fragments (Dias et al., 1994; Martinez and Man, 1998). For this reason, in the present study, meat samples were cooked at 100°C and 120°C in dry (hot air oven) and moist heat (water bath and autoclave) for 45 minutes to simulate cooking. Proper cooking was evident from discolored meat. Many times meat samples are brought to the laboratory for speciation one or two days after slaughter under unpreserved conditions. Looking to the reality of the situation that exists, different levels of autolysis was produced by allowing the meat samples to putrefy for variable periods (48 h or more) of time at room temperature in unpreserved conditions to stimulate the autolysis in meat. PCR successfully amplified small fragment of the cyt b gene from cooked and putrefied meat samples, indicating that partial degradation of DNA because of cooking or putrefaction of meat does not inhibit amplification of the cyt b gene region.

Mitochondrial DNA was used in the study as it offers two main advantages: first that mt DNA is present in thousands of copies per cell (as many as 2,500 copies), especially in the case of post –mitotic tissues such as skeletal muscle (Greenwood and Paboo, 1999). This increases the probability of achieving a positive result even in the case of samples suffering severe DNA fragmentation due to intense processing conditions (Bellagamba et al., 2001) and second that the large variability of mt DNA targets as compared with nuclear sequences facilitates the discrimination of closely related animal species even in the case of mixture of species (Prado et al., 2002).

Detection of adulteration from degraded DNA obtained from cooked and putrefied samples is one of the very important merits of this technique as a tool for meat species detection.

Sensitivity of the assay

To measure the detection limit of the duplex-polymerase chain reaction, DNA samples (cooked and putrefied) in 10 fold dilution i.e. 100, 10, 1, 0.1, 0.01, 0.001 and 0.0001 ng each were tested and positive signals up to 0.001 ng (i.e 1 pg)
Figures 1 and 2 showing gel electrophoresis pattern on 2% agarose.
Lane 1: Cattle DNA with common forward primers and cattle specific reverse primers
Lane 2: Buffalo DNA with common forward primers and buffalo specific reverse primers
Lane 3: Cattle DNA with common forward primers and buffalo specific reverse primers
Lane 4: Buffalo DNA with common forward primers and cattle specific reverse primers
Lane 5: Cattle and buffalo mixed DNA with common forward primers and cattle specific reverse primers
Lane 6: Cattle and buffalo mixed DNA with common forward primers and buffalo specific reverse primers
Lane 7: Cattle and buffalo mixed DNA with common forward primers and cattle specific and buffalo specific reverse primers
Lane 8: Cattle DNA with common forward primers and cattle specific and buffalo specific reverse primers
Lane 9: Buffalo DNA with common forward primers and cattle specific and buffalo specific reverse primers
Lane 10: 100bp DNA molecular weight marker.
of template DNA were observed. It can be concluded that cattle and buffalo meat could be reliably identified and differentiated using duplex PCR at optimized conditions, detecting up to 1 pg adulteration in cattle-buffalo meat mixture.

Also this method can be applied with equal efficiency to fresh, cooked and putrefied meat.

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Miguel, A. R., G. Teresa, G. Isabel, A. Luis, E.H.


ABSTRACT

A total of 38 buffalo fetuses were used in the present study and their CRL ranged from 0.9 cm to 80.0 cm CRL. In buffalo five pharyngeal pouches were noted at 0.9 cm CRL (26 days). The third pharyngeal pouch showed a common primordium for parathyroid III and thymus at 1.2 cm CRL (28 days). At 2.5 cm CRL (40 days) parathyroid and thymic primordia lost their connection with the pharynx. Thyroid primordium noted as a median diverticulum of the floor of the pharynx at 1.2 cm CRL (28 days). At 2.5 cm CRL (40 days) thyroid primordium showed two distinct lobes and they were connected by unpaired isthmus. At 19.5 cm CRL (115 days) formation of thyroid follicles as well as follicular epithelium was began and thyroid was highly vascular at this stage. At 31.0 cm CRL (143 days) thyroid follicles and follicular epithelium were distinct. At this stage colloid substance was characteristically seen in thyroid glands of buffalo. Many reticular fibers were also reported between the follicles at this stage.

Keywords: histogenesis, thyroid gland, parathyroid glands, buffalo

INTRODUCTION

The thyroid and parathyroid glands are derivatives of the embryonic pharyngeal region (Boyd, 1954). The embryonic development of thyroid and parathyroid glands have been studied in hamster (Ackerman and Knouff, 1964) and dog (Hendrickx, 1964).

But no information is available on development of thyroid and parathyroid glands in buffalo. Therefore, a comprehensive study on histogenesis of thyroid and parathyroid glands has been undertaken in buffalo. This will be contributory to the existing anatomical knowledge and it will provide a basis for future investigations.

MATERIALS AND METHODS

A total of 38 buffalo foetuses were used for the present study and their CRL (crown rump length) varied from 9.0 cm to 80.0 cm (26 days to 254 days). Buffalo foetuses of different gestational age were collected from non descript buffaloes, sacrificed in and around Tirupati, Andhra Pradesh. The CRL was measured as a curved line along the vertebral column between the most anterior part of frontal bone to the ischiatic tuberosity (Edward, 1965). The approximate age of the foetus was determined on the basis of their CRL by using
Soliman’s formulae (1975). After measuring the CRL, the small embryos of less than 6.0 cm CRL were fixed as a whole, while from large embryos thyroid and parathyroid glands were dissected out and fixed in 10 percent neutral buffered formalin.

The fixed tissues were processed for paraffin blocks by acetone benzene schedule. The paraffin sections of 5-6 μm were subjected to Mayer’s haematoxylin and eosin for normal histomorphology, Gridley’s method for reticular fibres and Verhoeff’s method for elastic fibres (Luna, 1968).

RESULTS AND DISCUSSION

In the present study 0.9 cm CRL (26 days) embryo was the youngest in which five pairs of pharyngeal pouches were noted between the brachial arches on the lateral wall of the pharyngeal entoderm. The last one of these (fifth) was small and appeared to be absorbed into the fourth pharyngeal pouch resulting in the formation of caudal pharyngeal pouch complex. The pharyngeal pouches in buffalo were numbered I to IV in cephalocaudal sequence (Figure 1). Similarly, Kingsbury (1935), Noden and De Lahunta (1985) in cattle and Prasad and Singh (1990) in goat stated that the fifth pharyngeal pouch never attained the morphological value of a definite pouch. The first and second pouches were small and shallower, whereas the third pouch was the largest of all.

At 1.2 cm CRL (28 days) stage the entoderm of the third pharyngeal pouch showed extensive proliferation of epithelial cells that led to formation of a common primordium for both parathyroid and thymus. The dorsal part of this primordium was differentiated into a solid bar of tissue that transformed into external parathyroid glands. This parathyroid tissue was referred to as parathyroid III, which denoted its origin (Latshaw, 1987). The ventral part of the primordium was initially tubular and transformed into a definitive thymus in the subsequent stage of development. Similarly, Latshaw (1987) in domestic animals reported dorsal solid and ventral tubular portions in the primordium of the third pharyngeal pouch. The entodermal cells of the dorsal diverticulum of the fourth pharyngeal pouch developed in a manner similar to their counter-parts of the third pouch that led to formation of parathyroid IV (Figure 2). Latshaw (1987) termed these parathyroids as caudal or internal parathyroids in ruminants. The ultimobranchial bodies were developed from the caudal pharyngeal pouch at 2.5 cm CRL (Figure 2).

At 2.5 cm CRL stage (40 days) the parathyroid and thymic primordia lost their connection with the pharynx. According to Hendrickx (1964), the connection between the pharynx and primordium of the third pharyngeal pouch was lost between 26 days to 28 days of gestation in dog. Later the two primordia were separated from each other and migrated to their definitive locations.

The epithelial cells of the parathyroid primordium were recognizably different in structure from those of thymic primordium. The parathyroid primordium appeared more lightly stained than the thymic primordium and was devoid of lymphocytes. The parathyroid III primordium was placed dorsal to the thymic primordium (Figure 3). Whereas parathyroid IV placed dorsal to the thyroid primordium and 3rd aortic arch and maintained its integrity as caudal or internal parathyroids (Figure 4). These findings were in accordance with the observations of Ackerman and Knouff (1964) in the embryonic hamster. Further, they mentioned
that parathyroid gland showed strong PAS reaction in contrast with the thymus.

**Thyroid gland:**

In the present study, primordium of thyroid gland first noted as a median diverticulum of the floor of the pharynx at 1.2 cm CRL (28 days). Whereas, Boyd (1950) noted thyroid primordium in 17-18 somite embryos of human. Histogenesis of the thyroid gland began at about 2.5 cm CRL (40 days). At this stage, the thyroid primordium showed two distinct lobes on either side of the developing trachea and they were connected by unpaired isthmus ventrally (Figures 3 and 4). The cords of entodermal cells from the thyroid diverticulum eventually branched into isolated growths and they transformed into thyroid follicles. At this stage cells were darkly stained and showed distinct nucleus. At 19.5 cm CRL (115 days) the thyroid primordium showed formation of follicles and follicular epithelium, at this stage thyroid was highly vascular (Figure 5). This may facilitate entrance of thyroid hormones into the bloodstream as opined by Latshaw (1987). In the present study, at 31.0 cm CRL (143 days) characteristic thyroid follicles were noted in the thyroid of buffalo and they were lined by cuboidal epithelium. At this stage, a distinct colloid substance was also observed in the follicles (Figure 6). These findings suggested that the thyroid of buffalo was functional from early foetal life for normal growth and development as opined by Latshaw (1987) in ruminants. Further, he stated that thyroxine cannot pass through the placenta and the thyroid is capable of forming thyroxine by about 35 days or earlier and functioning during the second trimester in ruminants. Formation of a capsule around the gland was noticed from the surrounding mesenchyme at 19.5 cm CRL (115 days). This capsule was distinct at 31.0 cm CRL (143 days) and in later stages of development. In the present study reticular fibres were few and were in formative stage at 115 days of gestation. However, they were characteristically seen at 143 days (Figure 7) and in subsequent stages of development.

![Figure 1. Photomicrograph of buffalo embryo of 0.9 cm CRL (26 days) showing branchial arch I (B1), branchial arch II (B2), branchial arch III (B3), branchial arch IV (B4), Pharyngeal pouch I (P1), Pharyngeal pouch II (P1I), Pharyngeal pouch III (PIII), and Pharyngeal pouch IV (P4), Aortic arch III (A3), and Aortic arch IV (A4). H&E x100.](image)
Figure 2. Photomicrograph of buffalo embryo of 2.5 cm CRL (40 days) showing developing oesophagus (O), Trachea (Tr), Parathyroid primordium (P3), Thymic primordium III (T3), Aortic arch III (A3), Parathyroid IV (P4), Ultimobranchial bodies (U) and Thyroid primordium (Th). H&E x100.

Figure 3. Photomicrograph of buffalo embryo of 2.5 cm CRL (40 days) at higher magnification showing developing oesophagus (O), Trachea (Tr), Parathyroid primordium (P3), Thymic primordium III (T3), Aortic arch III (A3), Erythroblasts (E) and Thyroid primordium (Th). H&E x200.
Figure 4. Photograph of 31 cm CRL (143 days) buffalo foetus showing Thyroid (T) Parathyroid glands (P) and Isthmus (I).

Figure 5. Photomicrograph of buffalo foetus of 19.5 CRL (115 days) showing developing thyroid follicles and follicular epithelium (F) and blood vessels (B) around the follicles. H&E x400.
Figure 6. Photomicrograph of Buffalo foetus of 31 cm CRL (143 days) showing thyroid follicles and follicular epithelium (F) and colloid substance (C) and distinct parafollicular cells (P). H&E x400.

Figure 7. Photomicrograph of buffalo foetus of 31 cm CRL (143 days) showing well developed reticular fibres (R) between the thyroid follicles (F). Gridley’s x 400.
of thyroid in buffalo. Few elastic fibers were noted in the developing capsule and blood vessels of the capsule at 115 days of gestation.

The ultimobranchial bodies were incorporated into the thyroid gland at 2.5 cm CRL (40 days) and these cells positioned between the developing thyroid follicles and these cells were termed as parafollicular cells by Latshaw (1987). These cells can be easily distinguished midst the substance of the thyroid by their large size and pale staining properties.

REFERENCES


*Continued from page 1

Figure 2. Photomicrograph showing lymphocytic, polymorphocytic, lymphoblastic, histocytic types which were inter mixed cell types. H&E ×70.
NUTRITIONAL STATUS AND PROBABLE CAUSE OF HAEMOGLOBINURIA IN ADVANCED PREGNANT BUFFALOES OF INDORE DISTRICT OF MADHYA PRADESH

R.K. Jain*, C.M. Saksule and R.K. Dhakad

ABSTRACT

In Indore district of Madhya Pradesh haemoglobinuria is a common problem in buffaloes fed on leguminous straws (gram/masoor) especially during the mid to advanced stage of pregnancy under field conditions. To find out the nutritional causes behind this problem, 60 advanced pregnant buffaloes were selected randomly from villages in and around Mhow tehsil. These animals were divided into two groups with 30 in each, (i) a wheat-straw fed group (WSFG), 30 (Avg. b wt. 538.96±7.25 kg) and (ii) a gram-straw fed group (GSFG), 30 (Avg. b wt. 535.28±7.01 kg). Average daily feed intake of each animal was recorded and proximate principles, major minerals (Ca and P) and carotene in available feed stuffs were determined to find out nutrient availability. Deficiency of various nutrients was calculated by comparing with the standard requirements. DCP, TDN and carotene were calculated from reported values. The average daily availabilities of DM, DCP, TDN, Ca, P and carotene were 7.84±0.08 kg, 184.50±4.78 g, 3.21±0.04 kg, 18.27±0.23 g, 4.91±0.07 g and 49.68±0.23 mg, respectively in WSFG. While, in buffaloes of GSFG, the average daily availabilities of DM, DCP, TDN, Ca, P and carotene were 7.74±0.09 kg, 372.53±4.22 g, 3.25±0.04 kg, 118.51±1.20 g, 3.07±0.03 g and 48.72±0.49 mg, respectively. Results indicated shortage of DCP (60 and 20%), TDN (38 and 38%), P (80 and 87%) and carotene (53 and 54%) in the WSFG and the GSFG, respectively. Ca supply was in deficit (41%) in the WSFG while it was in excess (282%) in the GSFG. It was concluded that there was shortage of almost all nutrients in both the groups but very low P intake (13% of required) with adverse Ca:P intake ratio (39:1) may be the probable cause of haemoglobinuria in buffaloes fed on gram straw.

Keywords: buffaloes, haemoglobinuria, gram straw, hypophosphatemia, phosphorus deficiency

INTRODUCTION

In Indore district of Madhya Pradesh haemoglobinuria is the common problem in pregnant buffaloes (mid to advanced stage) specially fed on leguminous straws (gram/masoor) during summer months under field conditions. The higher proportion of masoor straw in the ration create adverse Ca:P ratio due to the high level of Ca in this straw, leading to hypophosphatemia which may be the cause of haemoglobinuria in advanced pregnant buffaloes (Jain et al., 2009). Haemoglobinuria is an acute disease of high yielding dairy animals characterized by intravascular haemolysis, haemoglobinuria and anaemia (Radostits et al., 2000). The exact cause

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of this problem in different parts of the country is not fully understood. Dietary phosphorus deficiency and/or diets containing cruciferous plants are suspected causes of haemoglobinuria. In depth understanding of existing feeding practices in advanced pregnant animals would help not only in getting a comprehensive account of the nutrient supply to animals but it would be also suggestive for prevention of many pre and post parturient disorders in dairy buffaloes. The present study was conducted to assess the existing feeding practices in advanced pregnant buffaloes in and around Mhow tehsil of Indore district of Madhya Pradesh to find out the nutritional causes behind haemoglobinuria for its prevention and control.

MATERIALS AND METHODS

To study the nutritional status and probable cause behind haemoglobinuria in advanced pregnant buffaloes, a survey of existing feeding practices was carried out. For this purpose, sixty advanced pregnant buffaloes were selected randomly from six villages in and around Mhow tehsil. These animals were divided into two groups, thirty in each, on the basis of the type of straw being fed to them (i) a wheat-straw-fed group (WSFG), 30 (Avg. b wt. 538.96±7.25 kg) and (ii) a gram-straw-fed group (GSFG), 30 (Avg. b wt. 535.28±7.01 kg). The body weight of buffaloes was determined by body measurement (Sastry et al., 1982). Average feed intake of each animal was calculated by measuring feed offered and residue left for three consecutive days. Representative samples of various feed stuffs (wheat straw, gram straw, green grass and green sorghum) being fed to advanced pregnant buffaloes were collected for analysis of proximate principles (AOAC, 1990), calcium (Talpatra et al., 1940) and phosphorus (AOAC, 1995) to find intake of CP (crude protein), Ca and P, DCP (digestible crude protein) and TDN (total digestible nutrient) were calculated by multiplying proximate principles with the digestibility coefficients reported in the literature (Sen et al., 1978). Carotene was also calculated from reported values (Ranjhan, 1991). On the basis of average daily feed intake of each animal and chemical composition of available feed stuffs, the nutrient supply was determined. Deficiency/excess of various nutrients was calculated by comparing the nutrient supply with the standard nutrient requirements (Ranjhan, 1998). Statistical analysis were carried out by using student’s ‘t’ test (single mean) described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

It was observed that most of the farmers practice stall feeding and offer feeds twice in a day, i.e., morning and evening, to advanced pregnant buffaloes. The majority of farmers were using wheat straw and gram straw as dry roughage; only a few farmers fed some quantity of green fodder, i.e., locally available green grass and green sorghum. None of the farmers was feeding concentrate. The average chemical composition and nutritive value of available feedstuffs are presented in Table 1. The average daily intake, requirements and deficiency / excess of nutrients in buffaloes are shown in Table 2. The average daily availabilities of DM, DCP, TDN, Ca, P and carotene were 7.84±0.08 kg, 184.50±4.78 g, 3.21±0.04 kg, 18.27±0.23 g, 4.91±0.07 g and 49.68±0.23 mg, respectively, in the WSFG. While, in buffaloes of the GSFG, the average daily availabilities of DM, DCP, TDN, Ca, P and carotene were 7.74±0.09 kg, 372.53±4.22 g, 3.25±0.04 kg, 118.51±1.20 g, 3.07±0.03 g and
Table 1. Average chemical composition and nutritive value of available feedstuffs (on DM basis).

<table>
<thead>
<tr>
<th>Feedstuffs</th>
<th>CP (%)</th>
<th>DCP* (%)</th>
<th>TDN* (%)</th>
<th>Ca (%)</th>
<th>P (%)</th>
<th>Carotene** (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat straw</td>
<td>3.95</td>
<td>0.31</td>
<td>42.07</td>
<td>0.25</td>
<td>0.06</td>
<td>1.00</td>
</tr>
<tr>
<td>Gram straw</td>
<td>6.24</td>
<td>2.80</td>
<td>42.81</td>
<td>1.54</td>
<td>0.04</td>
<td>1.00</td>
</tr>
<tr>
<td>Green grass</td>
<td>7.60</td>
<td>3.40</td>
<td>12.00</td>
<td>0.42</td>
<td>0.07</td>
<td>114.10</td>
</tr>
<tr>
<td>Green sorghum</td>
<td>4.80</td>
<td>3.10</td>
<td>13.00</td>
<td>0.40</td>
<td>0.11</td>
<td>276.00</td>
</tr>
</tbody>
</table>

Note: * Calculated values, ** Values based on literature.

Table 2. Average daily intake, requirements and deficiency / excess of nutrients in buffaloes.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Body wt (kg)</th>
<th>DMI (Kg)</th>
<th>CP (g)</th>
<th>DCP (g)</th>
<th>TDN (kg)</th>
<th>Ca (g)</th>
<th>P (g)</th>
<th>Carotene (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requirement (Ranjhan,1998)</td>
<td>550</td>
<td>9.3</td>
<td>-</td>
<td>465</td>
<td>5.2</td>
<td>31</td>
<td>24</td>
<td>105</td>
</tr>
<tr>
<td>Wheat straw fed group (n = 30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>538.96 ±7.25</td>
<td>7.84**a</td>
<td>607.77a</td>
<td>184.50**a</td>
<td>3.21**a</td>
<td>18.27**a</td>
<td>4.91*b</td>
<td>49.68**a</td>
</tr>
<tr>
<td>Deficit (g/d)</td>
<td>-</td>
<td>1.46</td>
<td>-</td>
<td>280.50</td>
<td>1.99</td>
<td>12.73</td>
<td>19.09</td>
<td>55.32</td>
</tr>
<tr>
<td>Deficit (%)</td>
<td>-</td>
<td>15.69</td>
<td>-</td>
<td>60.32</td>
<td>38.26</td>
<td>41.06</td>
<td>79.57</td>
<td>52.68</td>
</tr>
<tr>
<td>Gram straw fed group (n = 30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>535.28 ±7.01</td>
<td>7.74**a</td>
<td>774.22b</td>
<td>372.53*b</td>
<td>3.25**a</td>
<td>118.51*b</td>
<td>3.07**a</td>
<td>48.72**a</td>
</tr>
<tr>
<td>Deficit (-) / Excess(+) (g/d)</td>
<td>-</td>
<td>(-)</td>
<td>1.56</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Deficit (-) / Excess (%)</td>
<td>-</td>
<td>16.77</td>
<td>-</td>
<td>19.88</td>
<td>37.50</td>
<td>282.29</td>
<td>87.20</td>
<td>53.60</td>
</tr>
</tbody>
</table>

a,b Figures with different superscript in a column differ significantly, **P<0.01; *P<0.05.
48.72±0.49 mg, respectively. Results indicated shortage of DCP (60 and 20%), TDN (38 and 38%), P (80 and 87%) and carotene (53 and 54%) in WSFG and GSFG, respectively when compared with standard requirements (Ranjhan, 1998). Ca supply was deficit (41%) in WSFG while excess (282%) in GSFG. There was shortage of almost all nutrients in both the groups but very low P intake (13% of required) with adverse Ca: P intake ratio (39:1) may be the probable cause of haemoglobinuria in buffaloes fed on gram straw. While, in the WSFG, the Ca:P intake ratio was narrow i.e. 3.7:1. When intakes of the WSFG was compared with the GSFG, it was observed that CP, DCP, Ca intakes in the GSFG were significantly higher and P intake was significantly lower than the WSFG. The findings reported in the study were similar to those reported by Jain et al. (2009). In advanced gestation, more phosphorus and calcium are required for developing foetus, and if, supplementary phosphorus is not provided, hypophosphatemia may result (Knochel, 1977). The level of Ca in the diet is considered to have little effect upon the absorption of P provided the P level is adequate. However, when animals are fed P-deficient diets, high levels of Ca may reduce the absorption of P (Young et al., 1966; Schneider et al., 1985; Jain, 1993) due to either precipitation of the P in nonabsorable forms within the intestine as the pH rises or the homeostatic mechanisms concentrating on regulating plasma Ca.

It may concluded from the present study that dietary-P deficiency along with very wide Ca : P ratio may result in decreased phosphorus absorption from the intestinal tract leading to hypophosphatemia and which may cause haemoglobinuria.

REFERENCES


Research, New Delhi, India.
STANDARDIZED KARYOTYPE AND IDIOGRAM OF MEHSANI BUFFALOES, *Bubalus bubalis* BY CONVENTIONAL STAINING, GTG-BANDING, CBG-BANDING AND AG-NOR BANDING TECHNIQUES

A. Kenthao¹, A. Tanomtong²*, P. Supanuam¹, C. Pinyotepratan³, P. Muangprom², K. Buranarom³ and L. Sanoamuang¹

ABSTRACT

Standardized karyotype and idiogram of the Mehsani buffalo (*Bubalus bubalis*) at Burirum Livestock Research Station, Thailand, was studied. Blood samples were taken from two male and two female buffaloes. After standard whole blood lymphocytes were cultured at 37°C for 72 h in the presence of colchicine, the metaphase spreads were prepared on microscopic slides and air-dried. Conventional staining, GTG-banding, CBG-banding and Ag-NOR banding techniques were applied to stain the chromosomes. The results showed that the diploid chromosomes number of Mehsani buffaloes was 2n=50; the fundamental numbers (NF) were 60 in both male and female. The types of autosomes were four large metacentric, six large submetacentric, eight large telocentric, eight medium telocentric and 22 small telocentric chromosomes. The X chromosome was a large telocentric chromosome and the Y chromosome was a small telocentric chromosome. In GTG-banding, each chromosome pair appeared clearly differentiated. CBG-banding showed dark bands on the centromere of all telocentric chromosomes (autosome pairs 6-24 and the X chromosome) but light band on the others (autosome pairs 1-5 and the Y chromosome). Ag-NOR banding exhibited six position of autosomes (four telocentric and two submetacentric chromosomes) in Mehsani buffaloes. The karyotype formula of Mehsani buffaloes was as follows: 2n (diploid) 50 = Lm⁴ + Lsm⁶ + Lt⁸ + Mt⁸ + St²² + sex chromosomes.

Keywords: karyotype, idiogram, Mehsani buffalo (*Bubalus bubalis*), chromosome

INTRODUCTION

The river buffalo, *Bubalus bubalis* (Figure 1) is an economically important livestock species in many Asian and Mediterranean countries, and its genetic improvement, especially in reproductive performance and quantity of meat and milk production, ranks high among the agricultural research needs of these countries (El Nahas *et al.*, 2001).

The first international conference which established standard karyotypes in domestic animals was the Reading Conference (1976). GTG-banded karyotypes at medium (cattle and buffalo) and low band (sheep and goat) resolutions were presented without diagrammatic representations of the banding patterns. Later, the use of R-banding techniques and of prometaphase preparations made it neces-

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sary to organize a second international conference (ISCNDA 1989). Karyotypes with more elongated chromosomes (450 band levels) were proposed using various banding techniques: GTG, QFQ, RBA and RBG for cattle (BTA), RBA and RBG for both sheep and goat (Di Berardino et al., 2001).

As is known, the buffalo (*B. bubalis*) includes two cytotypes commonly referred to as the river buffalo (2n=50) and the swamp buffalo (2n=48). Several cytogenetic studies have been carried out to define the conventional karyotype (Fischer and Ulbrich, 1968; Chandra, 1968; De Hondt and Ghanam, 1971) as well as the distribution of constitutive heterochromatin and G-banding pattern of this species (Gupta and Ray-Chaudhuri, 1978; Cribiu and Obeidah, 1978).

Previous cytogenetic studies of river buffalo include Dutt and Bhattacharya (1952); Chandra (1968); Fischer and Ulbrich (1968); De Hondt and Ghanam (1971); Bongso et al. (1977, 1982); Cribiu and Obeidah (1978); Gupta and Ray-Chaudhuri (1978); Di Berardino et al. (1979); Di Berardino and Iannuzzi (1981, 1984); Chavananikul (1989); Yadav et al. (1991); Iannuzzi (1994); Iannuzzi and Di Meo (1995); Iannuzzi et al. (1987, 1990, 1998, 2003); Di Meo et al. (2002); Tanaka et al. (1999, 2000); El Nahas et al. (2001); Patel et al. (2006); Chauhan et al. (2009) and Murali et al. (2009).

**MATERIALS AND METHODS**

Blood samples were collected from two male and two female Mehsani buffaloes, kept at the Buriram Livestock Research Station (BLRS), Thailand by aseptic technique. The samples were kept in 10 ml vacuum tubes containing heparin to prevent blood clotting and cooled on ice until arriving at the laboratory.

**Cell preparation**

The lymphocytes were cultured using the whole blood microculture technique adapted from Rooney (2001) and Campiranont (2003).

**Cell culture**

The 5 ml of RPMI 1640 medium was prepared with 2% PHA (phytohemagglutinin) as a mitogen and kept in blood culture flasks. A blood sample of 0.5 ml was dropped into a medium bottle and mixed well. The culture bottles were loosely capped, incubated at 37°C under a 5% carbon dioxide environment and shaken regularly in the morning and evening. When harvest time was nearly reached at 72 h of incubation, colchicine was added and mixed well, followed by further incubation for 30 minutes.

**Cell harvest**

The blood sample mixture was centrifuging at 3,000 rpm for 5 minutes and the supernatant was discarded. Ten milliliters of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture incubated for 30 minutes. KCl was discarded from the supernatant after centrifugation again at 3,000 rpm for 5 minutes. Cells were fixed in fresh cool fixative (3 methanol: 1 glacial acetic acid) gradually added up to 8 ml before centrifuging again at 3,000 rpm for 5 minutes, and the supernatant was discarded. The fixation was repeated until the supernatant was clear and the pellet was mixed with 1 ml fixative. The mixture was dropped onto a clean and cold slide by micropipette followed by the air-dry technique. The slide was conventionally stained with 20% Giemsa’s solution for 30 minutes.

**GTG-banding method**

G-banding technique was adapted from
Campiranont (2003). The slide was well dried and then soaked in working trypsin (0.025% trypsin EDTA) at 37°C until the termination of trypsin activity by washing the slide with sorensen buffer. The slide was stained with 20% Giemsa’s solution for 30 minutes.

**CBG-banding method**

Slides were heated at 60°C for 2-3 days, soaked in 0.2 N HCl for 10-15 minutes, rinsed with distilled water then soaked in 0.05 N Ba(OH)₂ for 15 minutes at 37°C, rinsed with distilled water at 60°C, and then soaked in 2X SSC at 60°C for 1-2 h. The slides were stained with 20% Giemsa’s solution for 30 minutes.

**Ag-NOR staining method**

Two drops of 50% silver nitrate and 50% gelatin were placed on slides, and then they were sealed with cover glasses and incubated at 60°C for 3 h. Then, they were soaked in distilled water until the cover glasses were separated. The slides were then stained with 20% Giemsa’s solution for 1 minute.

**Chromosomal checks, karyotyping and idiograming**

Chromosome counting was performed on mitotic metaphase cells under a light microscope. Twenty clearly observable and well spread chromosomes from the males and 20 from the females were selected and photographed. The length of short arm chromosomes (Ls) and the lengths of long arm chromosomes (Ll) were measured and total arm length of the chromosomes were calculated (LT, LT = Ls+Ll). The relative length (RL) and the centromeric index (CI) were estimated. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiograming.

**RESULTS AND DISCUSSION**

Cytogenetic study of the Mehsani buffalo using lymphocyte culture revealed that the chromosome number is 2ₙ (diploid) = 50 (Figures 2 and 6). This is the same chromosome number reported for river buffaloes in previous studies (Dutt and Bhattacharya, 1952; Chandra, 1968; Fischer and Ulbrich, 1968; De Hondt and Ghanam, 1971; Bongso et al., 1977, 1982; Cribiu and Obeidah, 1978; Gupta and Ray-Chaudhuri, 1978; Chavanakul, 1989; Yadav et al., 1991; Iannuzzi, 1994; Iannuzzi and Di Meo, 1995; Iannuzzi et al., 1987, 1990, 1998, 2003; Di Meo et al., 2002; Tanaka et al., 1999, 2000; El Nahas et al., 2001; Patel et al., 2006; Chauhan et al., 2009 and Murali et al., 2009).

The domestic buffalo (B. bubalis) has been classified into two general types according to geographical distribution: one is the river-type buffalo, raised in most areas from India to Egypt and in some southern and eastern European countries; the other is the swamp-type buffalo of South-east Asia (Mason, 1974). The karyotypes differ in the two types of buffalo, and their diploid chromosome numbers are 48 and 50 in the swamp type buffalo and the river type buffalo, respectively (Fischer and Ulbrich, 1968). The karyotypes of the two types of buffaloes differ due to tandem fusion translocation; the swamp-type chromosome 1 resulted from a telomere-centromere tandem fusion between the river type chromosome 4, and 9, with a loss of the centromere of river-type chromosome 9 (Bongso and Hilmi 1982; Di Berardino and Iannuzzi 1981; Tanaka et al., 1999).
This examination also revealed that the fundamental number (NF, number of chromosome arms) of the Mehsani buffalo was 60 in both the male and the female. This is the same NF for the river buffalo as reported by Chandra (1968); De Hondt and Ghanam (1971); Bongso et al. (1977) and Iannuzzi (1994). The family Bovidae includes several species demonstrating variable diploid chromosome numbers but having similar fundamental numbers (NF=60), which, with the exception of a few cases, vary between 58 and 62. The karyotype contains variable numbers of centric fusions, or Robertsonian translocations, which have changed the diploid number but not the NF (Wurster and Benirschke, 1968). These rearrangements of a basic karyotype consisting of one-armed chromosomes have later been confirmed by studies using banding techniques in various species of Bovidae (Evans et al., 1973; Buckland and Evans, 1978; Bunch and Nadler, 1980; Di Berardino and Iannuzzi, 1981, 1984).

The types of autosomes were four large metacentric, six large submetacentric, eight large telocentric, eight medium telocentric and 22 small telocentric chromosomes. These features are similar to the reports of Chandra (1968); De Hondt and Ghanam (1971); Bongso et al. (1977); Iannuzzi (1994) and Murali et al. (2009). The X chromosome of the Mehsani buffalo is a large telocentric chromosome, and the Y chromosome is the small a telocentric chromosome. These features are similar to the reports of Di Berardino and Iannuzzi (1981) and Di Meo et al. (2005) that revealed river buffalo have telocentric X and Y chromosomes. In comparison, in the other ruminant species in the family Bovidae in Thailand, the X chromosomes of swamp buffalo (B. bubalis), gaur (B. gaurus), banteng (B. javanicus), cattle (Bos taurus) and cattle (Bos indicus) are telocentric, submetacentric, submetacentric, submetacentric and submetacentric chromosome, respectively. The Y chromosomes of all these species are telocentric, metacentric, submetacentric, submetacentric and acrocentric chromosome, respectively (Wurster and Benirschke, 1968).

From GTG-banding technique, each chromosome pair appears with distinctively differentiated. The G-banded revealed that the number of bands on 1 set of haploid chromosomes, which includes autosomes, X and Y chromosomes, are 346 bands for the Mehsani buffalo (Figuers 3 and 7). The G-banded provide a clearly chromosome band which represent in black and white regions on chromosome. The level of band numbers is defined by a visible and in a haploid set which compose of autosomes, X and Y chromosome. Thus, the haploid set of the Mehsani buffalo consist of 24 autosomes include X and Y chromosome.

CBG-banding technique demonstrated dark bands (C-positive) on centromere of all telocentric chromosomes (pairs 6-24 autosome and X chromosome) but other appears as light bands or C-negative (pairs 1-5 autosome and Y chromosome) (Figures 4 and 8). The C-banding can provide a dark region on chromosome which represents the constitutive heterochromatin of chromosome that can be found at all centromeres and some telomeres of normal chromosomes. C-banding is being accepted technique for the sex chromosome studying, especially for the identification of Y chromosome because of its individual characteristics that normally cannot provide a dark region on the centromere (Campiranont, 2003).

In this investigation, the six nucleolar organizer regions, NORs (satellite chromosomes), which represent the chromosome marker, are located on the long arm near telomere of two pairs
Table 1. Mean of the short arm chromosome length (Ls), the long arm chromosome length (Ll), total arm chromosome length (LT), relative length (RL), centromeric index (CI), chromosome size and chromosome type from metaphase chromosomes of 20 cells in male and female the Mehsani buffalo (*Bubalus bubalis*), 2n (diploid) = 50.

<table>
<thead>
<tr>
<th>Chromosome Pair</th>
<th>Ls (cm)</th>
<th>Ll (cm)</th>
<th>LT (cm)</th>
<th>RL</th>
<th>CI</th>
<th>Size</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.359</td>
<td>0.820</td>
<td>1.179</td>
<td>0.071</td>
<td>0.695</td>
<td>L</td>
<td>sm</td>
</tr>
<tr>
<td>2</td>
<td>0.342</td>
<td>0.736</td>
<td>1.078</td>
<td>0.063</td>
<td>0.682</td>
<td>L</td>
<td>sm</td>
</tr>
<tr>
<td>3</td>
<td>0.453</td>
<td>0.554</td>
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<td>0.060</td>
<td>0.550</td>
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<td>m</td>
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<tr>
<td>4</td>
<td>0.347</td>
<td>0.638</td>
<td>0.985</td>
<td>0.053</td>
<td>0.647</td>
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<td>sm</td>
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<td>0.591</td>
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<td>1.000</td>
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<td>t</td>
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<tr>
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<td>t</td>
</tr>
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<td>0.499</td>
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<td>S</td>
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<td>0.327</td>
<td>0.327</td>
<td>0.024</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>21</td>
<td>0.000</td>
<td>0.313</td>
<td>0.313</td>
<td>0.023</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>22</td>
<td>0.000</td>
<td>0.293</td>
<td>0.293</td>
<td>0.021</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>23</td>
<td>0.000</td>
<td>0.269</td>
<td>0.269</td>
<td>0.019</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>24</td>
<td>0.000</td>
<td>0.240</td>
<td>0.240</td>
<td>0.017</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>X</td>
<td>0.000</td>
<td>0.892</td>
<td>0.892</td>
<td>0.065</td>
<td>1.000</td>
<td>L</td>
<td>t</td>
</tr>
<tr>
<td>Y</td>
<td>0.000</td>
<td>0.306</td>
<td>0.306</td>
<td>0.027</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
</tbody>
</table>

Remarks: L = large chromosome, M = medium chromosome, S = small chromosome, m = metacentric chromosome, sm = submetacentric chromosome, and t = telocentric chromosome.
Figure 1. Mehsani buffalo, *Bubalus bubalis* (Artiodactyla, Bovidae).
Figure 2. Metaphase chromosome plates (left) and karyotypes (right) of Mehsani buffalo (*Bubalus bubalis*) $2n$ (diploid) = 50 by conventional staining technique, showing sex chromosomes (arrows), scale bars 10 $\mu$m.
Figure 3. Metaphase chromosome plates (left) and karyotypes (right) of Mehsani buffalo *(Bubalus bubalis)*, 2n (diploid) = 50 by GTG-banding technique, showing sex chromosomes (arrows), scale bars 10 μm.
Figure 4. Metaphase chromosome plates (left) and karyotypes (right) of Mehsani buffalo (*Bubalus bubalis*) $2n$ (diploid) = 50 by CBG-banding technique, showing sex chromosomes (arrows), scale bars 10 μm.
Figure 5. Metaphase chromosome plates of male (top) and female (bottom) Mehsani buffalo (*Bubalus bubalis*) $2n$ (diploid) = 50 by Ag-NOR banding technique, showing nucleolar organizer regions, (arrows), scale bars 10 μm.
Figure 6. Idiogram of Mehsani buffalo (*Bubalus bubalis*) 2n=50 by conventional staining technique.
Figure 7. Idiogram of Mehsani buffalo (*Bubalus bubalis*) 2n=50 by GTG-banding technique.
Figure 8. Idiogram of Mehsani buffalo (*Bubalus bubalis*) 2n=50 by CBG-banding technique.
telocentric autosome (four positions) and on the short arm near telomere of one pair submetacentric autosome (two positions) (Figure 5). In contrast, Di Berardino and Iannuzzi (1981) indicated that NORs of the swamp buffalo and river buffalo appear on the long arm near centromere of the pair autosomes \(4_p, 8, 20, 22, 23\) (10 positions) and \(3_p, 4_p, 8, 21, 23, 24\) (12 positions), respectively. By comparing the two types of buffalo, it was concluded that all of the chromosomes are similar in banding patterns that chromosome 1 of swamp results from a telomere-centromere tandem fusion between two chromosomes identified as \(4_p\) and \(9\), respectively, in the river buffalo karyotype, thus accounting for the reduced diploid number of swamp buffalo; that the fusion causes the loss of NOR’s on the telomeres of chromosome 4, thus accounting for the reduced number of NOR chromosome pairs of swamp; that the presence of a pale C-banded are in the region of junction between chromosome 4 and 9 involved in the fusion suggests that the centromeric region of the later has been.

The chromosome of mitotic metaphase cells and the karyotypes of Mehsani buffalo in male and female by conventional staining, GTG-banding, CBG-banding and Ag-NOR banding technique are shown in Figures 2, 3, 4 and 5. The lengths of chromosomes in centimeters of (20 male and 20 female) cells, in mitotic metaphase were measured. The mean length of short arm chromosome (\(L_s\)), length of long arm chromosome (\(L_l\)), total length of arm chromosome (\(L_T\)), relative length (\(RL\)), centromeric index (\(CI\)), size and type of chromosome are presented in Table 1. The idiomgram of Mehsani buffalo shows gradually decreasing length of the autosomes (Figures 6, 7 and 8).

The Mehsani buffalo revealed that the chromosome marker is the chromosome pair 1, which is the largest telocentric chromosome. The important karyotype feature of Mehsani buffalo is the asymmetrical karyotype, which is all three types of chromosomes were found (metacentric, submetacentric and telocentric chromosome). The largest and smallest chromosomes show difference size (approximately 5 fold). The karyotype formula of Mehsani buffalo was as follows:

\[
2n (diploid) = L_{m}^4 + L_{sm}^6 + L_{l}^1 + M_{8}^1 + S_{12}^1 + \text{sex chromosomes.}
\]

ACKNOWLEDGEMENTS

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This study investigated the production efficiency of Swamp buffaloes and Mehsana river buffaloes under ordinary Thai conditions. The results showed that Swamp buffaloes raised under grazing with the roughage supplementation every morning and evening had significantly higher average weight gain and growth than other groups (P>0.01), while the heart girth and body length of Swamp buffaloes were similar (P>0.05). However, the Mehsana river buffaloes raised under grazing and under grazing with roughage supplementation every morning and evening gained more weight and had higher growth than buffaloes raised in the pen because they could eat more feed.

This study also investigated the production efficiency of Swamp buffaloes under river basin conditions. The results showed that buffaloes grazing on land made higher gains in weight on average than those grazing on land and in water or those grazing in water, but the average heart girths and body heights of all groups were close (P>0.05). Swamp buffaloes grazing on land had higher average higher weight gain and growth because the usual aquatic plants on which the buffaloes fed, such as water snowflake and tape grass, were low in dry matter.

Keywords: Swamp buffaloes, Mehsana river buffaloes, production efficiency, grazing, aquatic plants

INTRODUCTION

At this time when the number of buffaloes is declining, raising buffaloes as a primary occupation is very attractive. Buffaloes are easy to raise, the costs involved in raising them are low, and they can grow very well with low quality native forage. They can be used for cultivation under the self-sufficiency economy, and their dung can be soil fertilizer to reduce expenditure on chemical fertilizer and preserve the environment in line with the Thai government’s policy supporting the restructuring of production toward more sustainable and organic agriculture. Moreover, buffalo raising can reduce import of buffalo meat and live buffaloes from abroad. (Jintana, 2008)

MATERIALS AND METHODS

In the first experiment, the production efficiencies of Swamp buffaloes and of Mehsana river buffaloes were studied under ordinary Thai conditions.
conditions using 24 females 18-36 months of age for experimentation. They were divided into three groups: Group 1 animals were grazed, Group 2 were grazed and given roughage supplementation every morning and evening, and Group 3 were pen raised. The experiment lasted 120 days.

In the second experiment, the production efficiencies of Swamp buffaloes were studied under river basin conditions. Nine males, 18-36 months of age, were divided into three groups. Group 1 were grazed on land, Group 2 were grazed on land for 4 h a day and in water for 4 h a day, and Group 3 were grazed in water for 8 h a day. The experiment lasted 120 days.

The data of the two experiments were analyzed statistically using analysis of variance of randomized complete block design to compare the averages using Duncan’s new multiple range test. (Steel and Torrie, 1980).

**RESULTS AND DISCUSSION**

The production efficiency of Swamp buffaloes and Mehsana river buffaloes under ordinary conditions

*The Production Efficiency of Swamp buffaloes*

Group 2 buffaloes, given roughage supplementation, consumed 23.10 to 29.75 kg of fresh roughage (Napier grass) a day, and Group 3 buffaloes, raised in a pen, consumed 34.90 to 38.80 kg of fresh roughage a day (13.20 percent body weight). These groups of buffaloes ate native grass and Napier grass, which are low in nutrients (Table 1).

Group 2 buffaloes, which were given some roughage supplementation, gained more weight and had a higher rate of growth (P<0.01) than Group 1 buffaloes, which were grazed, because Group 2 consumed more feed. (Table 2)

The gains in heart girth, body length and body height of the three groups of buffaloes were very close (P>0.05) because they were at growth age and the amounts of food eaten were not very different. This amount of feed could not produce a difference (Table 2).

*The production efficiency of Mehsana river buffaloes*

The buffaloes of Group 2, given roughage supplementation, consumed 20-25 kg of fresh roughage (Napier grass) a day while the pen-raised Group 3 buffaloes, ate 40 - 45 kg (12.82 percent body weight) of fresh roughage a day. These buffaloes ate Ruzi grass, which is low in nutrients. (Table 1)

Group 1 buffaloes, raised by grazing, and Group 2 buffaloes, raised by grazing with roughage supplementation, gained more weight and had a higher rate of growth (P<0.01) than the pen-raised buffaloes of Group 3 because this group could not have the feed they liked (Table 3).

The gains in heart girth, body length and body height of the three groups of buffaloes were close (Table 3). The way the buffaloes were raised did not affect heart girth (P>0.05), but there was tendency for Group 3 buffaloes, raised in a pen, to have a higher heart girth than either the grazed Group 1 of the grazed and supplemented Group 2.

The average gains in body height of the buffaloes of Groups 1 and 2 were greater (P<0.01) than the average gain of Group 3 because this pen-raised group was able to have less feed (Table 3).
Table 1. Chemical Composition of Different types of Roughage (%).

<table>
<thead>
<tr>
<th>Kinds of Roughage</th>
<th>Dry Matter</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>NDF</th>
<th>ADF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Grass</td>
<td>27.20</td>
<td>5.07</td>
<td>0.97</td>
<td>7.55</td>
<td>71.47</td>
<td>43.48</td>
</tr>
<tr>
<td>Ruzi Grass</td>
<td>27.79</td>
<td>5.81</td>
<td>0.98</td>
<td>8.94</td>
<td>71.89</td>
<td>42.97</td>
</tr>
<tr>
<td>Napier grass</td>
<td>22.39</td>
<td>5.83</td>
<td>1.42</td>
<td>8.24</td>
<td>68.42</td>
<td>49.54</td>
</tr>
<tr>
<td>Tape grass</td>
<td>4.35</td>
<td>6.85</td>
<td>0.73</td>
<td>8.47</td>
<td>71.62</td>
<td>46.78</td>
</tr>
<tr>
<td>Water Snowflake</td>
<td>8.34</td>
<td>11.64</td>
<td>1.10</td>
<td>7.81</td>
<td>68.42</td>
<td>51.82</td>
</tr>
</tbody>
</table>

Table 2. Production efficiency of Swamp buffaloes.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of experimentation (days)</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Average weight gain (kg)</td>
<td>42.94</td>
<td>68.25</td>
<td>61.25</td>
</tr>
<tr>
<td>Rate of growth (g/day)</td>
<td>357.81</td>
<td>568.75</td>
<td>510.42</td>
</tr>
<tr>
<td>Heart girth gain (cm)</td>
<td>11.21</td>
<td>11.84</td>
<td>9.50</td>
</tr>
<tr>
<td>Body length gain (cm)</td>
<td>14.00</td>
<td>15.53</td>
<td>14.94</td>
</tr>
<tr>
<td>Body height gain (cm)</td>
<td>5.45</td>
<td>5.92</td>
<td>6.38</td>
</tr>
</tbody>
</table>

Different superscripts in a row show a significant difference (P<0.01).

Note: Group 1 = grazed, Group 2 = grazed with roughage supplementation every morning and evening and Group 3 = pen raised.

Table 3. The production efficiency of Mehsana river buffaloes.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of experimentation (days)</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Average weight gain (kg)</td>
<td>62.63</td>
<td>64.00</td>
<td>46.12</td>
</tr>
<tr>
<td>Rate of growth (g/day)</td>
<td>521.88</td>
<td>533.34</td>
<td>384.38</td>
</tr>
<tr>
<td>Heart girth gain (cm)</td>
<td>9.75</td>
<td>9.87</td>
<td>11.12</td>
</tr>
<tr>
<td>Body length gain (cm)</td>
<td>6.75</td>
<td>6.50</td>
<td>6.75</td>
</tr>
<tr>
<td>Body height gain (cm)</td>
<td>6.27</td>
<td>6.55</td>
<td>4.67</td>
</tr>
</tbody>
</table>

Different superscripts in a row show a significant difference (P<0.01).

Note: Group 1 = grazed, Group 2 = grazed with roughage supplementation and Group 3 = pen-raised.
Table 4. Comparison of production efficiency of Swamp buffaloes and Mehsana river buffaloes.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Grazing</th>
<th>Roughage Supplementation</th>
<th>Raising in the Pen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swamp</td>
<td>Mehsana</td>
<td>Swamp</td>
</tr>
<tr>
<td>Period of experimentation (days)</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Average weight gain (kg)</td>
<td>42.94</td>
<td>62.63</td>
<td>52.78</td>
</tr>
<tr>
<td>Rate of growth (g/day)</td>
<td>357.81</td>
<td>521.88</td>
<td>439.84</td>
</tr>
<tr>
<td>Heart girth gain (cm)</td>
<td>11.21</td>
<td>9.75</td>
<td>10.48</td>
</tr>
<tr>
<td>Body length gain (cm)</td>
<td>14.00</td>
<td>6.75</td>
<td>10.38</td>
</tr>
<tr>
<td>Body height gain (cm)</td>
<td>5.45</td>
<td>6.28</td>
<td>5.86</td>
</tr>
</tbody>
</table>

Different superscripts in a row show a significant difference (P<0.01).
The comparison of production efficiency of Swamp buffaloes and Mehsana river buffaloes

A comparison of the production efficiencies of the Swamp buffaloes and the Mehsana river buffaloes (Table 4) showed that the Group 2 buffaloes, given some roughage supplementation every morning and evening, had higher rates of growth (P<0.01) than either those of Group 1, raised by grazing without supplementation, or the pen-raised animals of Group 3 because Group 2 were able to have more feed. However, the average heart girth, body length and body height gains of the three groups were similar (P>0.05).

Both Swamp and Mehsana River buffaloes gained weight, and the average weight gain was similar (P>0.05). These two kinds of buffaloes were able to adjust themselves to living in humid weather (Table 5).

Both Swamp buffaloes and Mehsana river buffaloes should be raised by grazing with roughage supplementation every morning and evening so that they obtain enough nutrients. If they receive a lot of nutrients, their growth is greater.

The production efficiency of buffaloes under river basin conditions

Both water snowflake and tape grass contain good nutrients, but they have low dry matter and made the buffaloes full quickly (Table 1). As a result, the production efficiency of buffaloes under the river basin conditions was low. However, both water snowflake and tape grass can be fed to buffaloes during feed shortages.

Group 1 buffaloes, grazed on land, tended

---

Table 5. Comparison of production efficiency in Swamp and Mehsana buffaloes.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Swamp buffaloes</th>
<th>Mehsana buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of experimentation (days)</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Average weight gain (kg)</td>
<td>57.48</td>
<td>57.58</td>
</tr>
<tr>
<td>Rate of growth (g/day)</td>
<td>478.99</td>
<td>479.86</td>
</tr>
<tr>
<td>Body length gain (cm)</td>
<td>10.85</td>
<td>10.25</td>
</tr>
<tr>
<td>Body length gain (cm)</td>
<td>14.82a</td>
<td>6.67a</td>
</tr>
<tr>
<td>Body height gain (cm)</td>
<td>5.92</td>
<td>5.83</td>
</tr>
</tbody>
</table>

Different superscripts in a row show a significant difference (P<0.01).

Table 6. Production efficiency of Swamp buffaloes raised under river basin condition.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of experimentation (days)</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Average weight gain (kg)</td>
<td>58.67</td>
<td>49.50</td>
<td>48.00</td>
</tr>
<tr>
<td>Rate of growth (g/day)</td>
<td>488.89</td>
<td>412.50</td>
<td>400.00</td>
</tr>
<tr>
<td>Heart girth gain (cm)</td>
<td>8.33</td>
<td>9.42</td>
<td>8.63</td>
</tr>
<tr>
<td>Body length gain (cm)</td>
<td>16.55</td>
<td>12.52</td>
<td>11.05</td>
</tr>
<tr>
<td>Body height gain (cm)</td>
<td>3.22</td>
<td>3.38</td>
<td>3.37</td>
</tr>
</tbody>
</table>

Note: Group 1 = grazed on land, Group 2 = grazed on land and in water and Group 3 = grazed in water.
to have slightly higher weight and rate of growth (P>0.05) than either Group 2, grazed in water and in water, or Group 3, grazed in water (Table 6) because the first group was able to graze all day.

The gains in hearth girth, body length, and body height of the three group of buffaloes were similar (P>0.05).

REFERENCES


POLYMORPHISM IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN INDIAN BUFFALOES

A.K. Das and N.K. Majumder

ABSTRACT

Erythrocytic G-6-PD was studied for its activity and electrophoretic pattern in 1527 Indian buffaloes for its polymorphism. The overall G-6-PD activity was found highest in the normal phenotype as 915.55±6.61 miu. and lowest in the deficient phenotype as 260.46±7.76 miu. Whereas in heterozygote phenotypes, it was found intermediate as 592.66±9.44 miu. Electrophoretic variants were studied by starch gel and polyacrelamide gel electrophoresis. The electrophoretic pattern of erythrocytic G-6-PD was identical in all buffalo breeds, regardless of whether G-6-PD activity was normal or deficient.

Keywords: Indian buffaloes, G-6-PD, polymorphism, enzyme

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G-6-PD) is an important enzyme in the intermediary metabolism. It induces the glucose metabolism via the pentose phosphate pathway. Glucose is at first converted into glucose-6-phosphate, which by means of glucose-6-phosphate dehydrogenase, is then converted into 6-phospho gluconolactone. Genetic variants of this enzyme have been demonstrated in erythrocytic haemolysates of Malaysian water buffalo, horse, cattle, mouse and human (Tan et al., 1980, Mathai et al., 1966, Probeck, H.D. and Geldermann, H., 2009, Ruddle et al., 1968, Tinley et al., 2010).

Many studies reveal a G-6-PD deficiency in various animals. However, Naik et al. (1971) using electrophoretic resolution noticed that in dog and cattle, the enzyme band was similar regardless of whether the activity was normal or deficient. The activity of this enzyme seems therefore to be independent of electrophoretic pattern. A deficiency has also been observed in man (Fenu et al., 1982).

So far no G-6-PD polymorphism has been reported in the Indian buffaloes, so this work has been undertaken to find out the polymorphism of G-6-PD in Indian breeds of buffaloes.

MATERIALS AND METHODS

Blood samples from 1527 buffaloes of five different breeds, viz., Murrah, Jaffarabadi, Bhadawari, Surti, Nili-Ravi and non-descript buffaloes were tested. Blood samples were collected in an anticoagulant solution and immediately after collection shifted to the laboratory after packing in an ice pack. All the blood samples were screened for G-6-PD phenotypes by screening methods originally described by Bernstein (1962) by using
2, 6-dichlorophenol indophenol. On the basis of the screening test, three G-6-PD phenotypes were classified: normal, heterozygote and deficient. The G-6-PD activity levels were estimated by colorimetric methods using the technique described by Ells and Kirkman (1962).

The electrophoretic characterization of G-6-PD variants was done by starch gel and polyacrylamide gel electrophoresis. The starch gel electrophoresis was performed by the methods described by the WHO technical report series (1967). Starch gel electrophoresis for G-6-PD variants was standardized for concentration, pH of buffer, molarity of the solution, different haemolysate concentration voltage and running time. A 13% suspension of hydrolyzed starch at final concentration of 10 μM was used. The gel buffer solution was prepared by adding 150 ml of 0.5 M Tris chloride at pH 8.8 (25°C) to 15 ml of 0.27 M sodium EDTA at pH 7.0 finally added to 1350 ml of distilled water. The run was made at 2°C for 14-16 h. at a gradient of 160 V. Then, the gel was stained with the following solution: 10 ml 0.1M Tris-HCL buffer (pH 8.0), 10 mg glucose-6-phosphate (Na salt), 2 mg NADP (nicotamide adenine dinucleotide phosphate), 2 mg MTT, and 2 mg PMS. After covering the gel with freshly prepared staining solution, it was incubated at 37°C in a closed box. The G-6-PD variants developed as blue-violet zones in about 30 minutes.

Polyacrylamide gel electrophoresis (PAGE) was performed as by Davis (1964) using a set manufactured by Atto Corporation, Tokyo, Japan after standardization for running time and voltage applied to the gel. The electrophoresis was carried out at a constant current of 1 mA/gel for first 10 minutes and then 3 mA/gel for 6-8 h. The gel was taken out and was stained with a G-6-PD specific staining method for 4-6 h. in staining solution. When bands were clear the gel was preserved in cold water.

RESULTS AND DISCUSSION

Three G-6-PD phenotypes, normal, heterozygote and deficient were observed on the basis of the screening test. The overall proportions of normal, heterozygote and deficient G-6-PD phenotypes were found as 26.0, 66.27 and 7.73%, respectively, in Indian buffaloes. Whereas, the percentage of G-6-PD deficient animals were found highest in non-descript buffaloes (10.87%) followed by Jaffarabadi (6.61%), Surti (3.23%), Murrah (0.96%) and Bhadawari breed (0.81%) as shown in Table 1. On the basis of gene frequency and genetic distance, it appears that these breeds belong to three distinct groups. Group 1 consists of Jaffarabadi, non-descript and Surti, Group 2 consists of Nili-Ravi and Group 3 consists of Murrah and Bhadawari. The gene frequency of breeds of Group 1 and Group 3 were significantly different from each other, but they did not differ significantly within the group, whereas breeds of Group 3 did not differ significantly from either Group 1 or Group 2. G-6-PD deficient phenotypes were not observed in Nili-Ravi buffaloes. Significant differences in the frequency of G-6-PD deficiency were noted among the different breeds of buffaloes. Among Murrah breed the frequency was 0.26±0.02, which was found lowest, and for Jaffarabadi as 0.47±0.03 as highest.
The starch gel electrophoretic pattern of G-6-PD is shown in Figure 1 and polyacrylamide gel electrophoresis in Figure 2. In starch gel electrophoresis, all samples were alike in having one band, and in polyacrylamide gel electrophoresis, 2 bands regardless of whether activity was normal or deficient. Activity of the enzyme, therefore, seems to be independent of its electrophoretic pattern. A similar finding was also reported by Naik et al. (1971) in dog and cattle. The G-6-PD deficiency is a sex-linked trait in man and other animals (Mathai et al., 1966), but the inheritance pattern is not identifiable from the present study.

Further study is required for determining the mode of inheritance. Erythrocytic G-6-PD activity has been studied in many species of animal and has been shown to be homomorphic in some, i.e., it is either present or absent in all sampled populations of the species (Cheun, 1966; Naik and Anderson, 1970a). Other species, however, such as cattle, pig and dog are polymorphic. In spite of variation in enzyme activity, starch gel electrophoretic studies of the enzyme reveal no protein polymorphism in buffalo. This suggests that the mutation that has lead to loss of activity is not accompanied by a change in the electric charge of the protein molecule.

### Table 1. Frequency of G-6-PD deficient gene in different breeds of buffalo.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>No. Examined</th>
<th>Normal</th>
<th>Deficient</th>
<th>Percent Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murrah</td>
<td>209</td>
<td>0.74±0.02</td>
<td>0.26±0.02</td>
<td>0.96</td>
</tr>
<tr>
<td>Jaffarabadi</td>
<td>121</td>
<td>0.53±0.47</td>
<td>0.47±0.03</td>
<td>6.61</td>
</tr>
<tr>
<td>Bhadawari</td>
<td>123</td>
<td>0.73±0.02</td>
<td>0.27±0.02</td>
<td>0.81</td>
</tr>
<tr>
<td>Surti</td>
<td>93</td>
<td>0.60±0.03</td>
<td>0.40±0.03</td>
<td>3.22</td>
</tr>
<tr>
<td>Nili-Rabi</td>
<td>24</td>
<td>0.70±0.06</td>
<td>0.30±0.06</td>
<td>0.0</td>
</tr>
<tr>
<td>Non-Descript</td>
<td>957</td>
<td>0.54±0.01</td>
<td>0.46±0.01</td>
<td>10.86</td>
</tr>
<tr>
<td>Overall</td>
<td>1527</td>
<td>0.59±0.008</td>
<td>0.41±0.008</td>
<td>7.73</td>
</tr>
</tbody>
</table>

Figure 1. Starch gel electrophoretic pattern of G-6-PD in buffalo. Slots 1 and 2 are deficient, Slots 3, 4 and 5 are heterozygote and 6,7 and 8 are normal buffalo samples.

Figure 2. Polyacrylamide gel electrophoretic pattern of G-6-PD in buffalo. Slots 1,4 and 7 are deficient, 2,5 and 8 are heterozygote and slots 3,6 and 9 are normal samples.
The possibility of the enzyme being destroyed or altered in deficient samples, which sometimes occur in human samples, may be dismissed in view of the equal staining intensity of the bands (Figure 1). Another explanation for this finding could be the involvement of two closely linked loci, one controlling the activity and the other structure of the enzyme as per the model proposed by Jacob and Monod (1961). The higher frequency of G-6-PD deficiency in the Jaffarabadi and non-descript compared to the other breeds should be confirmed or denied with large samples. The high frequency of G-6-PD deficiency in these breeds might be the consequence of their close genetic relationship.

It has been shown that incidence of cancer is inversely proportional to G-6-PD deficiency in American Negroes (Naik and Anderson, 1970b). G-6-PD deficiency in man also affords protection against malarial infection (Motulsky et al., 1966). A similar phenomena has been suggested in sheep and goats to explain their resistance to trypanosomiasis (Khanolkar et al., 1963). It would be interesting to determine if buffalo that are deficient in G-6-PD activity might also be better adapted to areas that are endemic for trypanosomiasis, tick infection, and tick-borne blood protozoan disease. In this study we have found a good correlation between health status and G-6-PD activity in buffalo. Healthy buffalo shows higher G-6-PD activity as compared to emaciated buffalo.

REFERENCES


Naik, S.N. and D.E. Anderson. 1970a. Study of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the


The effect of tropical fasciolosis on the adrenal gland dysfunction was investigated in male Murrah buffalo yearlings. Eight animals were randomly assigned to two groups (Groups 1 and 2) of four animals each. Animals in Group 1 were administered per os with the primary infection dose of 800 viable *F. gigantica* metacercariae, whereas Groups 2 animals served as healthy controls. The Group 1 animals sequentially developed the characteristic clinical manifestations of tropical fasciolosis and had prepatent period of 92-95 days. A significantly higher serum cortisol concentration (p<0.05) was persistently observed from week-4 post-infection (PI) onwards in Group 1 animals. The highest cortisol level (31.2±4.2 nmol/L) suggestive of *F. gigantica* induced persistent stress, declined from week-10 PI onwards yet it remained higher in the disease hosts than the healthy controls. Further it was also associated with eosinophilia (14.0±1.8%) and leucocytosis (9.5±0.2x10^3/cmm). Interestingly, hypercortisolemia in Group 1 animals was the highest (82.97%) during early prepatency followed by 38.6% in late prepatency and 6.2% in the patency phase of the disease. Evidently, animals in Group 1 persistently suffered from variable degrees of stress in comparison with the healthy controls.

On necropsy, the histopathological picture of zona fasciculata revealed hypertrophic corticocytes with altered shape, arrangement and vacuolation in the infected animals (Group 1), confirming hyperactivity of the glands throughout the period of investigation. The infected animals had adult fluke recovery of 331.8±19.5 (41.47% of the infection dose) on the Day 112 PI, with a significant increase (p<0.01) in the hepatic tissue mass (35.14%). The complex pathophysiological events occurring during the different stages of bubaline fasciolosis vis-à-vis growth and development of the distome and impact of persistent disease stress on overall health have been discussed.

**Keywords**: *F. gigantica*, buffalo, adrenal glands, cortisol, stress

**INTRODUCTION**

Tropical fasciolosis, caused by *Fasciola gigantica*, has been recognized as a major constraint on the animal productivity in the South Asian countries, including India (Gupta and Singh, 2002; Garg et al., 2009). The distome exerts a variety of deleterious effects on the host, including anaemia, feed conversion and weight gain efficiency, lipolysis, persistent hypoxemia,
oxidative stress, besides elevated activities of certain enzymes suggestive of hepato-biliary trauma and cholestasis (Mehra et al., 1999; Yadav et al., 1999; Ganga et al., 2004; Edith et al., 2010). Recently, elevated serum cortisol concentration and depressed erythrocytic indices during the acute course of experimental bubaline fasciolosis have also appeared in the literature (Ganga et al., 2007). The overall deterioration in the body conditions and lack of energy in the *F. gigantica* infected host could be synergistic consequence of multiple factors, including progressive altered physiological activities of the adrenals. This study is a continued effort in the above direction. The findings reported herein on adrenal gland dysfunction are of pathophysiological interest. The report correlates the persistent cortisolemia with *F. gigantica* induced stress during different stages of acute fasciolosis and attempts to interpret the development of pathognomonic manifestations in the diseased host.

**MATERIALS AND METHODS**

**The animals**

Eight male buffalo yearlings of the Murrah breed, weighing between 200-250 kg, were procured from the field. The animals were stall fed on a balanced diet. They had access to cultivated fodder ad libitum and received concentrate mixture. They were maintained under a standard intensive system of management. Fresh drinking water was offered 2-3 times daily. Absence of parasitic infections was ensured through regular coprological examination for a period of at least 13 weeks. At the age of 12-15 months, they were randomly assigned to two groups of four animals each. On Day 0 of the experiment, each animal in Group 1 was administered per os the primary infection dose of 800 viable *F. gigantica* metacercariae (bubaline origin), as an electuary made of molasses and wheat flour. The animals in Groups 2 were administered electuary devoid of *F. gigantica* metacercariae and maintained as healthy controls. The health status of the experimental animals was critically monitored twice a day for the development of symptoms suggestive of illness and was on spot recorded along with their severity, frequency and duration, until necropsy of the animals at the end of the experiment.

**The Parasite**

Adult *F. gigantica* flukes were recovered at necropsy from the hepato-biliary system and gall bladder of the bubaline livers obtained from a local abattoir. The eggs discharged by the flukes in phosphate buffer saline (PBS) were used for in vitro culture, infection of *Lymnaea auricularia* snails and *F. gigantica* metacercariae were harvested on 4 cm² polysheets. The *Lymnaea auricularia* snails, collected from the endemic areas, were screened, in vitro acclimatized and bred. Each snail, aged 10-15 weeks, measuring 14-15 mm in length, was in vitro infected with 8-10 *F. gigantica* miracidia, and the *F. gigantica* strain was maintained in the author’s laboratory for the production of bubaline origin metacercariae for the experimental purpose. The *F. gigantica* metacercariae were stored at 4°C in sterile distilled water until use (Gupta and Yadav, 1994). These were orally administered to the target animals after a viability test. The viability of metacercariae was between 95-98% on the day of infection.

**Techniques**

The weekly blood samples were aseptically collected by jugular vein puncture from individual animals under the experiment for sixteen weeks.
The serum was separated from the blood for the estimation of cortisol, properly labeled and store at -20°C. Standard haematological techniques were used to monitor weekly alterations in haemoglobin (Hb), packed cell volume (PCV), total erythrocyte counts (TEC), erythrocyte sedimentation rate (ESR), total leucocyte counts (TLC) and eosinophils (Jain, 1986). Copro egg counts for each animal were estimated weekly following dilution technique (Sharma et al., 1989).

**Cortisol determination**

The serum cortisol concentration was determined via immunotech radio immuno assay (RIA) with a commercially available kit (M/s Beckman Company, France). The RIA of cortisol is a competition assay. A standard curve was plotted, while incubating the serum of the healthy controls and standard in monoclonal antibody coated tubes with I125 labeled cortisol tracer. Thereafter, the liquid contents of the tube were aspirated and bound radio activity was measured. A calibration curve was established and unknown values were interpolated from the standard curve.

On necropsy, the liver from each experimental animal was removed, thoroughly washed, weighed and examined for gross lesions and recovery of *F. gigantica* flukes from hepatobiliary system so as to precisely assess in situ fluke population. Only flukes or fluke fragments with a ventral sucker were counted. The flukes were thoroughly washed, the excess saline was removed by gentle blotting, and the mean weight of the flukes was recorded. The flukes were pressed in between a pair of microscopic slides, fixed in 10% warm formalin and the individual fluke length and width were measured and the mean values were derived.

On necropsy, the adrenal glands recovered from each experimental animal were thoroughly washed, blotted, weighed and measured. Tissues showing gross lesions were cut to appropriate size, fixed in warm 10% formal saline and processed for histopathological examination following standard protocol. Sections were cut into 5 μm thickness and stained with Mayer’s alum haematoxylin and alcoholic eosin prior to microscopic examination and recording pathological changes in the respective groups.

**Statistical analysis**

In order to facilitate precise interpretation of data, the weekly fluctuations data on haematology and cortisol profile during the course of investigation were clubbed together and the mean values were derived for four clinical stages of the disease viz. (a) preinfection values on week-0, (b) early prepatency levels from week-1 to 6 PI, (c) late prepatency values from week-7 to 13 PI, and (d) the mean values during patency from week-14 onwards, depending upon the growth, developmental stages of *F. gigantica* adolescercariae and their location in the hepatobiliary system of the host. Research data so generated were subjected to the student’s ‘t’ test (Snedecor and Cochran, 1989).

**RESULTS**

**Clinico-parasitological profile**

No untoward clinical signs were observed until the fourth week PI. Thereafter, the infected animals (Gr-I) sequentially exhibited pathognomonic signs of the disease described elsewhere (Yadav et al., 1999). The signs were of variable duration and were intermittent in nature. One out of the four animals died on Day 110 PI. Loss of subcutaneous fat (lipolysis), emaciation, marked muscular weakness and straight legged-
skeletal braced posture constituted terminal signs of the disease. Healthy controls did not develop any sign and were maintained perfect health status during the course of investigation.

The infected animals discharged *F. gigantica* eggs from Day 92 PI onwards with the mean EPG of 88.8±13.0 during the week. In these animals, the pre-patent period varied from 92-95 days. A progressive increase in faecal egg counts was evidenced during the patency; the highest mean EPG of 192.5±12.6 was recorded during week-16 PI. None of the healthy controls passed out *F. gigantica* eggs in their faeces during the course of study.

On necropsy, the carcass of the infected animals (Gr-I) was emaciated, icteric appearance and there was extensive gelatinization of subcutaneous fat. The liver was grossly enlarged and had exemplary appearance of *F. gigantica* infested liver. There was significant increase (p<0.01) in the hepatic tissue mass (35.14%) in the infected animals (Group 1) in comparison with the healthy controls. The abdominal cavity contained 0.75-1.5 litre of sero-sanguineous fluid, suggestive of ascites. The healthy controls (Groups 2) did not reveal any grossly visible pathological picture and were devoid of *F. gigantica*, suggestive of perfect health status during the course of study.

The numbers, mean length and width and weight of the flukes recovered from each infected animal have been tabulated in Table 1. On necropsy, 41.47% of the primary dose of infection was recovered as adult fluke population from hepatic parenchyma, bile ducts and gall bladder of the infected animals (Group 1). The in situ fluke population recovered from the infected animals had normal growth and development, and their length and width were within normal range for adult *F. gigantica*.

**Blood profile**

The weekly fluctuations in the serum cortisol levels during the course of infection in the respective groups of animals are shown in Figure 1. The pre-infection hormone levels in all the experimental animals were within normal range (12.9±1.6 to 15.3±1.9 nmol/L) and were comparable until week-2 PI. Thereafter, with the invasion of the developing adolescercariae in the hepatic tissues, the hormone levels started rising from the third week PI and remained significantly elevated (p<0.05-0.01) during the fourth through eighth week PI in the Group 1 animals than the healthy controls (Groups 2). The hormone concentrations were further analyzed in the respective groups during different stages of the infection and are presented in Figure 2. The cortisol levels were 82.97% higher in Group 1 animals during early prepatency (weeks 1-6 PI). Thereafter, the serum levels progressively declined to 38.6% during late prepatency (weeks 7-12 PI). The levels declined further during patency (weeks 13-16 PI), and were just 6.2% higher in Group 1 animals. The highest concentration (31.2±4.2 nmol/L) of the cortisol was observed during week-4 PI in the infected group of animals (Group 1). In healthy controls (Groups 2), the cortisol concentration fluctuated within normal range (12.9±1.6 to 14.4±1.8 nmol/L) and did not alter significantly.

The weekly data on erythrocytic indices in the respective groups have been summarized in Table 2. The pre-infection Hb, PCV and TEC in the infected group of animals (Group 1) were within normal range and were comparable with the healthy controls (Groups 2). Thereafter, these erythrocytic indices progressively declined from the fourth week PI onwards and touched the critical levels in Group 1 animals during week 8 PI. During early prepatency (weeks 1-6 PI), the falls were
Table 1. Dose of infection, fluke recovery and biometry in the infected animals.

<table>
<thead>
<tr>
<th>Group (n=4)</th>
<th>Dose of infection (mc)</th>
<th>No. of fluke recovered</th>
<th>Fluke recovery (%)</th>
<th>Mean length of fluke (mm)</th>
<th>Mean width of fluke (mm)</th>
<th>Mean weight of fluke (g)</th>
<th>Weight of liver (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>800</td>
<td>320</td>
<td>40.00</td>
<td>51.4</td>
<td>11.3</td>
<td>1.32</td>
<td>5.59</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>312</td>
<td>39.00</td>
<td>50.3</td>
<td>11.2</td>
<td>1.28</td>
<td>5.43</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>355*</td>
<td>44.38</td>
<td>53.1</td>
<td>12.0</td>
<td>1.35</td>
<td>5.85</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>340</td>
<td>42.50</td>
<td>54.0</td>
<td>11.8</td>
<td>1.41</td>
<td>5.50</td>
</tr>
<tr>
<td>Mean</td>
<td>800</td>
<td>331.8</td>
<td>41.5</td>
<td>52.2</td>
<td>11.6</td>
<td>1.3</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>±19.5</td>
<td>±2.2</td>
<td>±0.2</td>
<td>±0.04</td>
<td>±0.1</td>
<td>±0.2</td>
<td></td>
</tr>
</tbody>
</table>

mc: metacercariae

*One animal out of four died on Day 110 post-infection.

Table 2. The altered haematological indices (Mean ± S.E.) in the respective groups.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Groups</th>
<th>Hb (g/dL)</th>
<th>PCV (%)</th>
<th>TEC (×10⁶/cmm)</th>
<th>ESR (mm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Gr-I</td>
<td>12.97±0.9</td>
<td>39.88±1.0</td>
<td>6.32±0.5</td>
<td>34.0±8.5</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>13.03±0.8</td>
<td>40.13±1.8</td>
<td>6.35±0.5</td>
<td>35.2±7.8</td>
</tr>
<tr>
<td>2</td>
<td>Gr-I</td>
<td>12.25±0.2</td>
<td>39.3±0.1</td>
<td>6.11±0.2</td>
<td>34.75±4.9</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>12.75±0.3</td>
<td>40.2±0.3</td>
<td>6.54±0.2</td>
<td>36.0±2.8</td>
</tr>
<tr>
<td>4</td>
<td>Gr-I</td>
<td>9.2±0.4</td>
<td>28.75±1.4</td>
<td>4.8±0.2</td>
<td>34.0±4.3</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>12.9±0.3</td>
<td>39.9±0.3</td>
<td>6.5±0.2</td>
<td>34.25±5.0</td>
</tr>
<tr>
<td>6</td>
<td>Gr-I</td>
<td>5.65±0.2</td>
<td>17.08±0.8</td>
<td>2.8±0.1</td>
<td>59.25±7.0</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>12.95±0.2</td>
<td>39.8±0.4</td>
<td>6.54±0.1</td>
<td>34.75±2.8</td>
</tr>
<tr>
<td>8</td>
<td>Gr-I</td>
<td>5.43±0.2</td>
<td>16.85±0.3</td>
<td>2.8±0.1</td>
<td>55.0±8.17</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>13.05±0.2</td>
<td>40.25±1.0</td>
<td>6.76±0.1</td>
<td>37.0±1.8</td>
</tr>
<tr>
<td>10</td>
<td>Gr-I</td>
<td>5.53±0.1</td>
<td>18.25±0.1</td>
<td>2.74±0.1</td>
<td>64.5±4.9</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>13.1±0.3</td>
<td>40.25±0.8</td>
<td>6.72±0.2</td>
<td>37.5±3.4</td>
</tr>
<tr>
<td>12</td>
<td>Gr-I</td>
<td>5.73±0.2</td>
<td>19.5±0.1</td>
<td>3.1±0.1</td>
<td>42.25±9.2</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>13.15±0.3</td>
<td>40.25±0.4</td>
<td>6.7±0.1</td>
<td>33.75±7.0</td>
</tr>
<tr>
<td>14</td>
<td>Gr-I</td>
<td>6.05±0.2</td>
<td>21.25±0.7</td>
<td>3.21±0.1</td>
<td>45.75±6.4</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>13.2±0.4</td>
<td>39.95±0.6</td>
<td>6.57±0.1</td>
<td>34.25±3.0</td>
</tr>
<tr>
<td>16</td>
<td>Gr-I</td>
<td>6.28±0.2</td>
<td>22.25±0.8</td>
<td>3.24±0.1</td>
<td>41.0±6.2</td>
</tr>
<tr>
<td></td>
<td>Gr-II</td>
<td>13.13±0.2</td>
<td>39.7±0.5</td>
<td>6.54±0.1</td>
<td>36.25±2.3</td>
</tr>
</tbody>
</table>

The values bearing different alphabet as superscripts, differ significantly (p<0.05-0.01).
Figure 1. Serum cortisol levels (Mean ± SE) in the respective groups.

Figure 2. Serum cortisol levels (Mean ± SE) during different stages of the disease.
Figure 3. Total leucocyte counts (Mean ± SE) in the respective groups.

Figure 4. Eosinophils (Mean ± SE) in the respective groups.
30.4%, 28.86% and 27.7%, respectively, for Hb, PCV and TEC. However, during late prepatency (weeks 7-12 PI), the falls were comparatively more: 57.1%, 54.31% and 54.4%. The altered levels of these haematological indices exhibited slight improvement during patency (week 12 PI onwards), but persistently remained depressed and were significantly different (p<0.05-0.01) from the preinfection values. Overall falls of 57.8%, 57.09% and 56.4%, respectively, for Hb, PCV and TEC were recorded in Group 1 animals by the end of the experiment. However, the erythrocyte sedimentation rate (ESR) showed an increasing trend during the corresponding weeks, but the values were significantly (p<0.05-0.01) higher during weeks 6-12 PI. In the healthy controls (Groups 2), these values fluctuated within normal range and were not significantly different (Table 2). The infected animals (Group 1) revealed significant (p<0.05) leucocytosis with predominantly neutrophilia from the fourth week PI onwards and attained the highest level of 9.5±0.2x10^3/cmm by the end of the experiment (Figure 3). Likewise, eosinophils markedly increased in the infected group of animals (Group 1) and attained the highest level of 14.0±1.8% in the Group 1 animals by the end of the experiment (Figure 4).

The adrenal glands of the infected animals (Group 1) were grossly enlarged. There was an increase in the thickness of the cortex due to increased width of zona fasciculata. The corticocytes were hypertrophied, lost their
normal ovoidal/polyhedral shape and became elongated and columnar shaped. Their typical cord like arrangement pattern was disturbed. The cortical sinusoids were also comparatively dilated and engorged (Figure 5). The cytoplasm of the corticocytes evidenced single and/or multiple vacuoles. However, the outermost zona groemerulosa and the innermost zona reticularis of the cortex and the adrenal medulla did not reveal any appreciable histological changes. The adrenal glands of the healthy controls had normal shape, size and histological appearance.

**DISCUSSION**

The Group 1 animals receiving a primary infection dose of 800 *F. gigantica* metacercariae, sequentially exhibited characteristic signs of the tropical fasciolosis in buffaloes, higher faecal egg counts, depressed erythrocytic indices, leucocytosis and eosinophilia, pathognomonic hepatic lesions and in situ 41.47% fluke population of the dose of infection, analogous to earlier description (Yadav et al., 1999). The emaciated carcasses with gelatinization of subcutaneous fat on necropsy of Group 1 animals, strongly indicated the effect of elevated cortisol concentration on the overall metabolism operational in Group 1 animals and the magnitude of disease stress incidental to the distome, to meet extra energy requirements of the hosts having apyrexic inappetance with normal digestibility of nutrients (Mehra et al., 1999; Yadav et al., 1999). Besides, the periodical depressed erythrocytic indices, increased erythrocyte sedimentation rate (ESR) and leucocytosis with predominantly neutrophilia and eosinophilia in the Group 1 animals are in consonance with earlier reports on these aspects (Yadav et al., 1999; Ganga et al., 2007; Edith et al., 2010). The clinical manifestations and progress of the disease were synchronous with the growth, maturation of the infection dose and in situ fluke establishment in the infected animals, as elaborately discussed elsewhere (Yadav et al., 1999).

The adrenals are the most sensitive and versatile steroid hormone producing endocrine glands. They spontaneous interact with an altered in situ environment or a stimuli, and refluxed glucocorticosteroids, especially cortisol, into the host circulation to encounter the stimuli. An analysis of post-infection serum cortisol activity in the infected animals (Group 1) revealed that hypercortisolemia was the highest (82.97%) during early prepatency followed by 38.6% in late prepatency and 6.2% in the patency phase of the disease. These animals persistently had elevated hormone activity in the circulation in comparison with healthy controls, and this indicated variable degree and magnitude of adrenocortical response to the in situ distome population (stressor). The response was, however, variable depending upon clinical phase of the disease, in situ activities and location of the *F. gigantica* adolescercariae or adult fluke population.

The highest concentration of the hormone in Group 1 animals during the fourth week PI was suggestive of the highest degree of *F. gigantica* induced stress. Subsequent, progressive fall in the hormone activity synchronized well with the arrival and establishment of the causative organism in the hepatobiliary network, caessation of traumatic activity of the distome and partial resolution of the lesions in the hepatocytes and/or partial recovery of the animals from disease stress. On the contrary, the healthy controls (Groups 2) maintained along with the infected animals in the same environment did not suffer from any hypercortisolemia as
deduced from non-significant, all time within range fluctuations of the cortisol concentrations during the course of investigation. This rules out remotest possibility of the external/environmental/managerial factor(s) contributing towards the increased cortisol activity in Group 1 animals.

Further histopathological changes in zona fasciculata reported herein were also suggestive of hyperactivity of the corticocytes, persistently discharging cortisol into the host circulation, in proportion to nature and magnitude of the stimuli originating from the in situ host parasite interaction in Group 1 animals during different stages of the disease. The lesions did not completely resolve by the end of the experimental period and the animals continued to suffer subclinical stress incidental to the in situ flukes. Analogous hypercortisolemia and the histological changes in the zona fasciculata were also ascribed to Trypanosoma congolense infection in cattle (Ogwu et al., 1992). It is therefore speculated that the long-term secretion of cortisol (for 112 days) in Group 1 animals seems to have influenced the metabolism, and immune response of the host and consequently resulted in hepatomegaly, loss of collagen and elastin in the dermis, skeletal muscle weakness of the extremities and abdomen, lipolysis, etc., often evidence on necropsy in bubaline fasciolosis (Aiello and Mays, 1998). These findings on adrenal dysfunction during the course of investigation seems interesting to further explore to investigate and understand the impact of F. gigantica on the host as a syndrome rather than a disease confined to the liver.

The erythrocytic indices of Group 1 animals progressively decreased from week-4 PI onwards and significantly fell (p<0.05-0.01) during prepatency. It was, however, the highest during late prepatency, whereas erythrocytic sedimentation rates showed an increasing trend during the corresponding weeks. The events were synchronous with in situ migration, development of F. gigantica adolescercariae inflicting traumatic lesions/haemorrhagic tracts in the liver parenchyma. Partial improvement in the erythrocytic indices from week 12 PI onwards (during patency) indicated caessation of traumatic activities of the distome and its final establishment in the bile ducts. Analogous fluctuations were also documented earlier (Yadav et al., 1999). The altered erythrocytic indices during the acute course of bubaline fasciolosis seems to be a remarkably complex event in its origin and pathogenesis governed by several factors (Isseroff et al., 1979). The injurious effects of the fluke metabolites on circulating erythrocytes were recently confirmed by intraperitoneal inoculation of in vitro released F. gigantica metabolites for seven days in fluke free rats. A progressive and significant fall in erythrocytic indices was witnessed causing normocytic normochromic anaemia in the rats (Ganga et al., 2004a). Besides, it has also been documented that the in situ developing adolescercariae, at times accidentally feed on blood oozed out from the traumatic lesions in the liver (Radostits et al., 1994). Evidently, the ultimate consequence of the above on going events was a significant fall in oxygenated erythrocytes in circulation for a prolonged period of the experiment, causing generalized hypoxemia in the diseased host. The persistent eosinophilia reported herein, despite hypercortisolemia in the infected host, seems to be incidental to host-parasite interaction. The observed leucocytosis in Group 1 animals was response of the host defense mechanism against the invading distome.

In conclusion, the pathogenesis of tropical fasciolosis in buffaloes is a complex subject. More critically planned experiments with the main focus on F. gigantica induced hypercortisolemia
(persistent stress); physiological dysfunctions of the adrenal cortex through hypothalamus-pituitary-adrenal gland axis during the various stages of tropical fasciolosis are needed to precisely elucidate the persistent stress in large ruminant populations at risk of the disease. It would be interesting to investigate: (a) whether the *F. gigantica* induced dysfunction of adrenal cortex is a primary or secondary hyper adrenocorticism, (b) whether proliferation of corticocytes in zona fasciculata is induced and modified by the adreno corticotropic hormone or some other factors produced by corticotrophic cells in the pituitary gland of the host, and (c) whether *F. gigantica* induced anaemia is consequential to injurious effects of the fluke metabolites on erythrocyte membrane and/or depressed bone marrow activity coupled with prolonged hypoxemia. Investigations should also be conducted to appreciate the overall impact of the aetiological agent on altered metabolism vis-à-vis health status, growth and development, and productivity of the buffaloes in the endemic areas.

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