SHORT COMMUNICATION

Effect of graded levels of dietary crude protein on nutrient utilization and enteric methane emissions in growing Murrah buffalo calves

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Abstract: The present study was done to investigate the effect of graded levels of dietary crude protein on enteric methane (CH₄) production from Murrah buffalo calves. Fifteen Murrah buffalo male calves were divided into 5 groups (Av. BW=153.05) and were fed total mixed rations containing wheat straw, maize fodder and concentrate mixture in different proportions so that the dietary crude protein level was 5, 7.5, 10, 12.5 and 15% in groups T₁, T₂, T₃, T₄ and T₅, respectively. The trial lasted for 30 days. Dry matter intake increased from 2.49 to 4.40 kg/d. Dry matter digestibility increased (P<0.05) from 48.64 to 61.97%. CH₄ emissions decreased (P<0.05) from 34.48 to 12.73 g/kg DMI with increasing protein level in the ration. Hence, CH₄ emissions were lower (12.73-12.83 g/Kg DMI) in animals fed rations containing 12.5 to 15% CP.

Keywords: Buffalo calves, Enteric CH₄ emissions, Nutrient utilization

Concern of world environmental authorities towards increasing level of green house gases (GHGs) in the atmosphere is due to their global warming impacts. CH₄ is the second most important greenhouse gas after carbon dioxide due to its 25 times higher

global warming potential (Prathap et al. 2021). Ruminant livestock constitute the most important source of anthropogenic emissions of CH₄ (Broucek, 2014) and enteric fermentation and manure management (along with nitrous oxide) are the two responsible forms (Nampoothiri et al. 2018).

Due to prevailing feeding practices in the country where poor quality roughages forms major constituents of animal diet, production of acetate is more which contributes to higher CH₄ production and poor productivity of animals (Garg et al. 2012). Imbalanced feeding resulted in low milk production, poor growth and reproduction, shorter lactation length, longer calving intervals and excessive amounts of pollutants released into the environment (Gupta et al. 2019). Though biotechnological and management methods are quite tedious, manipulating methanogenic causes through feed interventions are more researched and adoptive ways (Prathap et al. 2021). Supplementation of concentrate or increasing the dietary crude protein in the ration is being practiced over the years to reduce CH₄ emissions (Muñoz et al. 2015). Thus, the present study was aimed to investigate the effect of using different crude protein in the ration on nutrient utilization and CH₄ production in Murrah buffalo calves.

Fifteen male Murrah buffalo calves (153.05 kg BW, 6-8 mon.) at Livestock Research Centre (LRC) of ICAR-National Dairy Research Institute (NDRI), Karnal, India, were distributed randomly in five groups of three each based on body weight and age. The animals were fed on graded levels of protein in the ration i.e. total mixed rations containing wheat straw, maize green and concentrate mixture in different proportions so that the dietary CP level was 5, 7.5, 10, 12.5 and 15% of DMI in groups T₁ (R:C=100:0), T₂ (R:C=80:20), T₃ (R:C=70:30), T₄ (R:C=65:35) and T₅ (R:C=55:45), respectively. The feeding trial lasted for 30 days, followed by a 7 day metabolic trial. Dry matter intake was recorded daily for each animal. The samples of feed, residue, feces and urine were analyzed for proximate principles (AOAC 2005) and NDF (Van Soest et al. 1991). Non-fibrous carbohydrates (NFC), total digestible nutrients (TDN), digestible energy (DE) and metabolizable energy (ME) values were calculated from feed composition using equations (NRC, 2001).

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Enteric CH₄ emission was measured for a total of 5 days (*i.e.* minimum of 5 samples per animal) using SF₆ tracer technique (Johnson et al. 1994). Collected samples (gases eructed from the mouth and nostrils) were analyzed for CH₄ and SF₆ gas using chromatograph (Nucon-5700, New Delhi) fitted with flame ionization detector (FID at 100°C) and electron capture detector (ECD at 250°C), respectively. Nitrogen was used as the carrier gas at a pressure of 1 kg/cm². The CH₄ emission rate was calculated as:

$$\text{CH}_4 (\text{g/day}) = \frac{(S_{\text{CH}_4} - B_{\text{CH}_4})}{(S_{\text{SF}_6} - B_{\text{SF}_6})} \times \frac{(M_{\text{CH}_4}/M_{\text{SF}_6})}{1000} \times Q_{\text{SF}_6} \times$$

Where, S_{CH₄} and B_{CH₄} are CH₄ concentrations in sample and background's canisters; S_{SF₆} and B_{SF₆} represents the concentrations of SF₆ in sample and background's canisters (ppt), respectively and Q_{SF₆} represents the release rate of SF₆ (mg/d).

Statistical analysis was done using completely randomized design, one-way classification as per the procedure given by Snedecor

and Cochran (1994). Significant differences among different treatments were identified using Duncan's Multiple Range Test and a *p*<0.05 was considered to be statistically significant. All the statistical analyses were done using SPSS version 16 (2010).

Concentrate mixture, maize green and wheat straw were fed to the five groups in the form of TMR in such a fashion so as to provide a total dietary CP level of 5, 7.5, 10, 12.5 and 15% in groups T₁, T₂, T₃, T₄ and T₅, respectively. Chemical composition and the energy content of the feeds have been presented in Table 1 and were in agreement with the earlier reports (Dixit et al. 2015; Budhani et al. 2016).

Nutrient intake and digestibility figures have been shown in Table 2. The DM intake was the lowest (*P*<0.05) in group T₁ (2.49 kg/d) and the differences among other groups were not significant, however, CP intake increased (*P*<0.05) with increasing levels of protein in the diet. Feed intake is partially dictated by physical capacity of the rumen, especially, in higher forage diets which might have reduced DMI (Nampoothiri et al. 2018).

Table 1 Chemical composition of experimental feeds (% DM basis)

Attribute	Concentrate mixture	Maize fodder	Wheat straw
Chemical composition (%)			
DM	91.36±0.51	24.14±0.78	93.05±0.04
OM	90.69±0.41	88.67±0.12	86.41±0.15
CP	20.86±0.04	8.14±0.54	3.02±0.47
EE	5.02±0.10	1.45±0.80	1.02±0.15
TA	9.31±0.63	11.33±0.05	12.59±0.85
NFC	37.53±0.18	21.44±0.10	10.72±0.01
NDF	25.83±0.16	56.63±0.24	70.66±0.81
ADF	13.67±0.77	28.41±0.35	42.61±0.84
Energy content			
TDN (%)	74.59±1.96	52.10±2.03	41.70±0.88
DE (MJ/Kg DM)	14.32±0.89	9.69±0.54	7.66±0.12
ME (MJ/Kg DM)	12.62±0.30	7.87±0.26	5.82±0.17

DM=Dry Matter; TA=Total Ash; OM=Organic Matter; CP=Crude Protein; EE=Ether Extract; NDF=Neutral Detergent Fiber; ADF=Acid Detergent Fiber; NFC=Non-Fibrous Carbohydrates; TDN=Total Digestible Nutrients; DE=Digestible Energy; ME=Metabolizable Energy

Table 2 Nutrient intake and digestibility in buffaloes given different levels of protein in diet

Particular	T ₁	T ₂	T ₃	T ₄	T ₅
BW (kg)	151.22±2.67	152.23±4.01	156.21±3.78	155.51±4.08	150.08±3.62
Nutrient intake					
DMI (kg)	2.49 ^a ±0.28	3.38 ^{ab} ±0.29	4.04 ^b ±0.24	4.26 ^b ±0.29	4.40 ^b ±0.59
CP intake (g/day)	100.69 ^a ±3.54	257.28 ^b ±36.85	400.28 ^c ±51.40	519.89 ^d ±64.44	587.49 ^d ±76.14
Digestibility of nutrients (%)					
DM	48.64 ^a ±0.77	50.45 ^a ±1.36	55.07 ^{ab} ±0.56	60.67 ^c ±1.57	61.97 ^c ±0.91
OM	50.90 ^a ±0.12	53.17 ^b ±0.94	57.01 ^b ±1.78	63.56 ^c ±0.49	65.61 ^c ±0.17
CP	45.45 ^a ±3.01	70.32 ^b ±1.24	75.47 ^{bc} ±2.67	80.18 ^c ±1.45	81.94 ^c ±1.08
NDF	50.23±2.98	53.74±2.69	53.19±4.78	56.77±3.29	50.62±3.94
ADF	43.69±1.99	46.95±5.01	43.78±1.33	45.45±1.45	43.86±3.28

^{abc}Means bearing different superscripts in the same row differ significantly (*P*<0.05)

Table 3 Enteric CH₄ emissions in calves as affected by crude protein content in the rations

Particular	T ₁	T ₂	T ₃	T ₄	T ₅
CH ₄ (g/d)	84.07 ^a ±1.95	66.93 ^b ±1.17	61.80 ^c ±0.71	54.07 ^d ±1.23	54.40 ^d ±2.06
CH ₄ (g/kg DMI)	34.48 ^a ±3.45	20.08 ^b ±1.52	15.39 ^b ±0.70	12.83 ^c ±0.99	12.73 ^c ±1.34
CH ₄ loss					
% of GE intake	11.75 ^a ±1.23	6.66 ^b ±0.74	5.04 ^{bc} ±0.44	4.32 ^c ±0.82	3.95 ^c ±0.37
% of DE intake	23.00 ^a ±3.39	11.58 ^b ±1.36	8.19 ^c ±1.57	6.61 ^d ±0.47	5.58 ^d ±0.67
% of ME intake	29.75 ^a ±3.97	14.34 ^b ±1.59	9.92 ^c ±1.88	7.87 ^d ±0.94	6.94 ^d ±0.36

^{abcd}Means bearing different superscripts in the same row differ significantly (P<0.05)

Enteric CH₄ emissions data have been presented in Table 3. On an average, CH₄ emission in groups T₃ to T₅ (12.73-15.39 g/kg DMI) found in agreement in case of growing Murrah calves i.e. 9.47-13.6 g/kg DMI (Nampoothiri et al. 2018), 12.2-13.3 g/kg DMI (Gupta et al. 2019) and 11.43-16.17 g/kg DMI (Budhani et al. 2016); however, CH₄ production in groups T₁ and T₂ were not comparable, might be due to low crude protein feed structure than the average feeding patterns of the rations. CH₄ loss represented similar loss of GE intake in the previous work (Appuhamy et al. 2016) resulting in reduced feed efficiency in the animals.

Thakur et al. (2021) found that increasing concentrate portion from 70-20% in the ration reduced the enteric CH₄ emission by 34.18% in crossbred kids. However, enteric CH₄ production was not affected by increasing CP content (14.1-18.1%) in the concentrate (Hynes et al. 2016). By selecting higher feed efficient animals, CH₄ production in the fermentation process of rumen can be reduced by decreasing CP content (130-175 g/kg DM) of the ration due to less urinary nitrogen losses (Kidane et al. 2018). Similar results have been reported (Niu et al. 2016) but with a higher level of CP (18.5%) in the diet.

Shifting the ration from concentrates to fibrous feed, higher acetate and butyrate levels, being the precursor for CH₄ production in the rumen levels caused higher CH₄ emissions (Dijkstra et al. 2011). Decreased CH₄ production by increasing CP content did not affect CH₄ emission in absolute terms i.e. L/day but there was reduction in CH₄ emission as a proportion of DMI, milk yield and GE intake (Sherasia and Garg 2016) as availability of grains causes formation of more propionate which does not provide H₂ for methanogenesis (Niu et al. 2016). However, accounting CH₄ emission per kg milk produced make sense only in the higher milk production animals (Muñoz et al. 2015) which negotiated the CH₄ production with higher milk yield. Higher grain content of concentrate led to increase in starch level in rumen, thereby, reducing ruminal pH and activity of protozoa and methanogens (Uddin et al. 2020).

Conclusions

Increasing proportion of dietary CP in the ration increased dry matter intake, nutrient intake and nutrient digestibility along with reduction in CH₄ emissions in growing Murrah buffaloes.

Therefore, 12.5-15% CP in the ration of growing Murrah buffaloes may be recommended based on the results of present study.

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