The objective of the present study was to evaluate the effect of feeding balanced rations on milk production efficiency and enteric methane emission in lactating buffaloes under tropical conditions. Early lactating buffaloes (n=61), yielding 5.0 to 9.0 kg milk per day, were selected from different agro-climatic regions of the country. Baseline methane emission of individual buffaloes was estimated by using sulphur hexafluoride tracer technique; thereafter the ration was balanced as per their nutrient requirements. After 30 days of feeding a balanced ration, enteric methane emission from buffaloes was estimated again. Analysis of feeding practices revealed that dietary intake of protein and energy were inadequate to their requirement. Calcium and phosphorus were also deficient by 37.6 and 53.0%, respectively. On feeding balanced rations, average milk yield, milk fat and 6% fat corrected milk (FCM) were improved (P<0.05) by 7.6, 4.8 and 11.6% in buffaloes. Milk production efficiency (FCM kg/kg DMI) of buffaloes improved (P<0.05) to 0.68 from 0.62 on feeding a balanced ration. Enteric methane emission (g d\(^{-1}\) and g kg milk yield\(^{-1}\)) was reduced (P<0.05) by 12.6 and 19.5%, respectively, in lactating buffaloes. Gross energy loss through methane was reduced (P<0.05) from 5.7 to 4.9% in buffaloes. The results of the field studies indicated that feeding nutritionally balanced rations not only increased milk production efficiency significantly but also reduced enteric methane emission from lactating buffaloes under natural feeding and management conditions. Large-scale implementation of a ration balancing programme can help in improving the production efficiency of milch buffaloes with the available feed resources in an environmentally sustainable manner.

**Keywords:** balanced ration, milk production, methane emission, lactating buffaloes, agro-climatic regions

**INTRODUCTION**

The Indian dairy sector has been on a steady path of progress since its independence and achieved an annual output of 127.9 million tones of milk during 2011-12 (DADF, 2012). This has made the country the highest milk producer in the world and provided milk and milk products for the burgeoning population. The water buffalo (Bubalus bubalis) is the mainstay of the dairy industry in India, contributing ~58% of the country’s milk production with an average yield of 1300 kg per lactation (GOI, 2007). This low productivity of buffaloes is mainly attributed to feeding of poor quality feed resources, specially crop residues and...
agro-industrial by-products, which are imbalanced in terms of critical nutrients like protein, energy, minerals and vitamins. Such feeding adversely impacts not only the productivity, health and welfare of animals, but also the environment. It is evident that enteric fermentation emitted 10.09 million tones of methane, which is ~74% of total methane emission from the agriculture sector in India (INCCA, 2010). The buffalo is the single largest emitter of methane due to its higher methane emission coefficient \( i.e. 50 \text{ kg head}^{-1} \text{ year}^{-1} \) (NATCOM, 2004).

Domestic demand for milk and milk products in India is projected at 200-210 million tonnes by 2021-22 (Anonymous, 2011). To meet the future demand for milk, enhancing milk production efficiency in an environmentally sustainable manner by making judicious use of available feed resources is the major challenge facing tropical countries like India. This can be achieved if the ration offered to an animal comprising various feed ingredients like brans, grains, oil cakes, dry and green roughages etc. are balanced for different nutrients. The National Dairy Development Board (NDDB) of India has developed user-friendly ration balancing software for preparing least cost balanced ration for dairy animals using locally available feed resources. In view of this, three field studies, each in western, northern and central regions, were undertaken to assess the impacts of feeding balanced rations on milk production efficiency and enteric methane emission in lactating buffaloes and the results are reported here.

**MATERIALS AND METHODS**

**Selection of buffaloes and feeding practices**

To evaluate the impact of feeding nutritionally balanced rations on milk production and enteric methane emission, sixty-one early lactating (up to 100 days of post-calving) buffaloes were selected from Junagadh district of Gujarat state (western region), Rae Bareli district of Uttar Pradesh state (northern region) and Nanded district of Maharashtra state (central region). All the selected buffaloes were in second to fourth stage of lactation. The feed intake of individual buffalo was measured and representative feed and fodder samples were taken for proximate principles to evaluate the nutrient intakes. Based on current feeding practices, the status of nutrient provision to the buffaloes in terms of crude protein (CP), metabolizable energy (ME) and essential minerals was assessed to better understand the deficiencies and/or excesses of nutrients in the ration. Thereafter, the ration of all buffaloes was balanced for CP, ME, calcium (Ca) and phosphorus (P) using ration balancing software developed by NDDB, which is based on Kearl (1982) standards for buffaloes. The balanced ration was fed to all the buffaloes for at least 30 days.

**Feed, fodder and milk analysis**

The feed and fodder samples were analyzed for proximate composition by the AOAC (2005) method. Daily milk yield before and after feeding a balanced ration was recorded in the morning and evening by visiting individual farmer’s house. Milk samples were collected and analyzed for milk fat level by MilkoTester, located at the village dairy cooperative society. Milk yield and milk fat level recorded before and after feeding the balanced ration was utilized for formulation of balanced rations. The body weight was calculated using Shaeffer’s formula as: 

\[
\text{BW (kg)} = \left(\left(\text{heart girth in inches}\right)^2 \times \text{length of the body in inches}\right)/300 \times 0.4536.
\]
Methane emission measurement

Methane emission measurement was done by the sulfur hexafluoride (SF₆) tracer technique (Johnson et al., 1994). A small permeation tube containing known release rate of SF₆ gas was inserted in the rumen of each of the experimental buffalo through mouth. The breath samples of all buffaloes fed on traditional ration were collected daily for 4 consecutive days in canisters (Figure 1) and brought to the laboratory for methane and SF₆ analyses. After 30 days of feeding the balanced ration, methane emission was measured again for 4 consecutive days. Methane and SF₆ concentrations were determined by a gas chromatograph (GC) instrument. All the breath samples were analyzed in triplicate using GC, fitted with a Porapack N column for CH₄ and molecular sieve 5A for SF₆. The column temperature was maintained at 50ºC and nitrogen (N) was used as a carrier gas, with flow rate of 30 ml/minutes. Standards were procured from Scott-Marrin Inc., Riverside, CA, USA and used to standardize the GC for CH₄ (10.4 ppmv and 101.9 ppmv) and SF₆ (39.2 pptv and 101.7 pptv). The methane emission rate was calculated as the product of the permeation tube emission rate and the ratio of CH₄ to SF₆ concentration in the sample as: Q CH₄ = Q SF₆ x (CH₄) / (SF₆), where Q CH₄ = CH₄ emission rate (g/min); Q SF₆ = Known release rate of SF₆ from permeation tube (g/min); CH₄ = CH₄ concentration of collected sample in the canister (μg/m³); and SF₆ = SF₆ concentration of collected sample in canister (μg/m³). The data were statistically analyzed by paired student’s t-test (Snedecor and Cochran, 1994) with the SPSS package (1999).

RESULTS AND DISCUSSION

Current feeding practices and ration balancing

Farmers in the western region generally feed wheat bran, cottonseed cake, jowar fodder, wheat straw and groundnut straw to their animals, while feeding practices in the northern region comprise wheat grain, wheat bran and wheat straw. Farmers feed green (mixed local grasses, maize and jowar fodder, guinea grass, hybrid napier and sugarcane tops), dry (jowar and soybean straw) roughages and cottonseed cake as energy source in the central region. Feeding of mineral mixture to the buffaloes was not practiced in studied area by the farmers. Analysis of the feeding practices revealed that the dietary intakes of ME (Mcal d⁻¹) was higher (28.8 vs 25.5; P<0.05) in the western region, whereas it was lower (19.7 vs 23.2; P<0.05) in the central region. Intake of CP (kg d⁻¹) was lower in the ration of milch buffaloes in the western (1.4 vs 1.6; P<0.05) and northern (0.9 vs 1.0; P<0.05) regions of India (Table 1). Adequate intake of CP and ME was observed in the central and northern regions, respectively. The level of Ca and P was also deficient in the ration of buffaloes by 37.6 and 53.0%, respectively, in different regions (Figure 2). From the feeding practices it was observed that the dietary intake of protein, energy and minerals were lower than the buffalo’s requirements. Similar findings were also observed by Tiwary et al. (2007) and Yadav et al. (2002) under Indian field conditions.

After collection of breath samples for baseline methane emission, the ration was balanced as per the requirement of individual buffalo for CP, energy, Ca and P. After feeding balanced rations to buffaloes, the intake of DM and energy were numerically higher. CP intake (kg d⁻¹) improved by 8.3% (P<0.05) on feeding a balanced ration.
Figure 1. Collection of a breath sample in a canister.

Figure 2. Percent deficiency of Ca and P in the ration.
Metabolic body weight was not affected by feeding balanced rations. The intakes of DM and energy were similar to those reported for buffaloes by Paul et al. (2003).

**Milk production efficiency**

Feeding a balanced ration improved (P<0.05) milk production, milk fat and 6% FCM yield in buffaloes (Table 1). Maximum improvement in milk yield was observed in the northern region (13.5%), followed by the central (8.2%) and western (4.7%) regions. On feeding a balanced ration, the level of 6% FCM increased by 8.9, 17.3 and 10.9% in the western, northern and central regions, respectively. Overall, balanced rations improved (P<0.05) milk yield and milk fat by 7.6 and 4.8%, respectively, in buffaloes. Milk production efficiency of fat corrected milk (FCM kg/kg DMI) of buffaloes improved (P<0.05) to 0.68 from 0.62 on feeding a balanced ration. Garg et al. (2013) reported that it is possible to increase the feed conversion efficiency (FCE) for milk production in cows and buffaloes through balanced feeding. This is useful to increase the profitability of milk producers and contributes to efficient use of scarce feed resources in developing countries while achieving targeted milk production. Shahjalal et al. (2000) and Castillo et al. (2001) also reported increased FCE for milk production by balancing the ration of milking animals.

In our study, improvement in milk yield and milk fat in buffaloes may have been due to the balancing of nutrients, which might have improved microbial protein synthesis, and also due to supply of minerals. Energy and protein are the most important limiting factors towards milk production and their supplementation in the diets of lactating ruminants increased milk yield significantly. Through balanced feeding, dietary energy and protein could be utilized in a more efficient manner in lactating buffaloes. The results are in agreement with those of Dutta et al. (2010); Kannan et al. (2010) and Khochare et al. (2010).

**Enteric methane emission**

Enteric methane emission from buffaloes after feeding a balanced ration was 175.2 g d⁻¹, which was lower (P<0.05) than the baseline emissions (Table 2). Methane emission (g kg milk yield⁻¹) was significantly (P value ranging from 0.05 to 0.01) reduced in all three regions by 18-21% in buffaloes. After feeding a balanced ration, the gross energy loss through methane was reduced from its initial level 5.7 to 4.9% (P<0.05) in buffaloes.

Due to imbalanced feeding of nutrients to animals, as practiced in India, the limitation for the growth of microbial cells is probably the inadequate concentration of ruminal ammonia and deficiency of minerals. This leads to a change in the rumen fermentation pattern towards production of more acetate and butyrate, leading to production of more hydrogen and carbon dioxide, the main substrate for methane production. The reduction in methane emission observed in the present study is attributed to the balancing of nutrients, which might have changed rumen fermentation towards more microbial cell production and lower volatile fatty acid (acetate and butyrate) production. The increased N supply after balanced feeding might have provided the required fermentable N for efficient microbial protein synthesis. The minerals supplied in the diet could also have enhanced the microbial cell growth since the ash content of microbial matter is 13%.

The reduction in enteric methane emission observed in the present study is consistent with the earlier reports (Mohini and Singh, 2010). Depending on the efficiency of utilization of ATP for microbial
Table 1. Effect of feeding balanced rations on nutrient intake and milk production efficiency.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Western region (n=22)</th>
<th>Northern region (n=13)</th>
<th>Central region (n=26)</th>
<th>Average (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before RB</td>
<td>After RB</td>
<td>Before RB</td>
<td>After RB</td>
</tr>
<tr>
<td>Average metabolic BW (kg w^{0.75})</td>
<td>91.8 ± 2.81</td>
<td>93.1 ± 1.38</td>
<td>91.6 ± 1.81</td>
<td>92.8 ± 2.01</td>
</tr>
<tr>
<td>DM intake (kg d^{-1})</td>
<td>12.7 ± 0.31</td>
<td>12.3 ± 0.36</td>
<td>10.2 ± 0.15</td>
<td>10.3 ± 0.13</td>
</tr>
<tr>
<td>DM intake (kg/100 kg BW)</td>
<td>2.9 ± 0.10</td>
<td>2.8 ± 0.09</td>
<td>2.5 ± 0.03</td>
<td>2.4 ± 0.03</td>
</tr>
<tr>
<td>DM intake (g/kg W^{0.75})</td>
<td>134.7 ± 0.78</td>
<td>127.9 ± 0.68</td>
<td>111.0 ± 1.38</td>
<td>110.9 ± 1.27</td>
</tr>
<tr>
<td>CP intake (kg d^{-1})</td>
<td>1.4^{a} ± 9.71</td>
<td>1.6^{b} ± 10.64</td>
<td>0.9^{a} ± 11.42</td>
<td>1.0^{b} ± 13.53</td>
</tr>
<tr>
<td>ME intake (Mcal d^{-1})</td>
<td>28.8^{a} ± 0.36</td>
<td>25.5^{b} ± 1.19</td>
<td>18.2 ± 3.15</td>
<td>18.3 ± 2.53</td>
</tr>
<tr>
<td>Milk yield (kg d^{-1})</td>
<td>8.5^{a} ± 0.49</td>
<td>8.9^{b} ± 0.40</td>
<td>5.2^{a} ± 0.14</td>
<td>5.9^{b} ± 0.13</td>
</tr>
<tr>
<td>Milk fat (%)</td>
<td>6.5^{a} ± 0.08</td>
<td>6.8^{b} ± 0.09</td>
<td>6.0^{a} ± 0.09</td>
<td>6.3^{b} ± 0.08</td>
</tr>
<tr>
<td>6% FCM yield (kg d^{-1})</td>
<td>9.0^{a} ± 0.28</td>
<td>9.8^{b} ± 0.24</td>
<td>5.2^{a} ± 0.11</td>
<td>6.1^{b} ± 0.10</td>
</tr>
<tr>
<td>Milk production efficiency</td>
<td>0.71^{a} ± 0.26</td>
<td>0.80^{a} ± 0.67</td>
<td>0.51^{a} ± 0.73</td>
<td>0.59^{b} ± 0.77</td>
</tr>
</tbody>
</table>

^{a,b}Values with different superscript in a row differ significantly (P<0.05).

^{c,d}Values with different superscript in a row differ significantly (P<0.01).

n = number of buffaloes; RB = ration balancing.
Table 2. Effect of feeding balanced rations on enteric methane emission.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Western region</th>
<th></th>
<th>Northern region</th>
<th></th>
<th>Central region</th>
<th></th>
<th>Average</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before RB</td>
<td>After RB</td>
<td>Before RB</td>
<td>After RB</td>
<td>Before RB</td>
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<td>Before RB</td>
<td>After RB</td>
</tr>
<tr>
<td>Methane emission (g head(^{-1}) d(^{-1}))</td>
<td>232.5 ± 5.93</td>
<td>199.6 ± 4.98</td>
<td>214.7 ± 7.10</td>
<td>192.2 ± 5.93</td>
<td>154.5 ± 5.46</td>
<td>133.9 ± 5.37</td>
<td>200.5 ± 6.16</td>
<td>175.2 ± 5.42</td>
</tr>
<tr>
<td>DM intake (kg d(^{-1}))</td>
<td>12.7 ± 1.25</td>
<td>12.3 ± 1.09</td>
<td>10.2 ± 0.15</td>
<td>10.3 ± 0.13</td>
<td>10.3 ± 0.23</td>
<td>11.6 ± 0.28</td>
<td>11.1 ± 0.54</td>
<td>11.4 ± 0.50</td>
</tr>
<tr>
<td>Methane emission (g.kg DM intake(^{-1}))</td>
<td>18.3 ± 2.23</td>
<td>16.2 ± 1.96</td>
<td>21.0 ± 0.65</td>
<td>18.7 ± 0.61</td>
<td>15.0 ± 0.63</td>
<td>11.5 ± 0.51</td>
<td>18.1 ± 1.17</td>
<td>15.3 ± 1.02</td>
</tr>
<tr>
<td>OM intake (kg d(^{-1}))</td>
<td>11.6 ± 1.21</td>
<td>11.3 ± 1.11</td>
<td>9.3 ± 0.15</td>
<td>9.4 ± 0.13</td>
<td>9.7 ± 0.20</td>
<td>10.0 ± 0.41</td>
<td>10.2 ± 0.52</td>
<td>10.2 ± 0.55</td>
</tr>
<tr>
<td>Methane emission (g.kg OM intake(^{-1}))</td>
<td>20.0 ± 2.23</td>
<td>17.7 ± 1.96</td>
<td>23.0 ± 0.78</td>
<td>20.5 ± 0.74</td>
<td>15.9 ± 0.74</td>
<td>13.4 ± 0.64</td>
<td>19.6 ± 1.25</td>
<td>17.2 ± 1.11</td>
</tr>
<tr>
<td>Methane emission (g.kg milk yield(^{-1}))</td>
<td>27.3 ± 3.21</td>
<td>22.4 ± 2.69</td>
<td>40.9 ± 3.62</td>
<td>32.4 ± 3.03</td>
<td>25.3 ± 1.59</td>
<td>20.4 ± 1.35</td>
<td>31.2 ± 2.80</td>
<td>25.1 ± 2.35</td>
</tr>
<tr>
<td>Gross energy intake (Mcal d(^{-1}))</td>
<td>55.0 ± 9.88</td>
<td>54.3 ± 8.19</td>
<td>40.6 ± 0.66</td>
<td>43.4 ± 0.57</td>
<td>41.0 ± 1.33</td>
<td>43.2 ± 1.34</td>
<td>45.6 ± 3.95</td>
<td>47.0 ± 3.36</td>
</tr>
<tr>
<td>Energy loss as methane (Mcal d(^{-1}))</td>
<td>3.1 ± 0.07</td>
<td>2.7 ± 0.06</td>
<td>2.9 ± 0.09</td>
<td>2.6 ± 0.08</td>
<td>2.1 ± 0.07</td>
<td>1.8 ± 0.07</td>
<td>2.7 ± 0.07</td>
<td>2.3 ± 0.07</td>
</tr>
<tr>
<td>Energy loss as methane (% of GE)</td>
<td>5.6 ± 0.14</td>
<td>4.9 ± 0.09</td>
<td>6.5 ± 0.20</td>
<td>5.8 ± 0.19</td>
<td>5.0 ± 0.24</td>
<td>4.1 ± 0.21</td>
<td>5.7 ± 0.19</td>
<td>4.9 ± 0.16</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Values with different superscript in a row differ significantly (P<0.05).

\(^{c,d}\) Values with different superscript in a row differ significantly (P<0.01).

RB = ration balancing.
cell synthesis, the amount of carbohydrate converted to microbial cells can be highly variable, which controls the production of methane and volatile fatty acids (Blummel et al., 2010). Therefore, feeding as per the nutrient requirements of animals provides an effective measure for reducing methane emission as recorded in dairy cattle in USA (Capper et al., 2009) and in India (Garg et al., 2013) due to improved feed utilization and enhanced overall production efficiency.

CONCLUSION

In the present study, balanced nutrition improved milk production efficiency and reduced enteric methane emission from lactating buffaloes. For improving productivity of lactating buffalo under small holding dairy systems in Indian tropical conditions, balanced nutrition approach using locally available feed resources at farmers’ doorstep would be considered as a prudent path for the future and pro-poor approach for the sustainable development of mixed crop livestock systems in developing countries.

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