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Effects of supplementing tropical dairy cows with different dietary protein sources on nitrogen excretion and manure greenhouse gas emissions

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Abstract

In Kenya, commercial concentrates and leguminous forages are widely used as protein sources in dairy production. However, little is known on their impact on manure greenhouse gas emissions. This study compared manure chemical composition, nitrogen excretion rates, and methane and nitrous oxide emissions in a controlled laboratory experiment. Fresh manure was collected from lactating dairy cows fed on a basal diet of *Brachiaria brizantha* cv. *Xaraes* hay (control, CON) and supplemented with either *Desmodium (Desmodium intortum)* hay (DES) or dairy cubes (CUBES) and incubated in 1 L mason jars at 20 °C for 84 days. Results showed that DES and CUBES supplementation increased nitrogen excreted both in faeces (1.81±0.02% DM, 1.68±0.10% DM) and urine (0.76±0.05%, 0.75±0.07%) compared to the CON (faeces: 1.35±0.02% DM, urine: 0.35±0.02%). These findings suggest that protein supplements affect manure composition, though in-situ studies are needed to understand their impact on manure emissions.

Keywords: *Brachiaria brizantha* cv. *Xaraes*, Condensed tannins, *Desmodium intortum*, manure incubation, methane, nitrous oxide, protein supplements

Introduction

Dairy farming is a crucial subsector of Kenya's agricultural sector, contributing significantly to the economy, food security, employment, and livelihoods of millions of farmers [1, 2]. With the increase in demand for dairy products due to population growth, rising incomes, and urbanization, livestock is becoming more important [3]. To meet the growing demand for milk, the dairy sector has been transitioning over time, moving towards more commercial and intensified systems [4, 5].

This transition involves higher stocking densities, improved genetic merit, and more concentrated diets [6]. However, this is likely to increase the volumes of manure [7] and influence manure composition [8]. Manure can negatively impact the environment if not well managed through nutrient pollution and greenhouse gas (GHG) emissions [9].

The East African smallholder systems utilize feeds high in fiber and low in N with little or no supplementation [10, 11] to support milk production. Locally grown leguminous supplements can improve production and reduce GHG emissions [12, 13]. As dietary N is the primary driver of N excretion [14-16], its variation affects the N excretion in the urinary or dung pathways, hence the urine-N: Faecal-N split. Increased N intake causes a shift from fecal-N to urine-N, which is critical because urinary-N is more susceptible to losses than faecal N [17] and can increase manure N₂O emissions [18, 19].

Tannins in leguminous feed supplements bind protein, protecting them from microbial degradation. Therefore, they can mitigate N losses by reducing urinary N excretion and associated N emissions [20, 21]. Despite the increasing use of N supplementation in Kenyan dairy production and the expected environmental consequences, there is little local data linking dietary N intake and manure GHG emissions.

To close this knowledge gap, the present study aimed to evaluate the effects of two protein supplements, *Desmodium intortum* (DES) and commercial dairy cubes (CUBES), on N excretion, manure composition, and CH₄ and N₂O emissions. It was hypothesized that Desmodium hay supplementation would (i) lower the urinary N excretion compared to CUBES and decrease the emissions of CH₄ and N₂O from manure during incubation and (ii) that supplementation with concentrates would increase manure N₂O emissions due to a higher urinary-N: fecal-N ratio.

Materials and Methods

Animal feeding Trial

For the manure incubation experiment, faeces and urine were collected from a controlled feeding trial involving twelve lactating Boran × Friesian crossbred cows, described in detail by ²². The cows were grouped into three groups, each consisting of four animals. They were fed *ad libitum* with either a control diet composed of Brachiaria hay (*Brachiaria brizantha* cv. *xaraes*), molasses, and urea or protein-supplemented diets that included Desmodium hay (*Desmodium intortum*) or commercial dairy cubes. The experiment was conducted to reflect the farmer's situation of supplementation with Desmodium or dairy cubes as an improvement over the basal diet of Brachiaria hay when fed alone. Each animal was housed and fed in partitioned open pens (1.90 m x 2.87 m), covered with shade-cloth sails and bedded with rubber mats ^[23]. The animals also had *ad libitum* access to water from automatic waterers. Manure was collected during the second feeding period of the experiment.

Manure incubation study

Faeces and urine collection

Faeces and urine were collected from each animal within a dietary treatment (N=4) over a 24-hour period. Faeces were scooped from the pen floor daily and stored in plastic buckets with lids. Urine was collected using non-invasive urine collection devices (i.e., urinals) designed for female animals. A silicon tube was attached to the base of the urinals to direct the flow of urine into 10 L plastic containers pre-filled with 200 ml of 5M HCl to lower urine pH from 7.0-8.7 to < 2 to prevent N loss through volatilization ^[24]. After collection, total faeces and urine from each cow were weighed separately, homogenized, and sub-sampled for analysis (see below for description on analysis methods).

Manure preparation and incubation

The faeces and urine of each cow were manually mixed in a mass ratio of 70:30 on a wet-weight basis. This ratio was equivalent to the average ratio of total dung to urine excreted on a mass basis by the experimental animals over 24 hours. To prepare 3 kg of manure, 2.1 kg of faeces were combined with 0.9 kg of neutralized urine (initially acidified with 5M HCL and then neutralized with 5M NaOH to a pH of 7.6). The mixture was gently stirred, and aliquots of 350 g were transferred into five replicates of labelled 1 L mason jars, which were then incubated at 20 °C for 12 weeks. Four jars were used for destructive manure sampling at different

intervals (after 1, 2, 4, and 8 weeks of incubation), and the remaining jar was used for continuous manual gas sampling and the final manure sampling after 12 weeks of incubation. At each sampling time point (day 0 and after weeks 1, 2, 4, 8, and 12), samples were taken for the same laboratory analyses as for faecal samples.

Laboratory analysis

The dry matter (DM) of faecal and manure samples was determined according to ^[25], and crude ash following ^[26]. The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of the dung and manure samples were determined by methods of ^[27] the concentration of condensed tannins was determined using the method described by ^[28] the total urinary N concentration was analyzed using the Kjeldahl method, while the total faecal and manure N and C were determined using elemental combustion on an Elemental analyzer ^[29].

Gas sampling and laboratory analysis

Gas sampling was done daily for the first 15 days of incubation, then four times a week for the next 15 days, and three times a week for the remaining eight weeks. To this end, the mason jars were fitted with lids and rubber seals to allow for air-tight closure during gas sampling, and a sampling port containing a rubber septum through which gas samples were drawn with a syringe. At each sampling date, jars were taken out of the incubation chamber, placed onto the lab bench, and left open for ventilation for 10 minutes. Lids were then closed and five gas samples of 30 ml each were drawn from each mason jar headspace using 60 ml plastic syringes through the sampling port and transferred to pre-evacuated 10 ml gas chromatography (GC) vials. The gas sampling protocol involved sampling at intervals of 2 min (0, 2, 4, 6 and 8 min) for the first four weeks and at intervals of 3 min for the last eight weeks (0, 3, 6, 9 and 12 min). After each time a gas sample was drawn from the jar, the same volume of air of known GHG concentration was injected into the Mason jar to refill the initial headspace volume and avoid under pressure build-up.

The concentrations of CH₄ and N₂O were determined using a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, CA, USA) equipped with an electron capture detector (ECD) for N₂O detection and a flame ionization detector (FID) with a methanizer for CH₄ detection ^[30]. The peak areas measured by the GC were converted into concentrations using calibration curve equations generated from standard gas concentrations (2.03 to 49.8 ppm for CH₄, and 329 to 2530 ppb for N₂O) relative to measured peak areas of standards. A linear equation was used for CH₄, while a power function was used for N₂O.

Calculation of manure-atmosphere GHG fluxes

Before gas flux calculation, the volumetric gas concentrations (ppm or ppb) for the second to last sampling time point were corrected to account for the air refilled during gas sampling for pressure equilibration (Equations 1, 2 and 3).

$$\text{Equation 1 } c_{\text{diluted}_t} = \frac{(c_{\text{measured}_t} \times \frac{AW}{22.41}) \times (V_{HS} - V_{spl}) + (c_{\text{refilled}} \times \frac{AW}{22.41}) \times V_{spl}}{V_{HS}} \times \frac{22.41}{AW}$$

$$\text{Equation 2} \quad \Delta C_t = C_{measured_t} - C_{diluted_t}$$

$$\text{Equation 3} \quad C_{corrected_{t+1}} = C_{measured_{t+1}} + \Delta C_t$$

In the first step (Equation 1), the dilution of the air concentration at time point t ($C_{diluted_t}$) was determined by using the relative contributions of the measured gas sample concentration at time point t ($C_{measured_t}$) and the concentration of the air used for refilling the headspace volume ($C_{refilled}$) weighed for their respective volumes (V_{HS} , headspace volume, V_{spl} , sample volume). For this, concentrations were converted from mixing ratios (ppb) to a mass-per-volume basis using the ideal gas law ($PV=nRT$). Because pressure (P), temperature (T) and the ideal gas constant (R , $8.314 \text{ J K}^{-1}\text{mol}^{-1}$) are the same for both concentrations, they can be disregarded, leaving only the volume (V) and the number of atoms or mixing ratios (n). Therefore, the mixing ratios can be converted to a mass-per-volume basis by multiplying with the atomic weights (AW) of C (12 g mol^{-1}) for CH_4 or $2 \times \text{N}$ (28 g mol^{-1}) for N_2O , and dividing by the ideal gas volume (V , 22.41 L mol^{-1}). In the second step (Equation 2), the change in concentration

due to the dilution (ΔC_t) was calculated by subtracting the diluted concentration at time point t ($C_{diluted_t}$) from the measured concentration at time point t ($C_{measured_t}$). Finally (Equation 3), the change in concentration was considered for the gas concentration at the next time point, $t+1$ to give the corrected concentration ($C_{corrected_{t+1}}$) by adding the change in concentration (ΔC_t) to the measured concentration at the next time point ($C_{measured_{t+1}}$).

Emissions were then computed from the change in concentrations with time using the measured concentration at the first time point ($t_0 = 0$ minutes, before any dilution steps had been conducted) and the corrected concentrations at the subsequent time points. For this, a linear regression approach was used to estimate the slope of headspace (gas concentration change over time). The slope was then used to calculate the flux rate following Equation 4 for CH_4 , and Equation 5 for N_2O :

$$\text{Equation 4 Gas flux (CH}_4\text{)} = \frac{dConc}{dt} \times \frac{P}{1013} \times \frac{273}{T+273} \times \frac{12}{22.41} \times \frac{V_{HS}}{DM} \times 60$$

$$\text{Equation 5 Gas flux (N}_2\text{O)} = \frac{dConc}{dt} \times \frac{P}{1013} \times \frac{273}{T+273} \times \frac{28}{22.41} \times \frac{V_{HS}}{DM} \times 60$$

Where $dConc/dt$ is the change in gas concentration over time (slope, ppm min^{-1}), P is the air pressure (hPa), 1013 is the sea level air pressure (hPa), T is the incubation chamber temperature ($^{\circ}\text{C}$), 22.41 is the ideal gas volume (L), 12 and 28 are the molecular weights of one atom of C (for CH_4) and 2 atoms of N (for N_2O), V_{HS} is the headspace volume of the glass jar (ml), which was obtained by subtracting the volume occupied by the manure from the volume of the jar.

The volume occupied by manure was determined from the measured bulk density and mass of the manure in the jar, where volume (ml) is equivalent to mass (g) over density (g ml^{-1}). DM is the manure dry matter (g), and 60 is the conversion factor from minutes to hours. Units of gas fluxes calculated were $\text{mg CH}_4\text{-C g}^{-1} \text{ DM h}^{-1}$ and $\mu\text{g N}_2\text{O-N g}^{-1} \text{ DM h}^{-1}$. For data quality checks, the R^2 of the calculated slopes were considered, and CH_4 and N_2O slopes with $R^2 < 0.7$ were considered not valid. The limit of detection (LOD) of the GC was also calculated based on ³¹, and fluxes below the LOD ($0.88 \text{ mg CH}_4\text{-C g}^{-1} \text{ DM h}^{-1}$ for CH_4 , and 4.28 for N_2O) were set at $0.001 \text{ mg CH}_4\text{-C g}^{-1} \text{ DM h}^{-1}$ and $0.001 \mu\text{g N}_2\text{O-N g}^{-1} \text{ DM h}^{-1}$. 72.3% of CH_4 , and 97.7% of N_2O flux measurements were set at 0.001. In the discussion section, we provide a more detailed explanation of CH_4 and N_2O flux dynamics (and why there were such large numbers of fluxes below the LOD). These gas fluxes were used to calculate cumulative GHG emission or uptake for the 84 days of storage using trapezoidal interpolation and finally expressed per 100 g of stored manure.

Statistical analysis

All statistical analyses were done using PROC GLM of SAS (2002). Data were first assessed for normality using the Shapiro-Wilk test. Diet effect on manure composition was evaluated using ANOVA in a completely randomized arrangement of treatments, with diet as the fixed effect. Tukey's test was used to test differences among the dietary treatments at each sampling time. Significant difference was declared at $p < 0.05$.

Results

Effect of supplementation on excreta chemical composition

This study found that the faecal total carbon (C) was 4% higher for cows on the DES diet ($44.0 \pm 0.24\%$ DM) compared with the CUBES diet ($41.9 \pm 0.21\%$ DM) and the CON diet ($42.1 \pm 0.11\%$ DM) (Table 1). Regarding total nitrogen (N), both faecal and urinary N were higher for the DES diet ($1.81 \pm 0.02\%$ DM, $0.76 \pm 0.05\%$) and CUBES diet ($1.68 \pm 0.10\%$ DM, $0.75 \pm 0.07\%$) compared with the CON diet ($1.35 \pm 0.02\%$ DM, $0.35 \pm 0.02\%$). The faecal lignin content for cows on the DES diet ($15.9 \pm 0.62\%$ DM) was 71% higher than those on the CUBES diet ($4.57 \pm 0.06\%$ DM) and the CON diet ($3.53 \pm 0.17\%$ DM). Additionally, the faeces of cows on the DES diet contained 89% more condensed tannins (CTs) than those on the CUBES diet.

Table 1: Water, carbon, nitrogen, C: N, condensed tannin and fibre content of faeces excreted by dairy cows fed three experimental diets (n=4, mean \pm SEM)

Parameter	Experimental diet			P-Value
	CON	DES	CUBES	
Water content (%)	83.2 \pm 0.38 ^a	81.6 \pm 0.61 ^a	82.7 \pm 0.48 ^a	0.126
Total Carbon (% DM)	42.1 \pm 0.11 ^b	44.0 \pm 0.24 ^a	41.9 \pm 0.21 ^b	<0.001
Total Faecal N (% DM)	1.35 \pm 0.02 ^b	1.81 \pm 0.02 ^a	1.68 \pm 0.10 ^a	0.0009
Total Urinary N (%)	0.35 \pm 0.02 ^b	0.76 \pm 0.05 ^a	0.75 \pm 0.07 ^a	0.0005
C: N	31.2 \pm 0.51 ^a	24.3 \pm 0.35 ^b	25.3 \pm 1.41 ^b	0.001
Hemicellulose (% DM)	24.3 \pm 0.61 ^b	16.7 \pm 0.31 ^c	26.4 \pm 0.51 ^a	<0.001
Cellulose (% DM)	28.6 \pm 0.87 ^a	29.2 \pm 0.74 ^a	26.1 \pm 0.77 ^a	0.055
Lignin (% DM)	3.53 \pm 0.17 ^b	15.9 \pm 0.62 ^a	4.57 \pm 0.06 ^b	<0.001
Condensed Tannins (% DM)	BLOD	0.82 \pm 0.07 ^a	0.09 \pm 0.06 ^b	<0.001

^{a, b, c} within a row, least square means with different superscripts differ ($p < 0.05$). BLOD below limit of detection.

Manure dry matter, nitrogen, carbon and condensed tannin concentrations during incubation

The initial dry matter (DM) of manure (i.e. mix of dung plus urine used in the lab incubation) from cows fed the DES diet was 9.6% higher compared with those fed the CON diet (Table 2). Over the 12-week incubation period, manure DM decreased by 8.49 \pm 0.95% for cows fed the DES diet, 8.87 \pm 1.17% for the CUBES diet and 10.1 \pm 1.33% for the CON diet.

The initial manure total N was 31% and 34% higher in manure from cows fed the CUBES diet (0.83 \pm 0.07 g jar⁻¹ DM) and DES diet (0.96 \pm 0.04 g jar⁻¹ DM), compared with the CON diet (0.63 \pm 0.01 g jar⁻¹ DM) (Table 2). The final concentrations were significantly different, with the DES diet resulting in a higher final total N concentration than the CUBES and the CON diet.

The initial total C content in the manure was significantly higher in manure from cows fed the DES diet (21.2 \pm 0.76 g jar⁻¹ DM) by 9.91% compared with the CUBES diet

(19.1 \pm 0.57 g jar⁻¹ DM) and by 15.1% compared with the CON diet (18.0 \pm 0.47 g jar⁻¹ DM) (Table 2). There were reductions in the total C concentrations by 6.83% for the DES diet, 10.57% for the CON diet, and 10.7% for the CUBES diet. Similarly, the initial lignin content was 67% and 73% higher in manure from cows fed the DES diet (5.41 \pm 0.25 g jar⁻¹ DM) compared with those fed the CUBES diet (1.80 \pm 0.12 g jar⁻¹ DM) and the CON diet (1.47 \pm 0.05 g jar⁻¹ DM), (Table 2). After the 12-week incubation period, the lignin content remained significantly higher in the incubated manure from cows fed the DES diet (5.21 \pm 0.33 g jar⁻¹ DM) compared with the CUBES diet (1.31 \pm 0.08 g jar⁻¹ DM) and the CON diet (1.03 \pm 0.04 g jar⁻¹ DM).

The initial manure CT content was 89% higher in manure from cows fed the DES diet (0.26 \pm 0.08 g jar⁻¹ DM) compared with the CUBES diet (0.03 \pm 0.02 g jar⁻¹ DM) and the CON diet (0.00 \pm 0.00 g jar⁻¹ DM). Over the incubation period, the CTs decreased, and at the end of incubation, 54% of CTs in manure from cows fed the DES diet had been degraded.

Table 2: Average manure Dry matter, total nitrogen, carbon, lignin and condensed tannins mass before and after incubation

	DM	TN	TC	Lignin	CT
	g jar ⁻¹	g jar ⁻¹ DM	g jar ⁻¹ DM	g jar ⁻¹ DM	g jar ⁻¹ DM
Start (day 0)					
CON	44.5 \pm 0.75 ^b	0.63 \pm 0.01 ^c	18.0 \pm 0.47 ^b	1.47 \pm 0.05 ^b	0.00 \pm 0.00 ^b
DES	49.2 \pm 1.22 ^a	0.96 \pm 0.04 ^a	21.2 \pm 0.76 ^a	5.41 \pm 0.25 ^a	0.26 \pm 0.08 ^a
CUBES	46.2 \pm 1.29 ^{ab}	0.83 \pm 0.07 ^b	19.1 \pm 0.57 ^b	1.80 \pm 0.12 ^b	0.03 \pm 0.02 ^b
End (Day 84)					
CON	40.0 \pm 0.70 ^b	0.63 \pm 0.01 ^c	16.1 \pm 0.33 ^b	1.03 \pm 0.04 ^b	0.00 \pm 0.00 ^b
DES	45.0 \pm 1.49 ^a	0.94 \pm 0.03 ^a	19.8 \pm 0.85 ^a	5.21 \pm 0.33 ^a	0.12 \pm 0.01 ^a
CUBES	42.1 \pm 1.07 ^b	0.87 \pm 0.09 ^b	17.1 \pm 0.33 ^b	1.31 \pm 0.08 ^b	0.00 \pm 0.00 ^b

^{a, b, c} Within a column, least square means with different superscripts differ ($p < 0.05$).

Effect of diet on greenhouse gas emissions from incubated manure

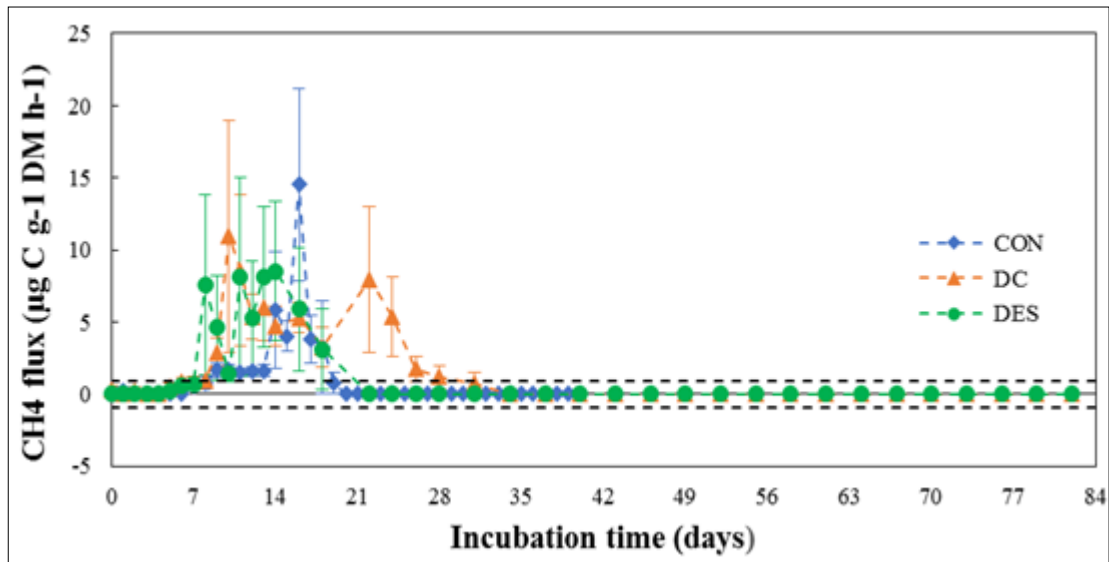
Manure CH₄ flux rates from manure for all experimental diets were initially below the limit of detection (LOD) of ± 0.88 in the first five days of incubation. Subsequently, CH₄ flux rates gradually increased and peaked between days eight and 16. The peak was at 8.54 mg C g⁻¹ DM h⁻¹ for the DES diet, 10.9 mg C g⁻¹ DM h⁻¹ for the CUBES diet, and 14.5 mg CH₄-C g⁻¹ DM h⁻¹ for the CON diet. After this period, the emission rates

declined and remained below the detection limit until the end of the incubation period, as shown in Figure 1.

The manure N₂O flux rates were below the LOD of ± 4.28 in the incubated manure from cows fed with the CON and DES diet throughout the study period. In contrast, manure from cows fed with the CUBES diet showed N₂O emissions ranging between 17.8-39.8 μ g N g⁻¹ DM h⁻¹ from day 24 until day 42 (week 6), with high variability between replicates as indicated by the large error bars (Figure 2).

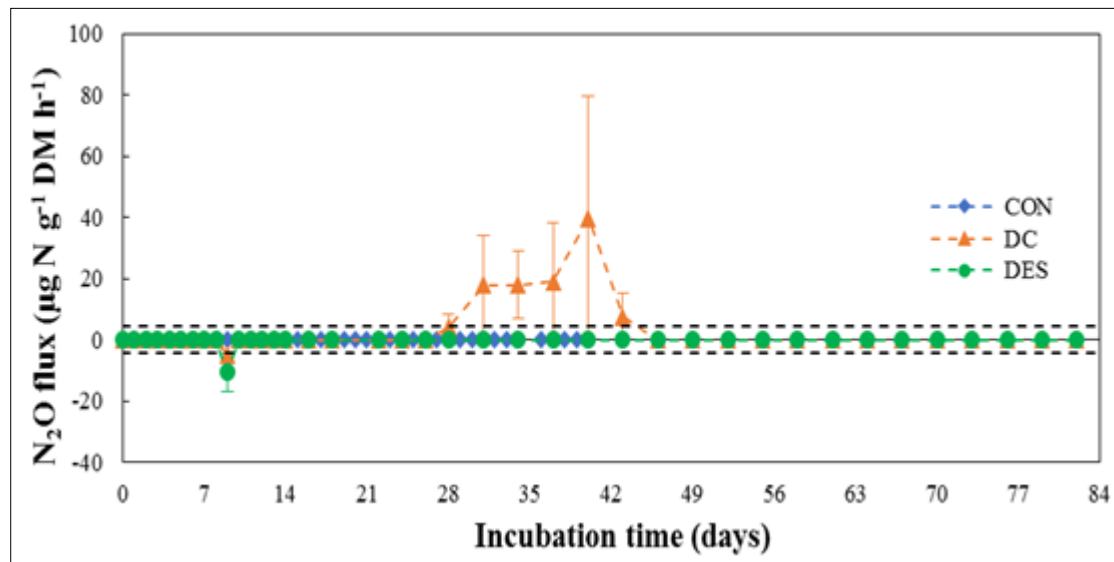
Table 4: Cumulative GHG emissions from incubated manure from cows fed three experimental diets

	Experimental diet			P-Value
	CON	DES	CUBES	
mg CH ₄ -C 100 g ⁻¹ stored manure for 12 weeks	35.1 \pm 8.32 ^a	62.1 \pm 33.0 ^a	49.1 \pm 13.7 ^a	0.6760
mg N ₂ O-N 100g ⁻¹ stored manure for 12 weeks	0.09 \pm 0.00 ^a	-4.20 \pm 2.51 ^a	195.2 \pm 136.0 ^a	0.1780



CON=Control, DES = Desmodium supplemented diet, DC = Dairy cubes supplemented diet. Flux values are mean±standard error mean (n=4). LOD =Limit of Detection ($\pm 0.88 \text{ mg C g}^{-1} \text{ DM h}^{-1}$).

Fig 1: Methane (CH_4) emissions from manure from cows fed three experimental diets during 84 days of incubation



CON=Control, DES = Desmodium supplemented diet, DC = Dairy cubes supplemented diet. Values are flux mean, Error bars=±standard error mean (n=4). LOD =Limit of Detection ($\pm 4.28 \text{ µg N}_2\text{O-N g}^{-1} \text{ DM h}^{-1}$)

Fig 2: Nitrous oxide (N_2O) emission from manure from cows fed three experimental diets during 84 days of incubation.

Discussion

In line with the hypothesis, the DES diet led to a shift in N excretion from urine to faeces (see for full details) [22], which has also been noted by other studies where dairy cattle were fed diets containing CTs [32, 33]. The higher faecal N as a result of feeding the DES diet can be explained by the reduced apparent total tract CP digestibility of the diet compared to CON and the CUBES diet (54.6% for DES vs 60.0% for CON and 70.3% for CUBES) [22]. Condensed tannins in the diet reduce the microbial degradation of protein in the rumen by forming CT-protein complexes [34, 35] and as a consequence, lead to lower urinary N excretion due to reduced ammonia absorption from the rumen [36].

In contrast, the CUBES diet led to higher urinary N excretion, which could be explained by the higher N intake and increased apparent total tract CP digestibility of the diet (70.3%) [22]. A similar observation was made in a study with dairy cows by [12], where concentrate supplementation increased N intake and urinary N excretion.

During the manure incubation period, we found that most of the N_2O fluxes were below the LOD, except for a short period of N_2O emissions in the CUBES treatment. For N_2O emissions to occur, several conditions must be met: the presence of enough NO_3^- for denitrification, the presence of labile C for heterotrophic denitrification, and the right moisture content (i.e., moderately moist but not too dry nor too wet) to give the right proportion of aerobic versus anaerobic microsites [37]. In fresh manure, most N is present as organic N, which first needs to be broken down into NH_4^+ and then nitrified to NO_3^- . Consequently, several studies have reported a time lag of N_2O emissions of several days [19, 38] to weeks [30], after the start of manure incubation, similar to what we have found in this study (emission window from day 28 to 42). Furthermore, the manure C: N ratio in the present study (24.3-31.2) was relatively high compared to other studies, which have simulated a more “Western” diet (e.g. C: N=23.8 in [39]; C: N=20.6 in [40]). Therefore, it seems plausible that in the present study, manure N-content was limiting DE nitrification in the control diet (C: N=31.2±0.5), whereas

sufficient N was present for N₂O production in the CUBES treatment (C: N=25.3±1.4). In addition, there might have been an additional effect of CTs on N₂O emissions, which might explain the fact that we did not see N₂O emissions in the DES-supplemented treatment (C: N=24.3±0.4), even though the C: N ratio was similar compared to the CUBES treatment. As mentioned earlier, the DES-supplemented diet led to a shift from urinary-N to faecal-N excretion. Previous studies in Kenya have found that urinary-N is more labile and promotes N₂O emissions from cattle urine patches, whereas dung patches led to lower or negligible N₂O emissions [18, 19]. In addition, the DES diet likely led to the formation of CT-protein complexes, which has been shown to inhibit microbial decomposition [41] and potentially led to a slower release of mineral N in the DES-supplemented diets, further suppressing N₂O emissions. Finally, the manure incubation simulated aerobic decomposition, as no water was added during incubation to replace water lost through evaporation, and manure dried out over time. Consequently, the conditions in the manure might have been too dry for denitrification [42, 43]. On methane emissions, it was found that CH₄ emissions were below LOD for the first five days of incubation. Fresh manure contains metabolically active methanogens from the hindgut and is high in degradable organic matter and moisture content, theoretically creating anaerobic conditions that favour the production of CH₄ [44]. Consequently, others have found that CH₄ fluxes were highest immediately after manure excretion and decreased as the manure dried out e.g. [19, 30]. It is possible that in our study, methanogens may have been disturbed by the manure preparation (mixing of dung for homogenization, stirring to mix urine and dung) for the incubation setup, for example, by mixing air into the manure during sample preparation, which suppressed methanogen activity. At the end of the first week, CH₄ emissions gradually increased and remained elevated until the end of the third week, after which they returned to levels < LOD until the end of the incubation. This could be explained by the drying of the manure over time, as well as the formation of a crust, which has the potential to consume and significantly reduce CH₄ emissions during storage via bacterial CH₄ oxidation [45, 46]. In this study, crust formation was observed after 18 days, and after 49 days (7 weeks) the manure in the jars had dried out completely (i.e., no further moisture loss was observed).

Conclusion

In conclusion, dietary treatments affected manure characteristics and their losses during incubation, with protein supplementation from *Desmodium intortum* significantly shifting nitrogen excretion from urine to faeces. However, cumulative manure GHG emissions were similar across the diets. Further research is recommended to evaluate the impact of tanniferous forages on manure emissions in on-farm settings.

Conflict of Interest

Not available

Financial Support

Not available

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