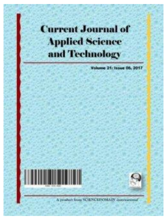


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# Effect of Diets with Different Roughage-to-Concentrate Proportions on Manure Methane and Nitrous Oxide Fluxes

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors VM and MM designed the study, performed the statistical analysis and wrote the protocol. Author BAM wrote the first draft of the manuscript and managed the literature searches. Authors MM and GM revised the manuscript. Author SP managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To measure methane and nitrous oxide fluxes from stored manure of buffalo calves fed different roughage to concentrate proportions.
**Place and Duration of Study:** The study was conducted at Livestock farm of NDRI Karnal Haryana from November 2014 to January 2015.
**Methodology:** Fifteen Murrah male calves (154.19± 17.77 kg; 6-12 months) were randomly assigned into three groups and were fed maize fodder, wheat straw and concentrate in three different proportions 20:60:20 (T20), 20:40:40 (T40) and 20:20:60 (T60), respectively. The dung samples from each calf were collected during the last week of the feeding trial and stored in heaps (height 61 cm; base radius 26 cm) under a plastic bucket for three months. The stored manure samples were collected every 2 weeks for composition analysis. Gas samples were collected through a sample port on top of each bucket 3 times per day for first 10 days then after every 2 weeks for the rest of storage period and analysed for methane ($CH_4$) and nitrous oxide ($N_2O$)

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concentrations. The amount of gas emitted was calculated as the product of gas concentration and flow rate of air passing through the exhaust. The emission rate was calculated by adding up gas emitted at each sampling and expressed in different units.

**Results:** Manure dry matter and organic matter (%) were not affected but nitrogen (%) was higher in T60 as compared to T20 and T40. Average $CH_4$ (mg/kg manure/d; mg/kg volatile solids/d) and $N_2O$ (mg/kg manure/d) emission rates were higher in T60 compared to T20. However, $CH_4$ emission expressed as g/animal/d and $N_2O$ as mg/animal/d did not (P>0.05) vary between the treatments groups. The fluxes increased up to the mid-storage period and then declined, more during the last week.

**Conclusion:** Overall, increasing dietary concentrate proportion increased manure $CH_4$ and $N_2O$ flux rates but the emissions were very low. Thus, the systems of manure storage in India are not much conducive for greenhouse gas emissions which must be taken into account during the inventory preparation.

*Keywords: Bubalis bubalis; flux rate; methane; nitrous oxide; storage.*

## 1. INTRODUCTION

Livestock manure management contributes makes a significant contribution to global greenhouse gas (GHG) emissions through carbon dioxide, methane ($CH_4$), ammonia and nitrous oxide ($N_2O$). It is estimated that manure management accounts for about 3-6% of total $CH_4$ and 7% of $N_2O$ emissions, globally [1]. In India, about 126 Gg of $CH_4$ and 80 Gg $N_2O$ per year are produced from livestock manure. The diet of an animal has a major effect on manure GHG emissions, as it changes the composition of excreted manure and also an effective strategy for reducing these emissions [2]. Straw and crop residue-based diets, rich in indigestible fibre are still largely fed to livestock in India and concentrate is supplemented to high yielding lactating animals only. Concentrate supplementation has been reported to reduce enteric $CH_4$ emission but may increase manure $CH_4$ production due to a greater degradable organic matter excreted [3]. However, [4,5] did not observe any significant effect of concentrate diet on manure-derived $CH_4$ and $N_2O$ emissions. The above observations were based on isonitrogenous diets and studies related to the effect of dietary protein levels on manure-derived $CH_4$ are very few.

Manure management also accounts for about 30–50% of $N_2O$ emissions from agriculture sector [6]. Nitrous oxide production is directly related to the dietary nitrogen intake and feeding protein in excess of animal requirements is wastage and contributes to environmental pollution [7]. Most of the ingested nitrogen (65-80%) by ruminants is excreted via faeces and urine. Reducing crude protein from 13% to 10% in steer diets decreased nitrogen excretion without affecting animal performance [8]. Thus, optimizing nitrogen intake with animal protein requirements reduces nitrogen loss and improves protein utilization efficiency [9]. The default emission factors of [10] are used to estimate concentrations of GHG in India which may not be accurate due to differences in livestock characteristic, animal feeding and environmental and storage conditions. The accurate quantification of GHG emissions is also required for preparation of an inventory and to prioritize the mitigation measures from this sector [11].

Considering the lack of data on manure GHG emissions from buffalo calves, the aim of the present research was to evaluate the effect of dietary forage-to-concentrate ratios on manure composition and $CH_4$ and $N_2O$ emissions from stored manure.

## 2. MATERIALS AND METHODS

### 2.1 Animals, Diets and Experimental Design

The experiment was conducted at the Livestock Research Centre of National Dairy Research Institute, Karnal located 29°42′ 20″N, 76°58′ 52.5″E under the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals [12]. Fifteen Murrah male calves (154.19 ± 17.77 kg; 6-12 months) were randomly assigned into three groups (T20, T40, T60) and housed in individual “tie-stalls’’. The three dietary treatments were fed 1) T20: maize fodder 200 g/kg DM, wheat straw 600 g/kg DM and concentrate feed mixture (CFM) 200 g/kg DM, 2) T40: maize fodder 200 g/kg DM, wheat straw 400 g/kg DM and CFM 400 g/kg DM, 3) T60: maize fodder 100 g/kg DM, wheat straw 300 g/kg DM and CFM 600 g/kg DM. Concentrate feed mixture consisted of maize grain (330 g/kg DM), groundnut cake (210 g/kg DM), mustard cake (120 g/kg DM), wheat bran (200 g/kg DM), deoiled rice bran (110 g/kg DM), mineral mix (20 g/kg DM) and common salt (10 g/kg DM). Diets were offered as total mixed ration in two equal sized proportions at 0800 and 1800 h. Drinking water was offered free of choice daily. Chemical composition of experimental diets is provided in Table 1.

## 2.2 Methane and Nitrous Oxide Fluxes

Faecal samples were collected from each calf during the last week of the feeding trial, proportional to excretion and stored at -20°C. After eight days of collection, samples were thawed, pooled together and stored in heaps (height 61 cm; base radius 26 cm) under a plastic bucket for three months from November 2014 to January 2015. The plastic bucket was having an air inlet and outlet for gaseous exchange. A power operated exhaust fan was fitted on one side of the bucket to facilitate the mixing of gases. The exhaust fan speed was regulated by a regulator to obtain an air velocity of approximately 0.5 m/s. The flow rate of air (volume/second) through exhaust was calculated as the product of air velocity (m/s) through exhaust and area of the exhaust ($m^2$). A sample port on the top of each bucket was used for gas collection. Gas samples were collected from each heap through sample port using gas tight-syringe (100 mL) 3 times per day for first 10 days and then after every 2 weeks for the rest of storage period. Samples were transferred to evacuated 30 mL vials fitted with a rubber stopper and transported to the laboratory for $CH_4$ and $N_2O$ analysis. Two buckets were left unfilled with manure to measure ambient air gas concentrations as control.

Methane was measured using gas chromatograph Nucon 5700 (Nucon Engineers, New Delhi) fitted with a flame ionization detector and stainless steel column packed with Porapak-Q (1.5m; 3.2mm; 2 mm). Nitrous oxide was estimated using another GC Nucon 5700 (Nucon Engineers, New Delhi) having an electron capture detector and molecular sieve column (3.3 m x 0.32 mm). Column and injector temperatures were 50°C and 40°C and detector temperature was set as 350°C. Nitrogen was used as carrier gas at a pressure of 98.06 kPa. The gases ($CH_4$ and $N_2O$) were identified from their retention times relative to standards (methane-35 parts per million and $N_2O$-2.5 parts per million) and concentrations were determined from their respective peak areas. Amount of gas emitted was calculated as the product of gas concentration and flow rate of air passing through the exhaust. Emission rate was calculated by adding up gas emitted at each sampling and expressed as mg/kg of manure/d, g/animal/d and $CH_4$ per kg volatile solids. The flux rate (g/kg manure/d) was calculated as

= (conc. of gas x flow rate/manure weight) x molecular weight of gas / Volume of a mole of gas at 20°C x 60 (s) x 60 (m) x 24 (h)

Manure samples were collected on day 0 and every 2 week from centre, top, bottom, and sides of heap, mixed and analysed for dry matter (DM), organic matter (OM) and nitrogen contents [13].

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

| Item | Treatments[1] | | |
|---|---|---|---|
| | **T20** | **T40** | **T60** |
| Dry matter (DM) | 778.03 | 774.05 | 840.79 |
| Organic matter | 906.82 | 906.25 | 908.04 |
| Crude protein (CP) | 79.23 | 114.61 | 142.82 |
| Ether extract | 17.90 | 24.91 | 31.14 |
| Total ash | 87.21 | 87.85 | 88.94 |
| Neutral detergent fibre | 645.72 | 526.07 | 426.01 |
| Acid detergent fibre | 416.63 | 330.17 | 259.74 |
| Neutral detergent insoluble CP | 64.61 | 52.94 | 45.32 |
| Acid detergent insoluble CP | 25.35 | 27.61 | 28.92 |
| Total digestible nutrients (%) | 53.39 | 59.84 | 64.90 |
| Metabolizable energy (MJ/kg DM) | 7.90 | 9.27 | 10.36 |

*[1] T20, 20:60:20 maize:wheat straw:concentrate; T40, 20:40:40 maize:wheat straw:concentrate; T60, 10:30:60 maize:wheat straw:concentrate.*

### 2.3 Statistical Analysis

Data were analysed by one way analysis of variance using the statistical analysis system 9.1 (SAS Inst. Inc., Cary, NC, USA). The differences between the means were considered significant at *P<0.05* by Tukey's method. The results are presented as means and standard error.

## 3. RESULTS AND DISCUSSION

### 3.1 Manure Composition

Manure dry matter and organic matter (%) were not affected but nitrogen (%) was higher (*P* = .05) in T60 as compared to T20 and T40 (Fig. 1, 2 & 3). Initial DM (%) was 17.93 (T20), 16.32 (T40) and 18.75 (T60) which increased to 23.80 (T20), 21.93 (T40) and 20.30 (T60), respectively after twelve weeks of storage. The organic matter degradation in stored manure was 6.84%, 7.17% and 9.21% for T20, T40 and T60, respectively. Manure composition depends upon diet composition, its digestibility and an optimal carbon-to-nitrogen ratio for effective OM degradation by rumen microbes. The absence of a significant effect on manure DM and OM may be because of their similar contents in the diets. In agreement with our results, [2] did not observe any significant effect of low forage:grain or high forage:grain diets on manure composition in feedlot steers, due to similar nitrogen and energy contents of the diets. Contrary to our results, manure DM content was 3% higher in heifers fed high concentrate (80%) compared to low concentrate (20%) diets [14].

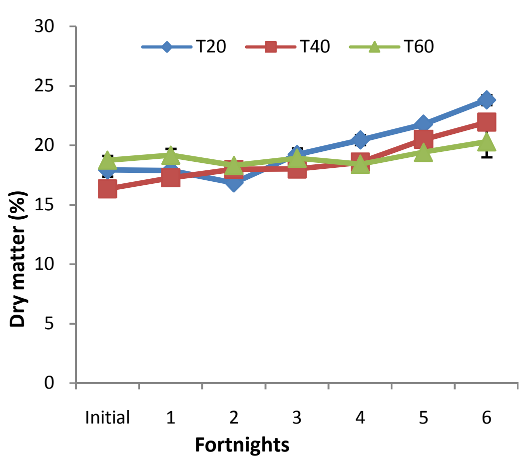


**Fig. 1.**

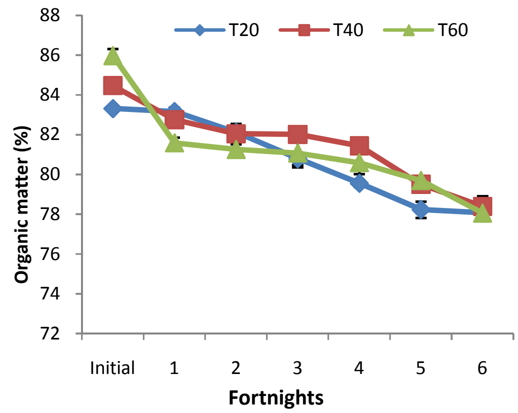


**Fig. 2.**

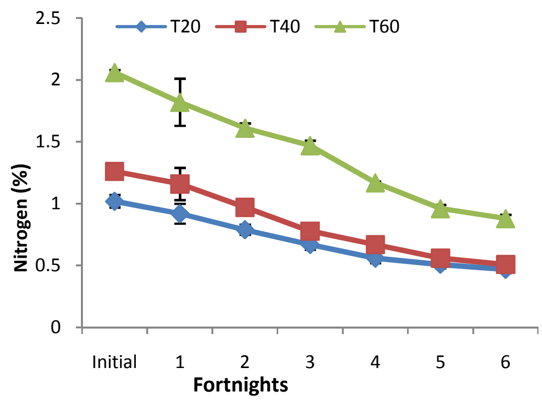


**Fig. 3.**

**Variations of dry matter (Fig. 1), organic matter (Fig. 2) and nitrogen (Fig. 3) contents of manure with storage time**

The initial nitrogen (%) was 1.02 (T20), 1.26 (T40) and 2.06 (T60), which decreased to 0.47 (T20), 0.51 (T40) and 0.88 (T60), respectively at the end of storage. Greater manure nitrogen in T60 could be due to higher CP content of the diet. Similar to the present findings, greater nitrogen excretion was found in steers fed 13% CP compared to 10% CP diet [8]. However, manure nitrogen content did not differ between high and low concentrate diets due to the high variability of faecal and urinary nitrogen content [15]. The differences could be related to composition of the diet, amount of concentrate and type, duration and conditions of storage.

### 3.2 Methane Flux Rate

Methane flux rate was affected by both diet and storage week (*P* = .05). Large variations were

**Table 2. Effect of dietary concentrate proportion on manure composition**

| Variable | Treatments[1] | | |
|---|---|---|---|
| | T20 | T40 | T60 |
| Dry matter (%) | 19.63 ± 0.93 | 18.64 ± 0.73 | 19.04 ± 0.26 |
| Organic matter (%) | 80.82 ± 0.87 | 81.52 ± 0.76 | 81.18 ± 0.92 |
| Nitrogen (%) | 0.71[a] ± 0.08 | 0.84[a] ± 0.11 | 1.24[b] ± 0.17 |

*[1] T20, 20:60:20 maize:wheat straw:concentrate; T40, 20:40:40 maize:wheat straw:concentrate; T60, 10:30:60 maize:wheat straw:concentrate.*

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

observed in the methane flux rate during the first ten days of storage (Fig 4). Methane flux rate increased from day one to eight in all the three treatments with T40 and T60 having higher (*P* = .05) flux rate than T20. The average $CH_4$ flux rate ranged 1.17-1.90 mg/kg manure/d over 12 weeks of storage period. The flux rate was much lower than 0.24 g $CH_4$/kg fresh manure reported from stored manure of heifers fed 55% concentrate diet [11]. The values were also lower than 0.0089 and 0.0106 g/kg fresh manure/d [16] but in range to 0.00011–0.0020 g/kg fresh manure/d from solid manure stored in cold conditions [17]. Overall, low methane emission values could be due be related to the absence of excreta and bedding material in the manure, storage during the winter and system of storage [18,19]. The presence of straw in manure provides additional fermentable fibre [9], urine acts as nitrogen source and emissions are higher in summer than winter. The manure $CH_4$ emission increases with rise in temperature [20], but in the present study, correlation between manure $CH_4$ and temperature was non-significant (Table 4). In addition, the use of dynamic chambers, in the present study may also have resulted in low gaseous concentrations. The dynamic chambers have an open air flow system which prevents the accumulation of gases and thus reduce gaseous concentrations [21].

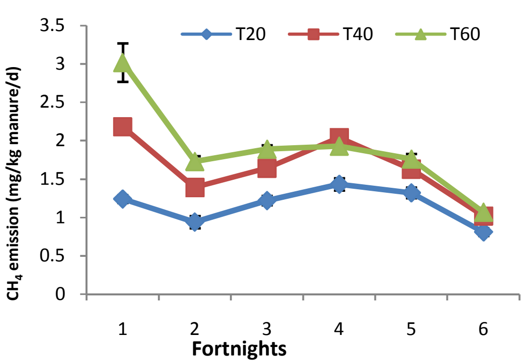


**Fig. 4.**

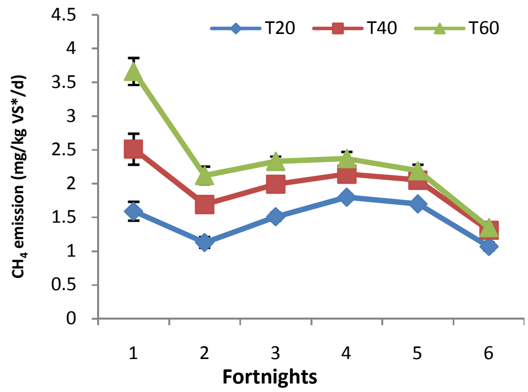


**Fig. 5.**

**Variation of methane ($CH_4$; Fig. 4 and 5) fluxes during twelve weeks of storage.**
**VS = volatile solids*

The flux rate was very high for the first 2 weeks by a sharp decline, after this $CH_4$ flux rate increased in all the treatments and then declined during the last week of storage of storage (Fig 5). The relatively higher $CH_4$ flux during the initial days may be attributed to greater degradation rate of hemicellulose and protein during early stages of composting [22] and release of $CH_4$ dissolved in the excreta [23]. Decline in flux rate after the peak is due to reduction in nutrient availability and water content [2] or changes in manure characteristics [4]. Average $CH_4$ emission rate expressed as mg/kg manure/d and mg/kg volatile solids/d was higher (*P* = .05) in T60 than T20 (Tables 2 and 3). Diet influences manure composition which in turn affects GHG emissions from it [24]. In partial agreement with present results, [3] found that manure-derived $CH_4$ was almost twice as high in dairy cows fed concentrate diet due to the higher neutral detergent fibre excretion by these cows. In a different type of dietary modification by above authors, high fibre concentrate diet reduced manure $CH_4$ emissions due to the resistance of excreted fibre to microbial fermentation [25] as may be with T20 treatment in the current study.

Greater $CH_4$ production with high concentrate diets may be due to reduced fibre digestibility which may have increased manure hemicellulose content and $CH_4$ production is positively correlated with hemicellulose content [26]. Methane emission depends on organic matter content of manure hence, methane emission increases with OM content and its degradation. The OM degradation was higher in T60 as compared to T20 and correlation of $CH_4$ flux with OM degradation was positive and significant (Table 4). In contrast, [5] and [2] did not find any significant effect of dietary forage-to-concentrate ratio on manure $CH_4$ emission in dairy cattle, due to similar manure composition and use of straw bedding in manure composting. In the present study, increasing dietary concentrate proportion resulted in higher CP content of the diet. There is an inconsistency of results on the effect of dietary crude protein on manure $CH_4$ methane production [27]. The [8], reported that feeding of different dietary CP levels (10% and 13% CP) to steers had no significant effect on the manure $CH_4$ emissions, which is contrary to our results. The authors suggested a similar starch source in both diets as a reason, which although similar in our study but varied in quantity.

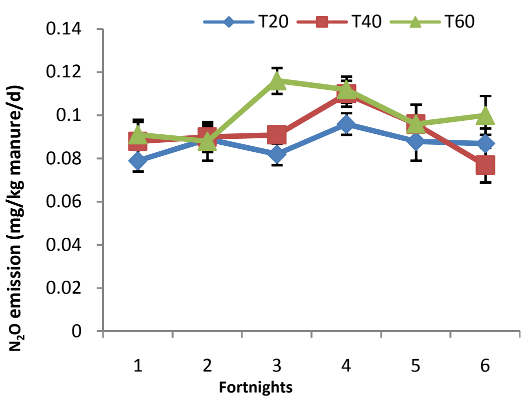


**Fig. 6.**

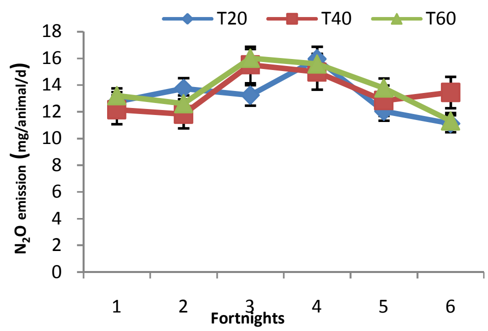


**Fig. 7.**

**Variations of nitrous oxide ($N_2O$; Fig. 6 and 7) fluxes during twelve weeks of storage.**

### 3.3 Nitrous Oxide Emission Rate

The nitrous oxide flux rate (mg/kg manure/d) for the first ten days of storage varied widely with no particular trend and was not affected by diet or storage period (Fig 6). During the twelve weeks of storage, average $N_2O$ emission rate (mg/kg manure/d) was higher ($P$ = .05) in T60 compared to T20 and T40 (Table 3) but $N_2O$ emissions expressed as mg/animal/day did not vary between the treatment groups. Nitrous oxide emissions ranged from 0.086-0.101 mg/kg manure/d over 12 weeks of storage lower than previous reported values 0.86–0.96 mg/kg manure from dairy cows measured by closed chamber technique [16]. The $N_2O$ emission values were also lower than 0.012-0.025 g/kg fresh manure from solid stored manure of calves fed diets containing different concentrate proportions [11]. In addition to the above cited reasons for low emissions, absence of additional straw in manure storage might have increased density of manure, decreased air diffusion through it, aerobic degradation of OM and consequent $N_2O$ production [11]. The $N_2O$ emission rate was higher for first 8 weeks possibly due to the greater nitrogen degradation during this period (Fig. 7).

**Table 3. Effect of dietary concentrate proportion on methane and nitrous oxide fluxes**

| Variable | Treatments[1] | | |
|---|---|---|---|
| | T20 | T40 | T60 |
| Methane emission (mg/kg manure/d) | $1.17^a \pm 0.04$ | $1.63^{ab} \pm 0.06$ | $1.90^b \pm 0.08$ |
| Methane emission (mg/kg VS*/d) | $1.47^a \pm 0.12$ | $1.95^{ab} \pm 0.17$ | $2.33^b \pm 0.30$ |
| Methane emission (g/animal/d) | 0.16 ± 0.01 | 0.24 ± 0.01 | 0.25 ± 0.02 |
| Nitrous oxide emission (mg/kg manure/d) | $0.086^a \pm 0.003$ | $0.092^a \pm 0.003$ | $0.101^b \pm 0.003$ |
| Nitrous oxide emission (mg/animal/d) | 13.14 ± 0.81 | 13.46 ± 1.13 | 14.41 ± 0.69 |

**VS= volatile solids, [1] T20, 20:60:20 maize:wheat straw:concentrate; T40, 20:40:40 maize:wheat straw:concentrate; T60, 10:30:60 maize:wheat straw:concentrate. [a, b] Means bearing different superscripts in the same row differ significantly (P< 0.05); number of animals sampled in each group (n = 5).*

**Table 4. Correlation of methane and nitrous oxide fluxes with diet chemical composition**

| Parameter | Methane flux rate | Nitrous oxide flux rate |
|---|---|---|
| Dry matter | -0.38 | -0.71* |
| Organic matter | 0.63* | -0.21 |
| Organic matter degradation | 0.52* | -0.22 |
| Nitrogen | 0.73* | 0.16 |
| Nitrogen degradation | 0.31 | 0.03 |
| Temperature | 0.08 | -0.32 |
| Humidity | -0.09 | -0.31 |

** P<0.05*

Manure $N_2O$ emissions are directly affected by nitrogen content of the diet. Higher $N_2O$ emission rate in T60 could be due to higher CP intake by these animals. This finding is in agreement with [28], who fed different levels of dietary CP (175, 150 and 125 g CP/kg DM) at 50:50 concentrate-to-forage ratio to lactating cows and, found higher $N_2O$ emissions with high dietary CP. In contrast, feeding of high, medium or low CP diets to dairy cows had no influence on $N_2O$ emissions [29]. Moreover, the application of slurry from dairy cows fed either high forage (75:25) or low forage (55:45) diets to grassland did not affect the daily $N_2O$ fluxes [15]. The correlations between $N_2O$ flux rate and chemical composition of manure were non-significant except DM (-0.71), which was negative and significant ($P$ = .05; Table 4) [30].

## 4. CONCLUSION

Results of this study showed that dietary concentrate proportion has significant effect on manure composition and greenhouse gas fluxes. Higher concentrate proportion increased $CH_4$ and $N_2O$ fluxes from stored manure but the emissions were very low. Thus, more research is needed for accurate quantification of manure GHG emissions under different feeding and management systems in India for its mitigation and inventory preparation.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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