



## Original Research Article

# Growth performance, and enteric and manure greenhouse gas emissions from Murrah calves fed diets with different forage to concentrate ratios

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## ABSTRACT

The present study investigated the effects of different dietary forage to concentrate ratios on animal performance, and enteric and manure greenhouse gas emissions in growing calves. Fifteen male Murrah calves ( $153.5 \pm 18.17$  kg; 6 to 12 months) were randomly assigned to 3 dietary treatments and fed corn fodder, wheat straw and concentrate in 3 different proportions: 20:60:20 (C20); 20:40:40 (C40) and 10:30:60 (C60), for a period of 120 days. Increasing dietary concentrate proportion had no significant ( $P > 0.05$ ) effect on dry matter intake (DMI) but increased crude protein (CP) and total digestible nutrient intake ( $P < 0.05$ ). Average daily gain and feed conversion efficiency were significantly higher ( $P < 0.05$ ) for C60 compared with C20 and for C40, these did not differ with C20 and C60 ( $P > 0.05$ ). The apparent digestibility of dry matter, organic matter and CP were higher ( $P < 0.05$ ), but acid detergent fiber digestibility was lower ( $P < 0.05$ ) for C60 compared with C20 whereas, ether extract and neutral detergent fiber digestibilities were not affected ( $P > 0.05$ ). Daily methane ( $\text{CH}_4$ ) emission (g/d),  $\text{CH}_4$  energy loss (MJ/d) and  $\text{CH}_4$  yield ( $\text{CH}_4$  g/kg organic matter intake [OMI],  $\text{CH}_4$  g/kg digestible OMI, and  $\text{CH}_4$  % of metabolizable energy intake) were significantly higher for C20 compared with C60 ( $P < 0.05$ ). Methane yield as g/kg DMI although lower for C60 compared with C20 but the difference was not significant ( $P > 0.05$ ). Manure  $\text{CH}_4$  (g/kg DMI) and nitrous oxide ( $\text{N}_2\text{O}$  mg/kg nitrogen) emissions were not affected ( $P > 0.05$ ), but  $\text{N}_2\text{O}$  emission on mg/kg DM basis was significantly higher ( $P < 0.05$ ) from the manure of calves fed C60 than that for C20. Thus, increasing dietary concentrate proportion improved animal performance, and reduced enteric  $\text{CH}_4$  emission (g/day) without any significant effect on manure  $\text{N}_2\text{O}$  (mg/kg nitrogen) and  $\text{CH}_4$  emissions.

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## 1. Introduction

Animal farming is an important source of anthropogenic greenhouse gases (GHG) including methane ( $\text{CH}_4$ ), nitrous oxide

( $\text{N}_2\text{O}$ ) and carbon dioxide (Steinfeld et al., 2006). The main sources from livestock are  $\text{CH}_4$  from enteric fermentation and  $\text{CH}_4$  and  $\text{N}_2\text{O}$  from manure management. Methane emissions also represent a loss of 3.9% to 10.7% of gross energy (GE) intake in ruminants (Appuhamy et al., 2016) thereby, limiting feed energy utilization. In India, enteric fermentation and manure management were responsible for 10,520 and 121 Gg of  $\text{CH}_4$ , respectively (Mohini, 2010). The diet composition and intake have great influence on ruminant  $\text{CH}_4$  production. The livestock feeding systems in India are mainly dependent on crop residues and high fiber diets that are deficient in nitrogen and digestible energy, limiting the animal performance. These high fiber diets rich in structural carbohydrates increase ruminal acetate-to-propionate ratio, thus produce more enteric  $\text{CH}_4$  but may limit manure  $\text{CH}_4$

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production due to the resistance of excreted cell wall to microbial fermentation (Boadi et al., 2004).

Concentrate supplementation has been suggested as an effective strategy to reduce enteric CH<sub>4</sub> emission from ruminants and can also improve growth performance and nutrient utilization efficiency (Muñoz et al., 2015). However, in India, concentrate feeding is often limited to high yielding lactating animals only. Diet composition, nutrient utilization and carbon-to-nitrogen ratio affect manure composition and in turn GHG emissions (Sun et al., 2008). The diets high in concentrate produce less enteric CH<sub>4</sub> than forage-based diets (Beuchemin and McGinn, 2005) however, manure CH<sub>4</sub> may be increased due to the presence of more degradable organic matter (Külling et al., 2002; Hindrichsen et al., 2006).

Nitrous oxide is the third most important GHG produced through the process of nitrification and denitrification of manure (Dijkstra et al., 2013), and possesses 265 to 298 times higher global warming potential over 100 years than carbon dioxide. Manure management produces about 75 Gg N<sub>2</sub>O/year, in India (Mohini, 2010). Nitrous oxide emissions are directly related to nitrogen intake in ruminants and approximately 2% of nitrogen excreted by the animals or applied to fields is emitted as N<sub>2</sub>O (Hao et al., 2004). Feeding protein in excess of animal requirements increases the environmental load of nitrogen (Hristov et al., 2011) and also protein source wastage. Menezes et al. (2016) found that decreasing protein content from 13% to 10% in Nellore bull diets had no significant effect on animal performance and enteric CH<sub>4</sub> emission but reduced nitrogen loss through manure. Thus, reducing dietary protein while maintaining animal performance may be the practical approach to reduce N<sub>2</sub>O emissions.

The intergovernmental panel on climate change (IPCC, 2006) Tier 1 methodology is followed for estimation of manure GHG emissions in country which may not be accurate due to differences in livestock characteristics, feeding systems, and climatic and manure storage conditions. The simultaneous measurements of both enteric and manure emissions are also important for better understanding of overall dietary effects (Hindrichsen et al., 2006). In addition, not much information is available on enteric and manure GHG emissions from the young stock of buffaloes under different dietary regimens. The hypotheses of the present research are: 1) increasing concentrate proportion in the diets of growing Murrah calves will decrease daily CH<sub>4</sub> emission (g/d) and CH<sub>4</sub> yield (g/kg DM) but will increase CH<sub>4</sub> emission from manure; 2) decreasing dietary CP content will have no effect on animal performance or manure CH<sub>4</sub> production but will reduce N<sub>2</sub>O emission from manure. Therefore, the objectives of this study were to determine the effects of dietary forage-to-concentrate proportions on animal performance, and enteric and manure GHG emissions from growing Murrah calves.

## 2. Materials and methods

The present study was conducted from June to October 2014 at livestock farm of National Dairy Research Institute, Karnal, located 29°42'20"N and 76°58'52.5"E at an altitude of 227 m above the sea level. This study was approved by the Institutional Animal Ethics Committee (IAEC) and followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2006).

### 2.1. Animals, diets and management

Fifteen Murrah male calves (153.5 ± 18.17 kg, 6 to 12 months) were randomly assigned to 3 groups ( $n = 5$  in each group). The dietary treatments were: 1) C20, corn fodder 200 g/kg DM, wheat straw 600 g/kg DM and a concentrate feed mixture (CFM) 200 g/kg DM,

2) C40, corn fodder 200 g/kg DM, wheat straw 400 g/kg DM and CFM 400 g/kg DM, 3) C60, corn fodder 100 g/kg DM, wheat straw 300 g/kg DM and CFM 600 g/kg DM. The CFM consisted of corn grain (330 g/kg DM), groundnut cake (210 g/kg DM), mustard cake (120 g/kg DM), wheat bran (200 g/kg DM), de-oiled rice bran (110 g/kg DM), mineral mixture (20 g/kg DM) and common salt (10 g/kg DM). The calves were offered experimental diets in a well-ventilated shed with facilities for individual feeding and free access to drinking water. The calves were offered total mixed rations prepared by hand mixing of chopped corn fodder (2 to 3 cm), thrashed wheat straw (2 to 3 cm) and CFM in the respective treatment proportions. Diets were offered *ad libitum* twice daily at 09:00 and 17:00 and feed offered was adjusted weekly to meet growing demands, allowing for minimal (10 g/kg) feed refusal. Feed offered and refused were weighed daily to calculate dry matter intake (DMI), respective samples were collected fortnightly and stored at 4 °C until further analysis. Body weight was measured fortnightly using computerized weight recording platform in the morning before offering feed and water, and each weighing was performed for 2 consecutive days. Feed conversion efficiency (FCE) was calculated as g of weight gain per kg of feed DMI.

### 2.2. Metabolism trial and enteric methane emission

A metabolism trial was conducted during the mid-experiment with 3 days adaptation and 7 days of collection period to determine apparent nutrient digestibility and nitrogen balance. Collection, weighing and recording of feed offered, refusal, feces and urine were done for 7 days daily at 08:00 before morning feeding. Feces voided during 24 h were collected in plastic containers separately for all animals and the total weight of daily feces was measured. After thorough mixing, an aliquot of feces (20 g/100 g) was collected and dried at 60 °C for 48 h, daily for 7 days. Dried feed, residue, and fecal samples from each calf were ground in a Willey mill (1 mm screen) and stored at 4 °C until further analysis. For nitrogen estimation, a sub-sample of wet feces (2 g/100 g) acidified with 10 mL (vol/vol) of 10% H<sub>2</sub>SO<sub>4</sub> was collected daily in an airtight plastic container. At the end of the collection period, an aliquot of wet feces was weighed and used for nitrogen estimation (AOAC, 2005). Total urine voided each day was collected in a plastic container after adding H<sub>2</sub>SO<sub>4</sub> (40 mL) to reduce the loss of nitrogen from volatilization, and thereafter a subsample (10 mL/100 mL) was taken for nitrogen estimation using the Kjeldahl nitrogen method. Apparent nutrient digestibility was calculated as nutrient intake (kg/d) minus fecal excretion of nutrient (kg/d) divided by nutrient intake (kg/d).

Enteric methane production was measured from each calf daily for 5 consecutive days by the sulfur hexafluoride (SF<sub>6</sub>) tracer technique (Johnson et al., 2007). The brass permeation tubes (35 mm × 11 mm) were filled with 600 mg pure SF<sub>6</sub> gas and kept at 39 °C for calibration. The SF<sub>6</sub> release rate was predetermined over 40 days by weighing each permeation tube weekly to produce a linear regression curve ( $R^2 > 0.999$ ); the average release rate of SF<sub>6</sub> was 2.2 ± 0.1 mg/d. These permeation tubes containing SF<sub>6</sub> were placed into the rumen of each animal by bolus gun approximately 4 days before CH<sub>4</sub> measurements for tracer gas to equilibrate in the rumen. The representative breath samples from each calf were collected in pre-evacuated (−82.7 to −89.63 kPa) yoke-shaped polyvinyl chloride canisters (<2 L; 250 mm length, 6.3 mm diameter) by means of a capillary tube fitted to halter. The air flow into the canister (initial flow rate 0.6 mL/min) was restricted through 915 mm long stainless steel capillary tubing so that the vacuum inside the canister was reduced by about 50% over 24 h. Canisters were changed every 24 h from each animal, transported to the laboratory and filled with 100 kPa overpressure of pure nitrogen

prior to CH<sub>4</sub> and SF<sub>6</sub> analysis. The canisters were checked for the pressure before analysis and discarded if the final pressure was beyond the expected range (–89.63 [initial pressure] to –41.36 [final pressure] kPa). Background concentrations of CH<sub>4</sub> and SF<sub>6</sub> were measured by suspending two canisters vertically 1.82 m above the ground near 2 ends of the animal shed.

A gas chromatograph (Nucon 5700, Nucon Engineers, New Delhi), fitted with an electron capture detector (ECD; at 250 °C) and molecular sieve column (3.3 m, 0.32 mm) was used to determine the SF<sub>6</sub> concentrations. Another gas chromatograph (Nucon 5700, Nucon Engineers, New Delhi), with a flame-ionization detector (FID; at 100 °C) and stainless steel column packed with Porapak-Q (1.5 m × 3.2 mm × 2 mm; mesh range 80 to 100) was used for CH<sub>4</sub> estimation. The column and injector temperatures were respectively 50 °C and 40 °C in both the instruments. Concentrations of CH<sub>4</sub> and SF<sub>6</sub> were determined from peak areas and identified from their different retention times relative to the known standards (CH<sub>4</sub>-35 parts per million and SF<sub>6</sub>-110 parts per trillion). All the samples were analyzed in triplicates. Nitrogen was used as carrier gas at a pressure of 98.06 kPa. The CH<sub>4</sub> output was calculated using following formula (Williams et al., 2011):

$$\text{CH}_4 \text{ (g/d)} = (S_{\text{CH}_4} - B_{\text{CH}_4}) / (S_{\text{SF}_6} - B_{\text{SF}_6}) \times (M_{\text{CH}_4} / M_{\text{SF}_6}) \times Q_{\text{SF}_6} \times 1,000$$

where S<sub>CH<sub>4</sub></sub> and B<sub>CH<sub>4</sub></sub> are CH<sub>4</sub> concentrations in sample and background canisters (parts per million), S<sub>SF<sub>6</sub></sub> and B<sub>SF<sub>6</sub></sub> represent the concentrations of SF<sub>6</sub> in sample and background canisters (parts per trillion), M<sub>CH<sub>4</sub></sub> and M<sub>SF<sub>6</sub></sub> are molecular weight of CH<sub>4</sub> (g) and SF<sub>6</sub> (g), respectively and Q<sub>SF<sub>6</sub></sub> represents the release rate of SF<sub>6</sub> (mg/d). Methane energy was calculated by multiplying methane emission (g/d) with an energy value of 55.76 MJ/g. Methane emission as a proportion of GE, metabolizable energy (ME), and nutrient intake was calculated by dividing the daily CH<sub>4</sub> production of each calf by their energy and nutrient intake, respectively, for the CH<sub>4</sub> sampling period.

### 2.3. Methane and nitrous oxide estimation from manure

Manure CH<sub>4</sub> and N<sub>2</sub>O emission potential was estimated by Hansen et al. (2004) with some modifications. Daily fecal samples (500 g) from each calf were collected in 2-L capacity glass bottles towards the end of the feeding trial, for consecutive 5 days. The bottles were flushed with nitrogen gas before and during filling to ensure anaerobic conditions in the headspace of bottles. Each bottle was sealed with a rubber stopper and an aluminium cap to make it airtight and kept at 39 °C for 24 h. The gas formed was collected in a syringe (100 mL) attached to the bottle by a three-way cork. After incubation, syringes were removed from the bottles and analyzed for CH<sub>4</sub> and N<sub>2</sub>O by gas chromatography, using FID detector for CH<sub>4</sub> and ECD detector for N<sub>2</sub>O as described in the above section.

### 2.4. Chemical analysis

The samples of feed, residue and feces were analyzed for crude protein (CP; # 984.13), ether extract (EE; #920.39), total ash (TA; # 942.050), neutral detergent fiber (NDF # 2002.04), acid detergent fiber (ADF # 973.18), and acid detergent lignin (ADL; # 973.18) (AOAC, 2005). Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were estimated by Licitra et al. (1996). The total digestible nutrients (TDN) and ME contents of the diets were calculated by NRC (2001). Total nitrogen in the urine sample (10 mL) was estimated by Kjeldahl method (AOAC, 2005) using a KELPLUS-Kjeldahl nitrogen analyzer (Pelican Equipments, India).

### 2.5. Statistical analysis

Data were analyzed by one-way analysis of variance using statistical analysis system 9.1 (SAS Inst. Inc., Cary, NC, USA) software with the following model:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

where Y<sub>ij</sub> = the jth observation on the ith treatment; μ = overall mean; T<sub>i</sub> = effect of the ith treatment; ε<sub>ij</sub> = residual error. The differences between the means were considered significant at P < 0.05 by Tukey's test. The results are presented as means and standard error.

## 3. Results

### 3.1. Chemical composition of diets

The chemical composition of complete diets is shown in Table 1. The contents of CP, ME and EE increased while NDF, ADF and ADL contents decreased with increasing proportion of concentrate in the diet. Diet C60 had highest CP and lowest NDF content whereas C20 diet had lowest CP and highest NDF content.

### 3.2. Dry matter intake, average daily gain and feed conversion efficiency

Dry matter intake, average daily gain and feed efficiency data are presented in Table 2. Concentrate proportion had no significant (P > 0.05) effect on DM intake (kg/d or g/kg metabolic body weight), but CP and digestible CP (DCP) intake (kg/d) increased with increasing concentrate proportion of the diet (P < 0.05). Total digestible nutrient (TDN) intake (kg/d), average daily gain (ADG; g/d) and FCE were higher for C60 compared with C20 (P < 0.05). For the diet C40, TDN intake and ADG did not vary (P > 0.05) with C20 and C60 but FCE was lower than C60 (P < 0.05).

### 3.3. Apparent nutrient digestibility

Apparent nutrient digestibility data are presented in Table 2. The apparent digestibilities of EE and NDF were not affected (P > 0.05) whereas, DM digestibility increased with increasing concentrate proportion in the diet (P < 0.05). The digestibility of CP was higher for C60 and C40 compared with C20 but ADF digestibility was lower (P < 0.05). The organic matter (OM) digestibility was higher

**Table 1**  
Chemical composition of complete diets (g/kg DM).

Item	Diets <sup>1</sup>		
	C20	C40	C60
Dry matter	778	774	847
Organic matter	906	906	908
Crude protein (CP)	79.2	114	142
Ether extract	17.9	24.9	31.0
Total ash	87.2	87.8	88.9
Neutral detergent fiber	645	526	426
Acid detergent fiber	416	330	259
Neutral detergent insoluble CP	64.6	52.9	45.3
Acid detergent insoluble CP	25.3	27.6	28.9
Acid detergent lignin	60.5	52.4	47.2
Total digestible nutrients, %	53.3	59.8	64.9
Metabolizable energy, MJ/kg DM	7.90	9.28	10.3

<sup>1</sup> C20, 20:60:20 maize:wheat straw:concentrate; C40, 20:40:40 maize:wheat straw:concentrate; C60, 10:30:60 maize:wheat straw:concentrate.

**Table 2**

Feed intake, daily gain, feed efficiency and nutrient digestibility in buffalo calves fed diets with increasing proportion of concentrate.

Item	Diets <sup>1</sup>		
	C20	C40	C60
Dry matter intake (DMI), kg/d	4.65 ± 0.275	4.66 ± 0.296	4.93 ± 0.324
DMI per kg metabolic body weight, g/kg W <sup>0.75</sup>	94.4 ± 1.73	93 ± 2.023	96.4 ± 2.563
Crude protein intake, kg/d	0.37 ± 0.024 <sup>a</sup>	0.53 ± 0.035 <sup>b</sup>	0.70 ± 0.052 <sup>c</sup>
Digestible crude protein intake, kg/d	0.21 ± 0.012 <sup>a</sup>	0.33 ± 0.029 <sup>b</sup>	0.47 ± 0.037 <sup>c</sup>
Total digestible nutrient intake, kg/d	2.51 ± 0.146 <sup>a</sup>	2.92 ± 0.181 <sup>ab</sup>	3.47 ± 0.231 <sup>b</sup>
Average daily gain, kg/d	0.47 ± 0.023 <sup>a</sup>	0.53 ± 0.035 <sup>ab</sup>	0.63 ± 0.053 <sup>b</sup>
Feed conversion efficiency, g gain/kg DMI	101 ± 5.714 <sup>a</sup>	114 ± 6.962 <sup>a</sup>	128 ± 2.308 <sup>b</sup>
Nutrient digestibility, %			
Dry matter	58.3 ± 0.593 <sup>a</sup>	62.2 ± 0.824 <sup>b</sup>	66.3 ± 1.165 <sup>c</sup>
Organic matter	63.4 ± 0.572 <sup>a</sup>	65.9 ± 1.053 <sup>ab</sup>	68.2 ± 1.081 <sup>b</sup>
Crude protein	67.9 ± 0.750 <sup>a</sup>	72.6 ± 2.347 <sup>b</sup>	73.5 ± 1.481 <sup>b</sup>
Ether extract	68.5 ± 1.061	70.0 ± 1.023	72.2 ± 0.155
Neutral detergent fiber	50.3 ± 1.346	46.8 ± 1.142	44.7 ± 2.052
Acid detergent fiber	49.8 ± 1.192 <sup>b</sup>	46.0 ± 0.580 <sup>a</sup>	41.7 ± 1.541 <sup>a</sup>

a, b, c Means bearing different superscripts in the same row differ significantly ( $P < 0.05$ ); number of animals sampled in each group ( $n = 5$ ).

<sup>1</sup> C20, 20:60:20 maize:wheat straw:concentrate; C40, 20:40:40 maize:wheat straw:concentrate; C60, 10:30:60 maize:wheat straw:concentrate.

**Table 3**

Nitrogen balance (g/d) in Murrah calves fed different dietary concentrate proportions.

Item	Diets <sup>1</sup>		
	C20	C40	C60
Nitrogen intake	58.8 ± 2.76 <sup>a</sup>	93.0 ± 4.89 <sup>b</sup>	118 ± 3.17 <sup>c</sup>
Fecal nitrogen	18.3 ± 1.42 <sup>a</sup>	26.0 ± 2.71 <sup>b</sup>	36.8 ± 5.16 <sup>c</sup>
Urinary nitrogen	24.0 ± 2.89 <sup>a</sup>	47.6 ± 4.37 <sup>b</sup>	56.6 ± 5.29 <sup>b</sup>
Total nitrogen loss	42.4 ± 3.36 <sup>a</sup>	73.7 ± 6.15 <sup>b</sup>	93.4 ± 5.65 <sup>c</sup>
Nitrogen retention	16.4 ± 0.75 <sup>a</sup>	21.3 ± 1.41 <sup>b</sup>	25.3 ± 1.22 <sup>c</sup>
Nitrogen retention, as % nitrogen intake	28.2 ± 1.96	23.4 ± 2.71	21.9 ± 2.46

a, b, c Within a row, means without a common superscript differ ( $P < 0.05$ ); number of animals sampled in each group ( $n = 5$ ).

<sup>1</sup> C20, 20:60:20 maize:wheat straw:concentrate; C40, 20:40:40 maize:wheat straw:concentrate; C60, 10:30:60 maize:wheat straw:concentrate.

( $P < 0.05$ ) for C60 compared with C20, and for C40, OM digestibility did not vary with either of two treatments ( $P > 0.05$ ).

### 3.4. Nitrogen balance

Nitrogen (N) intake (g/d), N output (fecal + urinary; g/d) and N retention (g/d) increased with increasing proportion of concentrate in the diet (Table 3) ( $P < 0.05$ ). Nitrogen retention (% of N intake) was not different ( $P > 0.05$ ) among the treatments.

### 3.5. Enteric methane emissions

Effect of diet on enteric CH<sub>4</sub> production is shown in Table 4. Daily CH<sub>4</sub> emission (g/d) was lower (−21.5%) for C60 compared with C20 ( $P < 0.05$ ). Methane yield (g/kg organic matter intake (OMI), g/kg digestible DMI, g/kg digestible OMI), and energy values (MJ/d) were higher for C20 compared with C60 ( $P < 0.05$ ). Methane yield (g/kg DMI) although lower (−30%) for C60 compared with C20 but the difference was not significant ( $P > 0.05$ ). For the diet C40, daily CH<sub>4</sub> emission and CH<sub>4</sub> yield did not vary with C20 and C60 ( $P > 0.05$ ). Increasing concentrate level did not affect the percentage of ingested GE converted to CH<sub>4</sub> but CH<sub>4</sub> as a proportion of ME intake was lower for C60 compared with C20 ( $P < 0.05$ ).

### 3.6. Methane and nitrous oxide emissions from manure

Table 5 presents the CH<sub>4</sub> and N<sub>2</sub>O emissions from calf manure. Dietary concentrate proportion had no significant ( $P > 0.05$ ) effect on total gas (mL/kg DM), CH<sub>4</sub> (g/kg DM) and N<sub>2</sub>O (mg/kg N)

production. Nitrous oxide emission on mg/kg DM basis was higher for C60 compared with C20 and C40 ( $P < 0.05$ ).

## 4. Discussion

### 4.1. Dry matter intake, average daily gain and feed efficiency

In the present study, DMI remained unaltered in the buffalo calves fed diets with different dietary concentrate-to-roughage proportions. This finding is in agreement with the results of

**Table 4**

Effect of dietary concentrate level on enteric methane emission in calves.

Item	Diets <sup>1</sup>		
	C20	C40	C60
CH <sub>4</sub> , g/d	66.4 ± 3.452 <sup>b</sup>	61.0 ± 2.990 <sup>ab</sup>	52.1 ± 2.617 <sup>a</sup>
CH <sub>4</sub> , g/kg DMI	13.6 ± 1.533	11.8 ± 0.924	9.47 ± 0.630
CH <sub>4</sub> , g/kg OMI	16.7 ± 1.213 <sup>b</sup>	14.0 ± 0.991 <sup>ab</sup>	11.4 ± 0.784 <sup>a</sup>
CH <sub>4</sub> , g/kg digestible DMI	23.4 ± 2.432 <sup>b</sup>	19.1 ± 1.633 <sup>ab</sup>	14.0 ± 0.905 <sup>a</sup>
CH <sub>4</sub> , g/kg digestible OMI	27.4 ± 1.705 <sup>b</sup>	21.4 ± 1.571 <sup>a</sup>	16.5 ± 1.062 <sup>a</sup>
CH <sub>4</sub> energy, MJ/d	3.72 ± 0.052 <sup>b</sup>	3.39 ± 0.047 <sup>ab</sup>	2.92 ± 0.039 <sup>a</sup>
CH <sub>4</sub> energy loss, %			
GE intake	4.35 ± 0.481	3.74 ± 0.294	3.01 ± 0.205
ME intake	9.14 ± 1.026 <sup>b</sup>	7.26 ± 0.572 <sup>ab</sup>	5.50 ± 0.371 <sup>a</sup>

DMI = dry matter intake; OMI = organic matter intake; GE = gross energy; ME = metabolizable energy.

a, b Within a row, means without a common superscript differ ( $P < 0.05$ ); number of animals sampled in each group ( $n = 5$ ).

<sup>1</sup> C20, 20:60:20 maize:wheat straw:concentrate; C40, 20:40:40 maize:wheat straw:concentrate; C60, 10:30:60 maize:wheat straw:concentrate.



**Table 5**  
Effect of dietary concentrate level on manure methane and nitrous oxide emissions.

Parameter	Diets <sup>1</sup>		
	C20	C40	C60
Total gas, mL/kg DM	4.19 ± 0.301	3.67 ± 0.275	4.16 ± 0.347
CH <sub>4</sub> , g/kg DM	0.61 ± 0.035	0.42 ± 0.051	0.53 ± 0.033
N <sub>2</sub> O, mg/kg DM	0.21 ± 0.005 <sup>a</sup>	0.23 ± 0.014 <sup>a</sup>	0.34 ± 0.021 <sup>b</sup>
N <sub>2</sub> O, mg/kg nitrogen	3.62 ± 0.303	3.38 ± 0.932	2.61 ± 0.154

<sup>a, b</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ); number of animals sampled in each group ( $n = 5$ ).

<sup>1</sup> C20, 20:60:20 maize:wheat straw:concentrate; C40, 20:40:40 maize:wheat straw:concentrate; C60, 10:30:60 maize:wheat straw:concentrate.

Granja-Salcedo et al. (2016) and Cantalapiedra-Hijar et al. (2009), who did not find any significant effect of concentrate-to-roughage ratios on DMI in steers and goats, respectively. Increased CP and TDN intake in calves fed diet C60 is due to higher dietary protein and energy densities. Variable responses of dietary energy and protein levels on DMI in ruminants have been reported. Feed intake remained unaltered (Pina et al., 2009), increased (Javaid et al., 2008) and decreased (Tauqir et al., 2011) with increasing dietary CP content. On the other hand, high energy diets, increased (Tauqir et al., 2011) and decreased (Ríos-Rincón et al., 2014) DMI in the calves. The feed intake in ruminants is regulated by both physical fill of reticulo-rumen and metabolic-feeding factors simultaneously (Detmann et al., 2014). Feed intake in high forage diets is controlled by the gut fill capacity whereas, energy density regulates feed intake in high concentrate diets (Haddad and Ata, 2009). The absence of an effect of concentrate level on DMI, in the current study, indicated that feed intake was gut fill-limited rather than energy-limited.

The higher ADG and FCE in the calves fed C60 diet is due to greater CP and TDN intake as well as improved nutrient digestibility (Cantalapiedra-Hijar et al., 2009). A quadratic response of dietary concentrate level on daily gain was observed by Papi et al. (2011), with highest daily gain at 50% and 70% concentrate levels. In contrast, feeding low, medium and high dietary protein and energy levels did not affect daily gain and feed efficiency in buffalo calves (Shahzad et al., 2011). The better daily gain with C60 diet (CP 142 g/kg DM and ME 10.3 MJ/kg DM) also indicated that nutrient requirements of Murrah calves were more precisely met compared with C40 (CP 114 g/kg DM and ME 9.28 MJ/kg DM) and C20 (CP 79.2 g/kg DM and ME 7.9 MJ/kg DM) diets. It is similar to the findings of Tauqir et al. (2011) who suggested 142 g/kg DM and 9.37 MJ/kg DM as optimum CP and ME requirements for growing male buffalo calves under 1 year of age. Similar to the present study, diet containing 145 g CP/kg DM and ME of 10.5 MJ/kg DM at 55:45 concentrate to roughage ratio was suggested optimum for growing Brahman × local crossbred calves (Rashid et al., 2015).

#### 4.2. Apparent nutrient digestibility

Concentrate supplementation may improve nutrient digestibility in low-quality roughage diets because of greater nutrient supply to rumen microbes and enhanced rumen fermentation (Cantalapiedra-Hijar et al., 2009). Apparent DM digestibility decreased with increased dietary forage-to-concentrate ratio which agrees with the findings of Na et al. (2017). Likewise, Santra and Karim (2009) also reported higher DM and OM digestibility with increasing dietary concentrate level. The enhanced DM and OM digestibility for C60 could be due to higher nonstructural carbohydrates in it which are more digestible than structural carbohydrates (Allen, 2000). The decline in apparent ADF digestibility for C60 was similar to the findings of Moorby et al. (2006) when

dietary forage proportion was decreased from 60% to 25% of DM in dairy cattle. Neutral detergent fiber digestibility for different diets remained similar and agrees with the findings of Granja-Salcedo et al. (2016) and Santos et al. (2015). High concentrate intake may be associated with lower rumen pH which inhibits fibrolytic bacterial growth and NDF digestibility (Moorby et al., 2006). In the current study, rumen pH was not probably reduced to the extent to impair the growth of fibrolytic bacteria and fiber digestibility. Contrarily, concentrate supplementation increased NDF and ADF digestibilities in the sheep (Asmare et al., 2010). Discrepancies in the digestibility results can be attributed to variation in animal species, levels of concentrate supplemented, composition of carbohydrate, and type and quality of basal forage.

#### 4.3. Nitrogen balance

In this study, increase in nitrogen intake with increasing concentrate proportion resulted in higher fecal and urinary nitrogen losses. Nitrogen retention also increased with increasing dietary concentrate level due to higher intake and digestibility of CP. Santos et al. (2015) fed 2 levels of dietary CP (10% and 14.25%) to lambs and found that higher protein level (14.25%) led to greater nitrogen intake, nitrogen excreted through urine and nitrogen retention without any effect on retained nitrogen/ingested nitrogen. Feeding increasing amount of dietary protein (8%, 11%, 13% or 16%) to heifers resulted in a linear increase in nitrogen intake, urinary nitrogen excretion and nitrogen absorbed (Hoffman et al., 2001). Nitrogen retention as a proportion of nitrogen intake is low with high concentrate diets due to quick production of high quantities of ammonia which cannot be utilized by the animals (Hristov and Jouany, 2005). In the present study, nitrogen retention (% nitrogen intake) was not affected by dietary concentrate level which suggested either reduced rumen ammonia production or enhanced microbial capture of ammonia because of synchronization of high energy and protein intake (Hristov and Jouany, 2005; Granja-Salcedo et al., 2016). Santos et al. (2015) observed lowest rumen ammonia-nitrogen concentration in the cattle fed 60% concentrate diet indicating greater use of ammonia for microbial protein synthesis. Moreover, higher microbial protein synthesis was observed in steers at 40% and 60% concentrate levels (Granja-Salcedo et al., 2016).

#### 4.4. Enteric methane emissions

The average CH<sub>4</sub> yield (9.47 to 13.6 g of CH<sub>4</sub>/kg DMI) was considerably lower than the previous reported values for buffalo calves; 12.45 to 18.11 g/kg DMI (Malik and Singhal, 2009), 15.97 to 18.34 g/kg DMI (Mohini and Singh, 2008), and 20.7 to 25.1 g/kg DMI (Prusty et al., 2017). The authors could not find a reason for such low methane yield compared to previous records for calves. The daily CH<sub>4</sub> emission (g/d) and CH<sub>4</sub> yield (CH<sub>4</sub> g/kg OMI, CH<sub>4</sub> g/kg digestible OMI and CH<sub>4</sub> % of ME intake) were significantly lower for C60 compared with C20. Numerically, CH<sub>4</sub> g/kg DMI was about 30% lower for C60 versus C20, but this difference was not statistically different may be because of relatively small number of calves per treatment and large variations between individual calves. The diet C60 was also associated with 5.3 g and 10.9 g less CH<sub>4</sub> per kg OMI, and per kg digestible OMI respectively, than C20. The results are in line with Niu et al. (2016), who reported 7.2% lower enteric CH<sub>4</sub> emission from dairy cows on reducing dietary forage from 53% to 38% of DM. Jiao et al. (2014) offered 4 concentrate levels (2.0, 4.0, 6.0, and 8.0 kg/cow per day) to grazing cattle, and found that daily CH<sub>4</sub> emission (g/d) was unaffected but CH<sub>4</sub> yield (CH<sub>4</sub> g/kg DMI, CH<sub>4</sub> % of GE and ME intake) decreased with increasing concentrate level.

The lower CH<sub>4</sub> production with concentrate supplementation is due to shift in the rumen fermentation towards propionate production resulting in lower acetate to propionate ratio, reduction in rumen pH, reduced methanogen population and rapid digesta passage rate (Granja-Salcedo et al., 2016). The dietary fat and CP contents increased with increasing concentrate proportion in the present study. The dietary fats have a suppressive effect on rumen CH<sub>4</sub> production but the inhibitory effect may not be significant below 5% dietary fat level (Patra, 2013), as may be in the current experiment. The effect of dietary crude protein on enteric CH<sub>4</sub> emission is highly variable (Todd et al., 2008) or insignificant (Niu et al., 2016). Niu et al. (2016) reported that feeding of different dietary CP levels (18.7%, 15.3%, 18.4% and 15.1%) to dairy cows had no significant effect on daily CH<sub>4</sub> emission or CH<sub>4</sub> emission intensity. Greater CH<sub>4</sub> production with high roughage diets is due to higher NDF intake, as rumen CH<sub>4</sub> is mainly produced from fermentation of structural carbohydrates. However, feeding higher concentrate level increased total CH<sub>4</sub> emission in grazing cattle (Lovett et al., 2005) and heifers (Silva et al., 2017), due to increase in total DMI. Methane yield (g/kg OMI and g/kg digestible DMI) was significantly lower for C60 compared to C20 because of higher nonstructural carbohydrates in it. The diets rich in non-structural carbohydrates increase ruminal propionate production which results in lower CH<sub>4</sub> production per unit of OM fermented (Johnson and Johnson, 1995).

Methane conversion rate (Y<sub>m</sub>, % of GE intake) was 3% to 4.3%, which is lower than previously reported values (3.93% to 5.73%) for buffalo calves fed wheat straw, concentrate and alfalfa fodder (Malik and Singhal, 2009). The Y<sub>m</sub> was also considerably lower than 6.3% suggested by Charmley et al. (2016) for less than 30% concentrate diets (C20) and (6.5 ± 1.0)% used by IPCC (2006) Tier 2, mainly due to low methane emissions. Similar to our results, CH<sub>4</sub> conversion rate (% of GE) was not affected by feeding high-grain and high-forage diets to steers (Beauchemin and McGinn, 2006). The lower energy losses as CH<sub>4</sub> in relation to ME intake indicated better feed utilization efficiency in high concentrate diet.

#### 4.5. Manure methane and nitrous oxide emissions

Manure CH<sub>4</sub> emission has received less emphasis as it comprises a minor proportion of total CH<sub>4</sub> emissions from an animal (Klevenhusen et al., 2011). The inferences for manure GHG emissions should be drawn with caution, as the present experiment studied only GHG emission potential and does not reflect any manure management practice being followed in the India. The manure CH<sub>4</sub> emission was not affected by increasing concentrate proportion of the diet, in line with Aguerre et al. (2012), who reported a non-significant effect of increasing forage to concentrate ratio (47:53 to 68:32) on manure CH<sub>4</sub> emission. Contrarily, Külling et al. (2002) reported that concentrate supplementation decreased enteric CH<sub>4</sub> output but increased manure CH<sub>4</sub> emission due to the higher amount of well digestible fiber excreted. Apart from the diet, manure emissions are also influenced by the system of storage, duration of waste management, storage temperature, and composition of manure and bedding material (Klevenhusen et al., 2011). Lack of a significant effect on manure CH<sub>4</sub> production, in the present study, can be attributed to shorter storage period (24 h). Manure CH<sub>4</sub> emission increased with storage time and peak emissions were observed around 35 days (Hindrichsen et al., 2006; Külling et al., 2002) and in some cases after 13 weeks (Klevenhusen et al., 2011) of storage. Thus, increasing concentrate supplementation to low-quality roughages improved animal performance and reduced enteric CH<sub>4</sub> emission without affecting manure CH<sub>4</sub> production.

Nitrous oxide emission values were lower than reported values of Boadi et al. (2004) from the manure of steers fed low forage:grain

(10:90) or high forage:grain (40:60) diets. The shorter storage period (24 h) in the current experiment might be responsible for overall lower N<sub>2</sub>O emissions. Nitrous oxide emission (mg/kg DM) was higher for C60 due to higher intake and fecal excretion of nitrogen. Similar to the present findings, Külling et al. (2002) reported higher N<sub>2</sub>O emission from manure of dairy cows fed high-protein diet. In contrast, feeding high or low-concentrate diets did not affect manure N<sub>2</sub>O emissions due to similar nitrogen content of the diets (Mathot et al., 2012; Boadi et al., 2004). Moreover, feeding 2 dietary CP levels (10% and 13% CP) to steers had no influence on manure N<sub>2</sub>O emission during storage, probably change in CP level was not enough to affect N<sub>2</sub>O emission (Chiavagato et al., 2015). But the CP levels in the current study had large variation between C20 (79.2 g/kg DM) and C60 (142.2 g/kg DM) diets. The N<sub>2</sub>O emission on mg/kg N basis was numerically lower for the C60 diet which indicated slightly better utilization of nitrogen.

## 5. Conclusions

In summary, data for both enteric and manure GHG emissions were obtained for growing buffalo calves. The higher dietary concentrate proportion improved animal performance, reduced enteric methane emission (g/day) but increased manure N<sub>2</sub>O emission (mg/kg DM) without affecting manure CH<sub>4</sub> production. The CH<sub>4</sub> conversion rate (% of GE intake) was considerably lower than the value (6.5 ± 1.0)% used in IPCC Tier 2. Thus, feeding higher concentrate proportion to growing calves for maximum growth resulted in lower enteric CH<sub>4</sub> emission and energy losses as CH<sub>4</sub>. The higher growth rate may also reduce the number of days to market and consequent CH<sub>4</sub> production.

## Conflicts of interest

The authors declare that there is no conflict of interest.

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