Supplementation of a tropical low-quality forage with *Calliandra calothyrsus* improves sheep health and performance, and reduces methane emission

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Ruminant production systems in the arid and semi-arid regions of Sub-Saharan Africa confront severe challenges due to recurring droughts and the intensifying effects of climate change (CC). These systems grapple with numerous stress factors, including poor animal nutrition, water scarcity, gastrointestinal (GIT) parasite burdens, and heat stress, which contribute to below optimal animal productivity and a high environmental footprint. Addressing these issues urgently, by creating livestock systems resilient to CC that also promote better animal health, enhanced productivity, and reduced environmental impact, is paramount to safeguarding the livelihoods of the rural population. This 50-day study aimed to evaluate the effects of improved feeding and nutritional management in sheep, focusing on GIT parasite infections, feed intake and digestibility, liveweight (LW) gain, and enteric methane (CH_4) emissions. We investigated the legume forage tree, Calliandra (*Calliandra calothyrsus*), as a high-quality feed source because of its rich crude protein content and its potential as a remedy for gastrointestinal tract (GIT) parasite infections, attributed to its high condensed tannin (CT) content. Twenty-eight Dorper lambs underwent random allocation across four treatments, each consisting of seven lambs. These treatments combined either a trickle infection or no infection with *Haemonchus contortus* with a supplementation (40% of diet on a dry matter basis or lack thereof) of the basal diet (Rhodes grass hay) with dried Calliandra leaflets. The treatments were: UnHay (uninfected, fed on hay-only), InHay (infected, *fed* on hay-only), InHay+Cal (infected, fed on hay plus Calliandra), and InHay+Cal+PEG [infected, fed on hay, Calliandra, and polyethylene glycol (PEG)]. The latter was to evaluate the potential effects of Calliandra's condensed tannins. The results show that lambs in the InHay+Cal treatment exhibited a higher packed cell volume (PCV) and lower faecal egg counts (FEC) compared to the InHay group. No effects of infection on the other measured variables were observed in unsupplemented lambs. Calliandra supplementation increased total feed dry matter intake (DMI) by 20% (61.8 vs. 51.7 g DM/kg LW0.75) (InHay+Cal vs. InHay) and enhanced LW gain (7.2 g/d) in the InHay+Cal group, whereas the unsupplemented infected group (InHay) experienced LW loss (-26.6 g/d). Calliandra supplementation to infected lambs reduced daily CH_4 emission by

15% (13.9 vs. 16.2 g/d) and CH_4 yield (g/kg DMI) by 30% (18.7 vs. 26.5 g/kg DMI), compared to emissions from unsupplemented infected lambs. Nonetheless, Calliandra supplementation decreased the digestibility of crude protein and fibre and raised the faecal nitrogen (N) output to N intake (FN/NI) ratio. The effects of PEG supplementation on CT activity remained inconclusive. The study concludes that a 40% replacement of a protein-deficient basal diet with Calliandra may be excessive. However, the findings underscore the considerable advantages of integrating Calliandra into farming systems. Such nature-based solution control GIT parasite infections and their lifecycle, bolster the nutritional value of a deficient basal diet, improve animal productivity cost-effectively, and mitigate enteric methane emissions both in absolute terms and intensity.

1 Introduction

According to the FAO database (FAO, 2018), the global population of sheep and goats is 1.18 billion and one billion, respectively. Africa has the second-highest population of small ruminants in the world at 352 million and 388 million heads of sheep and goats, respectively. These small ruminants play an important socioeconomic role as a source of nutrition, income, and other intangible benefits, such as savings, insurance against emergencies, and cultural and ceremonial purposes (Kosgey et al., 2008). They also play a complementary role to other livestock by utilizing feed resources in natural grasslands (Baker & Rege, 1994). Globally, the livestock sector greenhouse gas (GHG) emissions are equivalent to 7.1 Gt CO2-eq/year (14.5% of anthropogenic GHG emissions), of which the share of small ruminants is 6.5% (Gerber et al., 2013). Over 55% of emissions from small ruminants are attributed to enteric methane (CH_4) (Gerber et al., 2013).

The most significant production constraints facing small ruminant production systems in the tropics are gastrointestinal tract (GIT) parasite infection, shortage of feed and grazing land, and water scarcity due to recurrent droughts and climate change (CC) (Armson et al., 2021; Rahimi et al., 2021). Finding cost-effective solutions to these challenges is crucial for improving animal productivity, health, and welfare, and safeguarding the livelihoods of smallholder livestock keepers.

Feeding ruminants during dry seasons in the tropics is characterized by the provision of forages of poor feeding value (poor digestibility, low concentrations of crude protein (CP), and high concentrations of fibre and lignin). This is often below maintenance energy requirements of the animals and consequently leads to low productivity, loss of liveweight (LW), high enteric CH_4 emissions and results to high emission intensity (emissions per unit of animal product) (Kurihara et al., 1999; Chaokaur et al., 2015;

Goopy et al., 2020). In the tropics, there is a diversity of forage plants, especially legumes, which contain secondary compounds such as condensed tannins (CT) (Bhatta et al., 2013). Condensed tannins can directly affect rumen microorganisms, thereby decreasing CH_4 production (Tavendale et al., 2005; Berça et al., 2023). Furthermore, consumption of CT-containing forages can lead to reduced shedding of GIT nematode (GIN) eggs, hence reducing the contamination of pastures (Min et al., 2005) as well as lower host infection through reduced parasite numbers in the GIT (Brunet et al., 2008; Oliveira et al., 2013). There is currently a challenge with GIN resistance to most commonly used deworming drugs. This widespread increase in anthelmintic resistance (Greiffer et al., 2022) has prompted the search for alternative GIN control solutions, and efforts are also supported by increased consumer demand for more sustainable livestock production with reduced use of chemicals (Waller, 1999).

Supplementing grazing ruminants with legume forage trees, such as Calliandra (*Calliandra calothyrsus*), which is high in CP and CT, during the dry seasons may provide various benefits to livestock production systems, including reducing LW loss, providing nutrients to enhance animal resilience to GIN, and reducing CH_4 emissions (Kaitho et al., 1996; Min et al., 2005; Piñeiro-Vázquez et al., 2018). Calliandra is a multipurpose tree legume native to humid and sub-humid regions of Central America and Mexico. It was introduced to the Central Highlands of Kenya in the 1980s and has since been widely promoted and adopted as a supplement for ruminants on low-quality forages (Wambugu et al., 2001). Various studies conducted in Sub-Saharan Africa (SSA) have shown that supplementation with tropical tannin-rich forage legumes increases the productivity of animals fed on poor-quality diets (Kinuthia et al., 2007; Yisehak et al., 2014). Forage trees containing CT have also demonstrated anti-methanogenic activity when fed to ruminants (Tiemann et al., 2008; Piñeiro-Vázquez et al., 2018). However, to our knowledge, no research in SSA has evaluated the effects of these forages on the

health of animals in combination with enteric methane emissions. In this context, our study assesses the potential of Calliandra as a supplement to sheep consuming poor-quality Rhodes grass (*Chloris gayana*) hay evaluating its effects on voluntary feed intake, LW gain (LWG), nitrogen and energy partitioning, GIN infection (*Haemonchus contortus*) response, and enteric CH_4 emissions. We hypothesized that supplementation of poor-quality Rhodes grass hay with Calliandra would enhance sheep productivity (LWG) and health while reducing enteric CH_4 emissions.

2 Materials and methods

This study was conducted at the Mazingira Centre, International Livestock Research Institute (ILRI), Nairobi, Kenya. The experimental protocol was reviewed and approved by the ILRI Institutional Animal Care and Use Committee (IACUC; Reference number 2022-03).

2.1 Animal management and experimental design

Dorper lambs aged 10–11 months old, mixed sex, and 28.1 ± 2.7 kg liveweight (LW) were selected from a cohort of lambs born at the ILRI Kapiti Research Station & Wildlife Conservancy, located 60 km South-East of Nairobi. In this conservancy, sheep flocks are kept on a grassland interspersed with Acacia trees and shrubs. These lambs were then transported to the ILRI farm in Nairobi where they underwent a 3-week quarantine period. During this period, they were kept as a single group and fed Rhodes grass (*Chloris gayana*) hay before starting the experiment. At the beginning of the quarantine period, each lamb was given anthelmintic treatment using 10% albendazole (albafas) at a dose of 8 mg/kg LW. Subsequently, faecal examinations were conducted regularly to ensure that they attained a worm-free status and remained worm-free.

At the end of the quarantine period, the animals were acclimatized to their experimental diets for a period of seven days. Thereafter, the experiment was started lasting 50 days (Days 1–50). For the initial 40 days (Days 140), the lambs were housed in individual pens within an animal shed each equipped with feed and water bins. On Day 41, lambs were moved to individual metabolic crates and kept for seven days during which total faeces and urine were collected over the last five days (Days 43–47). Afterwards, the lambs were transferred to respiration chambers to measure CH_4 emission over the last three days of the experiment (Days 48–50).

The experiment used a completely randomized design. Twenty-eight Dorper lambs were subdivided in a balanced manner (LW and sex) into four experimental groups (seven animals each; three castrated males and four females). The lambs were then randomly allocated to four experimental treatments that combined infection status (infected or uninfected with *Haemonchus contortus*) and Calliandra supplementation (supplemented or unsupplemented), and fed a basal diet of poor-quality Rhodes grass hay. The experimental treatments were uninfected fed on hay only

(UnHay), infected fed on hay only (InHay), infected fed on hay plus Calliandra supplement (InHay+Cal), and infected fed on hay plus Calliandra supplement plus polyethylene glycol (PEG) supplement (InHay+Cal+PEG). InHay+Cal+PEG treatment was used to evaluate the anticipated role of CT in Calliandra on feed intake, digestibility, N metabolism, and CH_4 emissions, as PEG binds to tannins to form stable complexes (Makkar et al., 1995). The level of Calliandra supplementation was set to replace 40% of the voluntary feed intake (dry matter basis) of the Rhodes grass hay, whereas the level of PEG supplementation was set to neutralize the total concentration of CT in the diet (PEG : CT ratio of 1:1), based on results of preliminary laboratory analyses.

Given the availability of only three animal respiration chambers, the experimental animals were subdivided into ten batches (Eight batches of three animals each and two batches of two animals each). The batches entered the experiments in a staggered manner, every three days. Batches were balanced as much as possible for sex and experimental treatment, each batch included only one lamb from either of the four treatment groups.

2.2 Feeds and feeding

The Rhodes grass hay had 92.2% dry matter (DM) and on DM basis it contained 90.36% organic matter (OM), 73.0% neutral detergent fibre (NDF), and 5.6% crude protein (CP) (Table 1). Calliandra leaflets had a DM content of 91.8%, 94.1% OM, 17.4% NDF, 21.1% CP, and 9.2% total condensed tannins (CT) (Table 1). Calliandra leaves were harvested from a single farm in Kakamega County (northwest of Nairobi). Branches were cut and allowed to dry in the field over PVC sheets until some primary leaflets started self-detaching from the leaf rachis. Primary leaflets (pinnae) were then manually harvested. The harvested material was transported in jute sacks and stored under protection from rain and sun until the start of the experiment. Thus, the Calliandra material used in this study was composed of secondary leaflets in the pinnae.

TABLE 1 Mean (± standard deviation) chemical composition (dry matter (DM) basis) of Rhodes grass (*Chloris gayana*) hay and air-dried Calliandra (*Calliandra calothyrsus*) leaves.

Nutrient	Rhodes Hay (*n=7*)	Calliandra leaves (*n=7*)
Dry matter (%)	92.2 ± 0.51	91.8 ± 0.57
Organic matter (%)	90.3 ± 1.30	94.1 ± 0.16
Neutral detergent fibre (%)	73.0 ± 1.69	17.4 ± 0.69
Acid detergent fibre (%)	40.0 ± 1.48	11.7 ± 0.59
Crude protein (%)	5.6 ± 0.63	21.1 ± 0.31
Gross Energy (MJ/ kg DM)	17.4 ± 0.23	20.5 ± 0.53
Condensed tannins (%)	n.d.	9.2 ± 0.97

n.d., not determined.

During the seven-day acclimatization period, all animals were fed on chaffed Rhodes grass hay *ad libitum*. During the first five days of acclimatization, lambs in the supplemented experimental treatments (InHay+Cal and InHay+Cal+PEG) were gradually offered Calliandra. By the end of the acclimatization period, the supplemented animals had adjusted to their full allowance of Calliandra, with no refusals. Voluntary feed intake (VFI) of Rhodes grass was determined during the acclimatization period. The average VFI of the hay was determined on a metabolic LW basis ($LW^{0.75}$). The unsupplemented animals were fed hay at 1.1 VFI. Calliandra supplementation was set to replace 40% of the VFI of hay (DM basis); hence, the hay offered to Calliandra-supplemented animals was set at 0.7 VFI of hay. This feeding level remained consistent throughout the study.

Lambs in the InHay+Cal+PEG treatment were offered the Calliandra supplement sprayed with PEG. The amount of PEG used was based on preliminary sampling and analysis of Calliandra samples that had a CT concentration of 28.2 g CT/kg DM. The preliminary sampling was done 12 weeks prior to collection of the Calliandra forage used in the experiment (Both the preliminary and experimental samples were collected from the same farms). A solution of PEG was prepared daily using 250 g of PEG 6000 dissolved in 1250 ml distilled water and depending on the intake of Calliandra in the ration, a fixed volume of the solution was sprayed onto the Calliandra forage twice a day. The average daily dose of PEG solution was 50 ml (i.e., 10 g PEG/animal), providing a PEG : CT ratio of 1:1, a level slightly higher than 1:1 required to neutralize CT activity. Further analysis of CT in the Calliandra fed during the experiment (using specific standards) was carried out at the end of the study, which showed a higher concentration of total CT (92.1 g/kg DM), resulting in a PEG : CT ratio of 1:2.8.

The daily rations of hay and Calliandra were divided into two equal portions and fed at 0830 and 1630 h. The lambs were offered water *ad libitum* using a plastic bucket, and a mineral block was provided free of choice.

2.3 Experimental larvae preparation and infection

Larvae were prepared by infecting three adult donor sheep of the Dorper breed with a single dose of 5,000 *H. contortus* L_3 larvae suspended in distilled water, prior to deworming them by oral dosing with 10% albendazole (albafas) at a dosage of 8 mg/kg LW. After a 21-day prepatent period, following L_3 larvae dosing, the total faecal output was collected from each donor sheep. Larval production was performed according to the process described by Mwangi et al. (2023). In brief, the jars containing the faeces-vermiculite mix were incubated at 27°C (Lovibond, Germany). Hatched larvae were washed off the sides of the jars into cell culture flasks, and each flask was filled up to 60 ml (distilled water containing larvae) and stored at 4°C until they were administered to the lambs in the infection experimental treatments. The viability of the larvae was confirmed under a dissection microscope (Zeiss, Model Axiocam ERc 5s, Germany).

The larvae doses used for the experiment were calculated as described by Mwangi et al. (2023). Starting from Day 1 of the study, lambs in the infection experimental treatments (InHay, InHay+Cal and InHay+Cal+PEG) were dosed with 1000 *H. contortus* L_3 stage larvae over five consecutive days, leading to a total dose of 5,000 larvae per animal. The uninfected lambs were sham-infected with clean water as a control. The donor lambs were not included in the experiment.

2.4 Measurements and sample collection

2.4.1 Parasitological examination and haematology

A faecal sample for parasitological examination was collected per animal on Day 1 of the experiment, and weekly thereafter. The sample was collected directly from the rectum and placed in a plastic sampling bottle. Faecal egg counting was conducted using a modified McMaster technique (Whitlock, 1948).

At the times of faecal sampling, blood samples were collected via jugular venipuncture into EDTA sampling bottles for hematological examination. Packed cell volume (PCV, %) was determined using a hematology analyzer (Nihon Kodehn, MEK 6550K, Japan).

2.4.2 Feed intake

The lambs were fed individually throughout the study. A sample of the feeds on offer (hay and Calliandra) were collected daily during meal weighing. These samples were stored at -20°C, weekly, and subsequently oven-dried for DM determination. Feed refusals were collected daily from each animal before the morning feeding and treated similarly to the feeds on offer, pooled weekly per animal and then oven-dried.

Feed intake by individual animals (feed on offer minus refusals, for each feed item) was calculated on DM basis for the pen period (Days 1−40), during their time in metabolic crates (Days 43−47) and respiration chambers (Days 48−50). Lambs supplemented with Calliandra showed no refusal of Calliandra forage.

2.4.3 Liveweight and liveweight gain

Liveweight (LW) measurement of each lamb was recorded before morning feeding on Day 1, and weekly thereafter until the end of the experimental period (Day 50) using an outdoor automatic digital weighing scale (Gallagher, Model 3B4092, Hamilton, New Zealand).

2.4.4 Total faeces and urine outputs (balance period)

A total collection of daily outputs of faeces and urine was conducted over Days 43−47, while the lambs were kept in individual metabolic crates. Collections were not carried out over the first two days in metabolic crates (Days 41 and 42 due to disruption in feed intake caused by animal movement from pens to crates). The metabolic crates allowed for separate collection of faeces and urine for each animal. Faeces passed through the crate floor to a mesh below, while urine passed through this mesh and flowed into a

Perspex tray, eventually being collected in a bottle containing 70 ml of 10% HCl. This setup ensured the urine pH remained below 3 at the conclusion of the 24-hour collection period and prevented ammonia volatilization. The urine tray was sprayed with fresh water five times daily during daylight to prevent nitrogen loss from urine droplets. Roughly 0.5 L of water was used for this, and its addition did not influence urine nitrogen and energy calculations.

Every morning before feeding, the daily faecal and urine outputs were collected. Faecal outputs from each lamb were weighed and recorded, a 10% sub-sample was stored at -20°C in a zip lock bag. Simultaneously, the day's total urine (plus water) output was sieved and its volume recorded, and a 10% sub-sample was stored at -20°C in an airtight bottle. The daily faecal and urine samples were then pooled by individual animals for future processing for determination of DM (faeces) and subsequent chemical analyses.

2.4.5 Methane emissions from enteric fermentation

Enteric CH_4 emission from each lamb was measured over three consecutive days (Days 48 to 50) using three open-circuit respiration chambers (No Pollution Industrial Systems Limited, UK) as described by Mwangi et al. (2023). During this measurement period, each lamb spent one day in each of the three chambers. On each day, the CH_4 measurement was conducted over 23.5 hours (starting at 0830 h and finishing to 0800 h following day), with chambers opened twice a day for 15 min each for feeding and watering (morning and afternoon) and cleaning (before morning feeding). Emissions calculations were extrapolated to 24-h periods.

During measurement, the internal temperature of the chambers was maintained at 22°C with a relative humidity of 47%. The ventilation rate of the chambers was set to 8 l/s. A cavity ringdown laser absorption spectrometer (Picarro G2508 analyzer, Santa Clara, USA) was used to measure the CH_4 concentrations (in ppm) at intervals of approximately five minutes (including the incoming background gas sample). CH_4 emissions were calculated using the difference between inlet and outlet gas concentrations multiplied by volumetric air flow and corrected to standard temperature and pressure (25°C and 101,300 Pa).

A whole-system recovery of known volumes of pure CH_4 into the respiration chamber system was carried out before the start of the methane measuring period and at the end of the measurement period using a gas phase titration unit (Environics 4020, Environics Inc., Tolland, USA). The CH_4 recovery rate was 98–106%.

2.4.6 Rumen sampling and pH measurement

Once the lambs exited from the respiration chambers (0900 h, before feeding), rumen samples were collected from each sheep using an orogastric tube. With the animal properly restrained and the neck extended forward, a PVC tube speculum was placed in the mouth. A flexible orogastric tube was gently passed through the speculum to the base of the tongue. While the animals were allowed to swallow, the tube was slowly pushed through the oesophagus and into the rumen. Sample extraction was performed once the tube was in the rumen using a pump connected to the tube. The first 10–20

ml of rumen sample collected was discarded, and a second sample (~40 ml) was collected, and pH was measured immediately using a pH and conductivity meter (Jenway 3540 model, UK).

2.5 Laboratory analyses

Samples of feeds on offer (pooled on a weekly basis) and within-animal pooled samples of feed refusals and faeces were oven-dried (Genlab oven, Model SDO/425/DIG, UK) at 50°C for 72 h. The dried samples were ground to pass a 5 mm sieve using a Wiley mill (Model MF 10B, IKA Werke, Willmington, N.C., USA) and then passed a 1 mm sieve using a hammer mill (MF 10 basic, IKA, Werke GmbH & CO. KG, Staufen, Germany). The total DM was determined by drying the samples at 105°C in an oven (Genlab oven, model SDO/425/TDIG, UK) for 24 h while ash was determined by combustion in a muffle furnace (Nabertherm GmbH, Germany) at 550°C for six hours according to the methods of the Association of Official Analytical Chemists (AOAC, 1990 methods no. 924.05). Feed, refusals, and faecal samples were analysed for NDF and ADF using the methods of Van Soest et al. (1991) with an Ankom 200 fibre analyzer (model A2001, USA). The total nitrogen (N) content in urine samples was determined using the micro-Kjeldahl procedure (AOAC, 1990, method no. 988.05). The total N content in feed, refusals, and faecal samples was determined using an organic elemental analyzer (Vario Max, model vario Solid Sampler, Germany). Total condensed tannins (CT) in Calliandra were preliminarily analysed in the Competence Division Method Development and Analytics Laboratory of Agroscope (Posieux, Switzerland) using the modified butanol-HCl procedure described by Terrill et al. (1992), using cyanidin to set the standard calibration curve. Subsequent analyses of CT in Calliandra, conducted at the end of the study, were performed at the Texas A&M Agrilife Center (Stephenville, TX) using the simplified butanol-HCl method and specific plant standards, as described by Wolfe et al. (2008).

Gross energy (GE) content of feed on offer, feed refusal, and faeces was determined according to the methods of Harris (1970) using a bomb calorimeter (Parr 6200, model A1290DDEE, UK). The gross energy contents of the urine samples were determined by a method described by Zhao et al. (2016), where the urine was soaked in a filter paper with a predetermined GE, the soaked filter paper was dried (50°C, 2 h), weighed, and its GE determined in a bomb calorimeter. To calculate the GE of urine, the GE value of the filter paper was subtracted from the GE value of the urine-soaked filter paper.

2.6 Calculations

Daily feed intake of DM and its constituents (OM, N, NDF, ADF, GE on a DM basis) by individual animals was calculated from the amounts on offer and refusals. Feed intake was calculated for the periods when the animals were in the individual pens, metabolic crates, and respiration chambers. Daily feed intake was expressed both daily (g/d) and as g/kg metabolic LW (g/kg LW0.75).

The average daily liveweight gain (LWG, g/d) was calculated both for the pen period (Days 1–40) and entire experimental period (Days 1–50).

Apparent total tract digestibility (%) of feed DM and its constituents (OM, N, NDF, ADF, GE; DM basis) was calculated from the ingested and excreted amounts in faeces of each component during the balance period (Days 43–47). For the same period, nitrogen partitioning into faeces, urine, and retained was calculated. Partitioning was expressed as % of N intake.

Daily gross energy intake (GEI) during the balance period was calculated using the DMI and GE concentrations. The partitioning of GEI into faeces, urine, and CH_4 was calculated, as well as digestible energy (DE) and metabolisable energy (ME). Digestible energy (DE) was calculated by subtracting faecal energy (FE) from GEI (DE=(GEI-FE)), while ME was calculated by subtracting FE, urine energy (UE) and energy lost in CH_4 from the GEI (ME=GEI-(FE+UE+CH_4E). The methane energy for the balance period was calculated using the CH_4 energy conversion factor (Ym, MJ CH_4/MJ GEI) determined during the chamber period.

Given that each animal rotated throughout the three respiration chambers (one day per chamber), the average daily CH_4 emissions from each animal were calculated by averaging the emissions over the three days. Calculations of the daily emissions in each chamber accounted for the ventilation rate and net concentrations at each data point, gas conditions, and CH_4 recovery rate. Daily CH_4 emissions were expressed on an absolute basis (g/d), CH_4 yield [g/kg DMI, g/kg organic matter (OM) intake, OMI; g/kg digestible DMI (DDMI) and g/kg digestible OMI (DOMI)], and Ym (CH_4 energy/GEI).

2.7 Data analysis

Data were analysed using a linear mixed-effects model in R statistical software (version 4.1.2; R Development Core Team, USA). The data sets were first tested for normality by producing residual vs fitted plots, normal quantile-quantile plots, and density plots of the residuals in R., Visual inspection of the plots was done to confirm the normal distribution of data. The model for each variable was fitted using the "lmerTest" package in R. The treatment was set as the fixed effect, while the sex of the lambs and batch number were set as random effects. ANOVA type 3 was used to test the significance of the fixed factors. The least square means were calculated using the "lsmeans" package and Tukey's built-in "Multicompview" package in R was used to separate the means per treatment. The significance level was set at P<0.05.

3 Results

3.1 Packed cell volume and faecal egg count

The packed cell volumes (PCV) on Day 1 were comparable across treatments (P>0.05), a trend that persisted until Day 21 post-infection (Table 2). However, by Day 50, the PCV of infected, unsupplemented lambs (InHay) was significantly lower than that of lambs in other treatments (P<0.05). At this stage, there was no difference in PCV between Calliandra supplemented lambs, regardless of PEG supplementation (InHay+Cal and InHay+Cal+PEG) (Table 2). The faecal egg counts (FEC) on Day 1 confirmed that all lambs were worm-free (Table 2). By Day 21 post-infection, there was no significant difference in FEC among the infected lambs (P>0.05). Yet, on Day 50, FEC in InHay lambs was significantly higher than in Calliandra-supplemented lambs (P<0.05) (Table 2). Throughout the study, uninfected lambs remained worm-free.

3.2 Feed intake, liveweight and liveweight gain

Experimental treatments significantly influenced feed DMI (g/d and g/kg $LW^{0.75}$) (Table 3). Calliandra-supplemented lambs had a significantly higher DMI than unsupplemented lambs (P<0.05), but PEG supplementation did not alter DMI (P>0.05). Moreover, infection status did not influence feed intake in unsupplemented lambs (Table 3). There was no significant difference in lamb LW across treatments on days 1, 40, and 50 (P>0.05) (Table 3). However, treatments significantly affected LWG during both the pen period (Days 1–40) and the whole experimental period (days 1–50) (Table 3). Notably, Calliandra-supplemented lambs exhibited positive LWG, while unsupplemented lambs experienced weight

TABLE 2 Mean ($\pm$ standard error of means, sem) packed cell volume (PCV, %) and faecal egg counts (FEC, eggs/g) on Days 1, 21 and 50 of the study for lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
PCV Day 1 (%)	32.8	29.5	33.5	36.6	1.86	0.071
PCV Day 21 (%)	33.8	29.2	30.4	31.0	1.75	0.171
PCV Day 50 (%)	29.7[a]	20.5[b]	25.8[a]	27.1[a]	1.84	0.002
FEC Day 1 (eggs/g)	0	0	0	0	–	–
FEC Day 21 (eggs/g)	0	827	1040	655	300	0.249
FEC Day 50 (eggs/g)	0	8550[a]	1284[b]	3302[b]	1082	<0.001

[a,b]Mean values within a row with different superscript letters differ significantly (P<0·05).

TABLE 3 Mean ($\pm$ standard error of means, sem) intakes of dry matter (DM) in Rhodes grass hay, Calliandra and total intake (g/d and per unit of metabolic weight, $LW^{0.75}$) over the entire experiment (50 days) by lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
Dry matter intake						
Rhodes grass (g/d)	626[a]	644[a]	439[b]	425[b]	21.3	<0.001
Calliandra (g/d)	0	0	314	307	6.59	<0.001
Total (g/d)	626[a]	644[a]	75[b]	73[b]	24.1	0.001
Total (g/kg $LW^{0.75}$)	51.6[a]	51.7[a]	61.8[b]	61.1[b]	1.09	<0.001
Liveweight						
LW Day 1 (kg)	27.9	28.8	28.1	27.5	1.12	0.843
LW Day 50 (kg)	27.1	27.5	28.4	28.0	1.04	0.807
LWG 1–50 (g/d)	-16.68[a]	-26.60[a]	7.16[b]	11.30[b]	9.65	<0.001

[a,b]Mean values within a row with different superscript letters differ significantly (P<0·05).

loss. Supplementation with PEG did not influence the LWG of Calliandra-supplemented lambs. PEG supplementation had no effect on LWG in Calliandra-supplemented lambs (InHay+Cal +PEG vs. InHay+Cal) (P>0.05). Furthermore, infection status did not affect LWG in unsupplemented lambs (P>0.05) (Table 3).

3.3 Nutrient intakes, apparent feed digestibility, nitrogen and energy partitioning (balance period)

Calliandra-supplemented lambs (InHay+Cal and InHay +Cal+PEG) had higher (P<0.05) intakes of DM, OM, and CP than the unsupplemented lambs (UnHay and InHay) (Table 4). Unsupplemented lambs had higher (P<0.05) NDF intake than Calliandra-supplemented lambs, while there was no significant treatment effect on ADF intake (Table 4). Similarly, PEG supplementation had no effect on DMI or its constituents in Calliandra-supplemented lambs. Infection had no effect on the

feed intake of Calliandra-unsupplemented lambs (UnHay and InHay) (Table 4).

The apparent total tract digestibility of DM, OM, CP, NDF, and ADF differed significantly (P<0.05) among the experimental treatments (Table 5). Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG) had similar digestibility. However, lambs not receiving PEG (InHay+Cal) had significantly lower crude protein (CP) digestibility (P<0.05) than those that did receive PEG (InHay+Cal+PEG). While the digestibility of DM, OM, NDF, and ADF was numerically and slightly higher in PEG-supplemented lambs, it wasn't statistically significant (P>0.05) compared to lambs without PEG. Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG) had significantly lower (P<0.05) feed digestibility than the unsupplemented ones (UnHay and InHay). However, there were no discernable differences (P>0.05) in feed digestibility between the uninfected and infected lambs that were solely on a hay diet (UnHay vs. InHay) (Table 5).

The experimental treatments exhibited significant differences (P<0.05) in nitrogen (N) intake and its partitioning (g/d) into faeces

TABLE 4 Mean ($\pm$ standard error of means, sem) dry matter intake (DMI), organic matter (OMI), crude protein (CPI), neutral detergent fibre (NDFI) and acid detergent fibre (ADFI) by lambs over the balance period fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
Nutrient intake						
DMI (g/d)	571[b]	594[b]	752[a]	724[a]	25.7	<0.001
DMI (g/kg $LW^{0.75}$)	47.2[b]	47.5[b]	61.6[a]	60.4[a]	2.01	<0.001
OMI (g/d)	512[b]	531[b]	689[a]	664[a]	23.1	<0.001
CPI (g/d)	35.3[b]	38.2[b]	91.1[a]	89.0[a]	2.32	<0.001
NDFI (g/d)	413[a]	423[a]	374[b]	356[b]	16.7	0.020
ADFI (g/d)	220	224	210	199	9.06	0.196

[a,b]Mean values within a row with different superscript letters differ significantly (P<0·05).

TABLE 5 Mean ($\pm$ standard error of means, sem) digestibilities (%) of dry matter (DMD), organic matter (OMD), crude protein (CPD), neutral detergent fibre (NDFD), acid detergent fibre (ADFD) and gross energy (GED) by lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay +Cal+PEG	sem	*P*-value
DMD	52.8[ab]	53.7[a]	49.4[b]	50.7[ab]	1.04	0.009
OMD	56.8[ab]	57.6[a]	52.3[c]	53.4[bc]	0.98	<0.001
CPD	42.4[ab]	44.8[a]	35.3[b]	44.1[a]	2.84	0.043
NDFD	65.3[a]	66.2[a]	55.4[b]	58.7[b]	1.00	<0.001
ADFD	63.3[a]	63.8[a]	49.6[b]	53.9[b]	1.24	<0.001

[a,b,c]Mean values within a row with different superscript letters differ significantly (P<0.05). Digestibilities were measured during the balance period (Days 43–47).

(FN), urine (UN), and retained (RN). Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG) had similar (P>0.05) daily intake and partitioning of N. However, lambs not given PEG had higher (P<0.05) faecal partitioning than those given PEG (InHay+Cal vs. InHay+Cal+PEG) (Table 6). As expected, lambs without Calliandra supplementation (UnHay and InHay) had lower (P<0.05) daily N intake, faecal partitioning, and retained N compared to Calliandra-supplemented lambs (InHay+Cal vs. InHay+Cal+PEG) (Table 6). There was no significant difference (P>0.05) in daily N partitioning into urine between the unsupplemented lambs (UnHay and InHay) and the Calliandra-supplemented lambs without PEG (InHay+Cal). However, lambs given both Calliandra and PEG (InHay+Cal+PEG) had the most pronounced (P<0.05) daily partitioning of N into urine. The infection status did not influence (P>0.05) the daily N intake or partitioning in unsupplemented lambs (UnHay vs. InHay) (Table 6).

The ratio of faecal N to N intake (FN/NI) was the highest (P<0.05) in lambs that received Calliandra supplementation without PEG (InHay+Cal), with no differences (P>0.05) between the other treatments (UnHay vs. InHay vs. InHay+Cal+PEG) (Table 6). In contrast, the ratio of urinary N to N intake (UN/NI) did not differ (P>0.05) between the Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG). However, these lambs had lower (P<0.05) UN/NI ratios than the unsupplemented lambs (UnHay and InHay). Infection status did not impact the FN/NI or UN/NI ratios of unsupplemented lambs (P > 0.05) (Table 6). The retained N to N intake ratio (RN/NI) did not significantly vary (P>0.05) across experimental treatments. However, lambs in the combined Calliandra and PEG treatment (InHay+Cal+PEG) showed a higher RN/NI, particularly when compared to unsupplemented lambs (P=0.12) (Table 6).

Experimental treatments differed in the daily intake of GE (GEI) and its daily partitioning (megajoules/d, MJ/d) into faecal energy (FE), urinary energy (UE), CH_4 energy (CH_4 E), digestible energy (DE), and metabolisable energy (ME). Overall, both GEI and its partitioning were higher (P<0.05) in Calliandra-supplemented lambs (InHay+Cal and InHay+Cal+PEG) than in those without supplementation (UnHay and InHay) (Table 6). PEG supplementation did not affect (P>0.05) the daily GEI or its partitioning in Calliandra-supplemented lambs (InHay+Cal+PEG

vs. InHay+Cal). Similarly, the infection status did not affect (P>0.05) the daily GEI and its partitioning in unsupplemented lambs (UnHay and InHay) (Table 6). The ratio of digestible energy (DE) to GE (DE/GEI) differed among the experimental treatments. However, the ME/GEI ratio did not differ (P>0.05) between the experimental treatments (Table 6).

3.4 Enteric methane emission

Daily CH_4 emissions CH_4 yield, and CH_4 conversion factor (Ym) differed significantly among treatments (Table 7). Calliandra-supplemented lambs emitted less CH_4 compared to their unsupplemented counterparts (Table 7). PEG supplementation had no discernible effect on these measures. Infection had no effect (P>0.05) on CH_4 emissions in unsupplemented lambs (Table 7).

3.5 Rumen pH

Rumen pH did not differ significantly (P>0.05) across treatments. The average pH for the respective treatments were: UnHay (7.06), InHay (7.06), InHay+Cal (7.15), and InHay+Cal+PEG (7.12).

4 Discussion

4.1 Effects of *Haemonchus contortus* infection on animal health, feed intake and digestibility, nutrient partitioning, liveweight gain and CH_4 emission

This study found reduced *Haemonchus contortus* infection with Calliandra supplementation. However, there were no effects of infection on feed intake and digestibility, nutrient partitioning, liveweight gain, or CH_4 emission. These findings confirmed previous observations in lambs infected with *H. contortus* (Mwangi et al., 2023). However, Mwangi et al. (2023) found that LW loss by infected lambs was significantly higher and twice as high than the LW loss in uninfected lambs, similarly in the current study, the LW loss in the infected lambs fed solely on hay was 37% higher

TABLE 6   Mean ($\pm$ standard error of means, sem) intakes of nitrogen (N) and gross energy (GEI) and their partitioning by lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay+Cal+PEG	sem	*P*-value
Nitrogen (N) partitioning						
N intake (NI, g/d)	5.65[a]	6.12[a]	14.58[b]	14.25[b]	0.37	<0.001
Faecal N (FN, g/d)	3.16[a]	3.37[a]	9.42[c]	7.95[b]	0.23	<0.001
Urine N (UN, g/d)	1.69[b]	1.81[b]	2.34[ab]	2.79[a]	0.23	0.005
FN/NI	0.58[ab]	0.55[b]	0.65[a]	0.56[b]	0.03	0.043
UN/NI	0.31[a]	0.30[a]	0.16[b]	0.20[b]	0.03	<0.001
Retained N (RN)						
g/d	0.79[b]	0.94[b]	2.75[a]	3.48[a]	0.43	<0.001
RN/NI	0.11	0.15	0.19	0.24	0.06	0.122
Energy partitioning						
Intake (GEI, MJ/d)	9.95[b]	10.31[b]	14.0[a]	13.5[a]	0.45	<0.001
Faecal (FE, MJ/d)	4.61[b]	4.73[b]	7.18[a]	6.75[a]	0.20	<0.001
Urine (UE, MJ/d)	0.46[ab]	0.30[b]	0.55[a]	0.61[a]	0.09	0.042
Methane (CH$_4$ E, MJ/d)	0.83[ab]	0.89[a]	0.77[ab]	0.74[b]	0.03	0.011
Digestible (DE, MJ/d)	5.34[b]	5.57[b]	6.82[a]	6.76[a]	0.30	<0.001
Metabolizable (ME, MJ/d)	4.04[c]	4.36[bc]	5.53[a]	5.41[ab]	0.34	0.003
DE/GEI	0.536[ab]	0.539[a]	0.488[c]	0.500[cb]	0.01	0.001
MEI/GEI	0.40	0.42	0.39	0.40	0.02	0.461

[a,b,c]Mean values within a row with different superscript letters differ significantly (P<0·05).
Partitioning was measured during the balance period (Days 43–47).

than that of their uninfected counterparts, however this difference was not statistically significant.

A decrease in PCV and increase in FEC are reliable indicators of GIN infection (Xiang et al., 2021). In this study, parasite infection in the infected treatment groups was successful. Our results also indicate the effects of infection on PCV and FEC were evident from 3-week post-infection.

Haemonchus contortus infection results in the destruction of the abomasal mucosa and increased mucus production (Ramos-Bruno et al., 2021), consequently compromising feed intake and digestibility, nutrient absorption, and feed conversion efficiency (Kyriazakis, 2014; Hoste et al., 2016; Sahoo and Khan, 2016). A recent study conducted by Xiang et al. (2021) concluded that lambs infected with *H. contortus* (10,000 L$_3$ larvae) had decreased feed

TABLE 7   Mean ($\pm$ standard error of means, sem) daily methane emission (g/d), methane yield [expressed per kg of intake of dry matter (DMI), organic matter (OMI), digestible DMI (DDMI), digestible organic matter intake (DOMI)] and methane conversion factor (Ym, MJ CH$_4$/MJ GEI) by lambs fed on Rhodes grass hay (control diet, Hay) and either uninfected with *H. contortus* (UnHay), infected with *H.contortus* (InHay), infected and supplemented with Calliandra forage (InHay+Cal) or infected and supplemented with Calliandra forage and polyethylene glycol (InHay+Cal+PEG).

	UnHay	InHay	InHay+Cal	InHay+Cal+PEG	sem	*P*-value
g/d	15.1[ab]	16.2[a]	13.9[ab]	13.5[b]	0.60	0.011
g/kg DMI	25.6[a]	26.5[a]	18.7[b]	18.6[b]	0.69	<0.001
g/kg OMI	29.0[a]	29.9[a]	21.0[b]	20.9[b]	0.83	<0.001
g/kg DDMI	46.8[a]	48.0[a]	38.2[b]	36.8[b]	1.54	<0.001
g/kg DOMI	50.1[a]	51.0[a]	41.5[b]	40.3[b]	1.82	<0.001
Ym (MJ CH$_4$/MJ GEI)	0.082[a]	0.085[a]	0.057[b]	0.057[b]	0.002	<0.001

[a,b]Mean values within a row with different superscript letters differ significantly (P<0·05).
Methane emission measurements conducted over three days (Days 48–50).

intake and nutrient digestibility (especially of protein and lipids), reduced LWG, disturbed protein absorption, altered plasma amino acid profiles, and changes in gastrointestinal microbial community composition. In contrast, a meta-analysis conducted by Méndez-Ortíz et al. (2019) on the effects of gastrointestinal nematodes on feed intake and LWG by lambs revealed that 41% and 27% of the studies did not observe a negative effect of GIN on feed intake and LWG, respectively. In the present study, however, there was no effect of *H. contortus* infection on feed intake, feed digestibility, nitrogen retention, or diet metabolisability and the rumen pH was not altered. In line with the above, various authors (Sykes and Coop, 1977; Kyriazakis et al., 1996; Hoste et al., 2016) have suggested that the effects of *H. contortus* on feed intake and animal performance are much less pronounced and transient than those of infection by other gastrointestinal nematodes.

It is plausible to assess the nutritional cost of infection in terms of nitrogen and energy metabolism (Retama-Flores et al., 2012; Méndez-Ortíz et al., 2019; Ramos-Bruno et al., 2021). For example, Ramos-Bruno et al. (2021) suggested that the nutritional cost of infection depends on the animal's ability to withstand the parasitic impact (resilience) or the ability to mount an immune response (resistance). In line with the latter, Blackburn et al. (2013) suggested that the host immune response rather than direct parasite damage is the major cause of loss in productivity. In the present study, infection did not affect feed digestibility, N retention, or diet metabolisability. Therefore, there was no significant impact of infection on animal performance. The LW loss by infected and uninfected lambs could be explained by the poor quality of the Rhodes grass hay.

GIN infection in small ruminants substantially alters the anatomy, function, microbial structure, and composition of the rumen and abomasum (Li et al., 2016; El-Ashram et al., 2017; Corrêa et al., 2020, 2021; Xiang et al., 2021), resulting in decreased daily emissions of CH_4 (arising from reduced feed intake) but increased CH_4 yields (emissions per gram of intake). In fact, studies involving parasite-naïve sheep or mixed GIN infection (Fox et al., 2018; Lima et al., 2019; Corrêa et al., 2021; Fernandes et al., 2022) showed depressed feed intake and high CH_4 yields associated with parasite infection. The lambs in this study, sourced from a flock kept on rangelands, had previous exposure to *H. contortus* and other GIN. Thus, the lack of GIN infection effect on feed intake, digestibility, and CH_4 emissions in the current study supports the hypothesis of Mwangi et al. (2023) that prior exposure of lambs to GIN confers them with a degree of resilience to the negative effects of infection on feed intake and digestibility (and consequently CH_4 emission) and animal performance.

4.1.1 Effects of Calliandra supplementation in alleviating *H. contortus* infection

Consumption of tannin-rich forages has been associated with the effects of these compounds on different stages of the GIN life cycle. *In vitro* studies involving condensed tannin (CT) extracts (including those of Calliandra) have reported that CT is effective in inhibiting the egg, larvae, and adult stages of *H. contortus* and disrupts its life cycle (Wabo et al., 2011; Hoste et al., 2012; Mustabi

et al., 2019). Similarly, *in vivo* studies (Cresswell, 2007; Manolaraki et al., 2010) have reported decreased egg production when CT-containing forages were fed. For example, Cresswell (2007) fed fresh Calliandra forage (114 g total CT/kg DM) to lambs infected with 3,200 *H. contortus* L_3 larvae, which showed 24–68% lower egg production, compared with control animals. Reduced egg production has been attributed to the reduced fertility of female worms (Manolaraki et al., 2010; Martínez-Ortíz-de-Montellano et al., 2010). Reduced establishment rates of the infective stage of larvae have also been reported when CT-containing diets are fed to goats (Paolini et al., 2003; Brunet et al., 2008). Furthermore, tannin-rich fodder may also disrupt GIN egg development becoming infective L_3 larvae, thereby disrupting the life cycle of nematodes and reducing pasture contamination (Molan et al., 2000; Min et al., 2005). In the present study, the FEC in Calliandra-supplemented lambs was 46–63% lower than that in unsupplemented lambs, confirming the anthelmintic effect of CT in Calliandra.

In the present study, *H. contortus* infected lambs fed only hay had significantly lower PCV than their counterparts supplemented with Calliandra (InHay vs. InHay+Cal). This can be partially attributed to the improved N nutrition provided by Calliandra. Studies involving sheep infected with *H. contortus* and supplementation of a basal diet (low in CP) with higher protein sources (Abbott et al., 1988; Wallace et al., 1996; Bricarello et al., 2005) have reported improved animal health (e.g., higher PCV and total plasma proteins). However, in this study, Calliandra supplementation, which contained a high concentration of CT, likely turned the CP at least partially unavailable for the host. Although the amount of retained N (RN) was higher in supplemented lambs, RN/NI did not differ between the supplemented and unsupplemented lambs (InHay+Cal vs InHay).

4.1.2 Effects of Calliandra supplementation on feed intake and liveweight gain

The total DMI of lambs supplemented with Calliandra was significantly higher (by 17–20%) than that of their unsupplemented counterparts (InHay+Cal vs. InHay or UnHay). However, care must be exercised when interpreting this difference. Unsupplemented lambs ate more of the basal diet (hay) than their Calliandra-supplemented counterparts, that is, the intake of Calliandra effectively substituted the intake of hay. The astringent properties of CT reduce feed intake and animal performance when the CT content in the diet is >50 g/kg DM (Barry and McNabb, 1999; Naumann et al., 2017). In the present study, the concentration of total CT in the InHay+Cal diet was approximately 39 g/kg DMI, with no apparent impact on feed intake. However, literature shows both negative effects of CT on feed intake (and digestibility) at concentrations <50 g CT/kg DM, and no impact at >50 g CT/kg DM. Thus, it is likely (Méndez-Ortiz et al., 2018) that the impact of CT on feed intake depends more on the structure of CT rather than its concentration. The astringency arising from CT-protein complexes is responsible for low palatability; the greater the protein bound by CT, the greater the astringency and the lower the palatability (Naumann et al., 2017). The level of astringency depends on the ability of CT to bind to proteins (McMahon et al.,

2000; Waghorn, 2008; Naumann et al., 2017). A reduced rate of fibre digestion associated with CT activity slows the clearance of feed residues from the rumen, thereby reducing voluntary feed intake (Waghorn, 2008).

The improved total feed intake found in this study confirms reports from other studies where low-quality forages were supplemented with tannin-rich forages (Yisehak et al., 2014; Korir et al., 2016; Gaviria-Uribe et al., 2020). For example, Yisehak et al. (2014) reported improved feed intake by sheep when *Albizia gummifera*, a leguminous fodder tree, was supplemented at 30% of the basal diet consisting of forage from natural mixed species. Korir et al. (2016), in their study with cattle fed on wheat straw, reported an improved feed intake when a straw basal diet in cattle was supplemented with Calliandra dry leaves (20 g/kg LW). The increase in feed intake observed as a result of legume forage supplementation is also in agreement with other studies where ruminants fed on a low-protein diet were supplemented with non-forage protein sources (for example, McGuire et al., 2013). The increase in feed intake when sources of protein are supplemented is attributed to increased ruminal microbial activity and increased rumen turnover (Köster et al., 1996). The higher fibre content of poor-quality diets reduces voluntary feed intake (Minson, 1990; Bosa et al., 2012). Hence, it is likely that the increased intake observed when higher-quality forages are supplemented results from an increased rate of passage (Barahona et al., 1997). In this study, the basal diet contained 73.0 and 5.6% NDF and CP, respectively, whereas the corresponding contents of Calliandra were 17.4 and 21.1%, respectively.

In the present study, Calliandra-supplemented lambs had a higher LWG than their unsupplemented counterparts (InHay+Cal vs. InHay or UnHay), which is consistent with the findings of other studies. For example, Yisehak et al. (2014) reported a LWG of 14 g/d when Bonga lambs were supplemented with *Albizia gummifera*, while their unsupplemented counterparts fed on hay alone gained 3 g/d. In another study, Nherera et al. (1998) reported 44 g/d LWG in goats fed on maize stover and supplemented with Calliandra which was higher than goats supplemented with Leucaena. Similarly, Palmer and Ibrahim (1996) reported that sheep supplemented with Calliandra at inclusion of 27% on DM basis had higher LWG than their counterparts fed on a tropical grass alone (25 vs. -27 g/d). In Kenya, Kinuthia et al. (2007) observed that weaner goats supplemented with 200 g air-dried Calliandra leaves over seven months had higher LWG than those fed Rhodes hay alone (57 vs. 17 g/d). The superior LWG of Calliandra-supplemented lambs observed in the present study could be attributed to improved feed intake and nutrition (increased N retention and metabolisable energy).

4.1.3 Effect of Calliandra supplementation on nutrient intakes, apparent feed digestibility, and nitrogen and energy partitioning

During the balance period, Calliandra-supplemented lambs had significantly higher daily intakes of DM, OM, and CP than their unsupplemented counterparts. The reasons for these higher intakes are discussed in the preceding subsection. The unchanged intake of NDF and ADF with supplementation can be attributed to the low

level of fiber in the Calliandra. In this study, the Calliandra consisted not of whole leaves, but of their primary leaflets, which had low NDF content (Table 1).

In this study, lambs supplemented with Calliandra had 9% lower OM digestibility than their unsupplemented counterparts (InHay+Cal vs. InHay). However, the decrease in CP and ADF digestibilities were much higher (22%). The latter is in close agreement with the findings of Tiemann et al. (2008), in which lambs fed on a tropical grass and supplemented with Calliandra (CT=175 g/kg DM) at 30% of the daily diet (DM basis) had depression in CP and ADF digestibilities of the order of 38%, compared to those receiving no supplement. Similarly, Fassler and Lascano Aguilar (1995) and Hove et al. (2001) reported a much greater effect on ADF digestion than NDF digestion when CT-rich tropical legumes were supplemented to small ruminants.

In the current study, the only measured aspect of CT was its concentration, and the estimated total concentration of CT in the diet of supplemented lambs (InHay+Cal) was ~39 g/kg DM, which is far below the suggested limit of tolerance of 50 g/kg DM) (Barry & McNabb, 1999). However, as stated earlier, the anti-nutritional effects of CT cannot be ascribed to its concentration alone (Waghorn, 2008; Naumann et al., 2017; Besharati et al., 2022). Waghorn (2008) concluded that the effects of CT on ruminal proteolysis, crude protein digestibility, OM digestibility, and N balance are well understood, and that the degree to which CT interferes with these processes is a function of the astringency, concentration, and chemical and physical properties of CT. The negative effects of CT on CP digestibility and, to a minor degree, on fibre digestibility are common features reported in the literature (e.g., Jayanegara et al., 2012; Cabral Filho et al., 2013; Méndez-Ortiz et al., 2021). Besharati et al. (2022) concluded that CT mainly affects CP digestibility by creating hydrogen bonds that are stable in the pH range of 3.5–8.0, whereas Naumann et al. (2017) concluded that at a high tannin: protein ratio, the protein becomes coated with CT, leading to insoluble precipitates, which is especially true in CT-rich Calliandra (Rira et al., 2022). High concentrations of dietary CT increase dietary faecal nitrogen excretion; however, tannin consumption may also increase endogenous N loss (Besharati et al., 2022). Rira et al. (2022) reported that tannins interfere with the microbial colonisation of plant material, especially by fibrolytic bacteria, thereby affecting fibre digestion. McMahon et al. (2000) from a review of *in vitro* studies concluded that CT could inhibit cellulose digesting ruminal bacteria and fungi. Furthermore, the strong chelating properties of CT may reduce the availability of metal ions necessary for microbial metabolism (McMahon et al., 2000).

Compared to unsupplemented lambs (InHay+Cal vs. InHay), Calliandra supplementation significantly increased faecal N partitioning (FN/NI) and reduced urinary N (UN/NI), whereas retained N (RN/NI) remained unaltered. This is in close agreement with previous reports (McSweeney et al., 2001; Tiemann et al., 2008; Besharati et al., 2022). For example, Tiemann et al. (2008) with sheep supplemented with Calliandra, found increased FN/NI and lowered UN/NI, whereas RN/NI was

negative. Similar findings were also observed when *Gymnopodium floribundum*, a Polygonaceae plant species, was supplemented to sheep in a diet providing 86 g CT/kg DM (Méndez-Ortiz et al., 2021). The increased faecal N partitioning is in line with the depressed digestibility of CP, whereas decreased urinary N excretion likely reflects the effect of CT on ruminal proteolysis by complexing proteins, making them less available to proteolytic microorganisms (Mueller-Harvey, 2006). The latter suggests that CT may have an additional role in reducing urinary sources of NH_3 and N_2O (Śliwiński et al., 2004; Waghorn, 2008). In the present study, despite the higher N loss in faeces, the retained N (g/d) of Calliandra-supplemented lambs was significantly higher than that of the unsupplemented lambs.

In this study, Calliandra-supplemented lambs had higher daily energy intake (GEI), daily faecal energy (FE), and urinary energy (UE) outputs than their unsupplemented counterparts (InHay+Cal vs. InHay). The DE/GEI ratio was lower for the supplemented lambs, whereas the ME/GEI ratio did not differ between these groups of lambs. The former indicates the effects of compromised feed digestibility discussed earlier. Our findings are in partial agreement with those of Tiemann et al. (2008), who observed a 12% decrease in DE/GE and ME/GE ratios in Calliandra-supplemented lambs compared with the control (grass only). Similarly, Méndez-Ortiz et al. (2021) reported depressed DE/GE (by 26%) and ME/GE (by 29%) ratios in lambs supplemented with *Gymnopodium floribundum* compared to unsupplemented lambs. The differences between these studies may be due to the nature and concentration of CT (Waghorn, 2008). In our study, the digestible (DE) and metabolisable (ME) energies available for supplemented lambs were higher than those for their unsupplemented counterparts.

4.1.4 Effect of Calliandra supplementation on enteric methane emission

In this study, daily CH_4 emissions (g/d), CH_4 yield (per unit of intake), and Ym were decreased by Calliandra supplementation (InHay+Cal vs. InHay). Our findings confirm those from other *in vitro* and *in vivo* studies that reported significant reductions in CH_4 emissions when CT-containing forages were fed (Pinares-Patiño et al., 2003; Puchala et al., 2005; Tiemann et al., 2008; Jayanegara et al., 2012; Rira et al., 2022). Forages rich in CT can reduce daily CH_4 emissions. For example, Pinares-Patiño et al. (2003) and Piñeiro-Vázquez et al. (2018), working with sheep and heifers, respectively, reported 62–65% reduction in CH_4 yield (emissions per unit of feed intake) with diets comprising >80% CT-rich legumes. Tiemann et al. (2008) reported a 17% decrease when lambs were supplemented with Calliandra at a rate of 30% daily DMI. The negative effects of CT on CH_4 emissions can be associated with the reduced digestion of CP and fibre, hence reducing the availability of reducing equivalents for methanogenesis, as well as direct anti-methanogenic action (McMahon et al., 2000; Tavendale et al., 2005; Rira et al., 2022). In fact, Rira et al. (2022), from *in situ* studies, reported that archaea diversity was reduced in high CT-containing *Calliandra calothyrsus*, and concurrently, *in vitro* studies revealed that CH_4 production was low. Furthermore, Kelln et al.

(2020) indicated that CT (Catechins) act as H_2 sinks, reducing the availability of H_2 to archaea.

4.3 Effect of PEG treatment in reducing condensed tannins activity in Calliandra supplemented lambs

Polyethylene glycol (PEG) is a non-ionic detergent with high affinity for CT (Bento et al., 2005). Because of PEG's ability to bind CT, the addition of PEG to diets can reduce the effect of CT and has led to its use in testing the effects of CT on feed intake, rumen function, and metabolism of N and energy (de Frutos Fernández et al., 2004), and methane emissions (Wang et al., 2018). The ability of PEG to bind CT has been suggested to improve the feeding value of CT-rich plant species, especially in the tropics (Waghorn, 2008). In the current study, the PEG dose was calculated based on a preliminary analysis of the CT concentration in the Calliandra forage material collected prior to collection of the actual experimental Calliandra forage, which showed a total CT of 28.2 g/kg DM. However, analysis of samples of the Calliandra forage used in the experiment (carried out at the end of the study) in a different laboratory yielded a higher concentration of total CT (92.1 g/kg DM). The reason for this large difference in the analytical results may be due to differences in CT concentration between the samples or due to the different analytical procedures used. Studies in Sub-Saharan Africa have reported total CT concentrations in Calliandra in the range of 10–200 g/kg DM (Dzowela et al., 1995; Hove et al., 2001). The large range can be attributed to various factors, including the environment where the sample was collected, sample drying, and method of analysis (Wolfe et al., 2008). Based on the lack of differences in the effects of InHay+Cal and InHay+Cal+PEG treatments on infection response, DMI, LWG, feed digestibility, N and gross energy partitioning, and CH_4 emission, we are confident that the initial analyses of CT concentrations underestimated the real concentrations, resulting in undercalculation of PEG dosage required to neutralize CT activities. Other studies consider Calliandra to be a high CT-rich legume (Perez-Maldonado and Norton, 1996; Rakhmani et al., 2005; Cresswell, 2007; Tiemann et al., 2008; Rira et al., 2022).

In this study, underestimating CT concentration in Calliandra led to a lower PEG dosage in the InHay+Cal+PEG treatment. The intended ratio of PEG: CT was 1.1, while an actual PEG : CT ratio of 1:2.8 was achieved. Consequently, the PEG level used was insufficient to fully counteract the CT properties. In effect, the mean values of PCV and FEC, feed intake, LWG, nutrient intake in feed, feed digestibility, N partitioning to urine and retained N, energy partitioning, and CH_4 emission did not differ between Calliandra-supplemented treatments with and without PEG supplementation (InHay+Cal vs. InHay+Cal+PEG). On the other hand, CP digestibility and partitioning of N intake to faecal N were higher and lower, respectively, in PEG-supplemented lambs than in lambs receiving no PEG supplement (InHay+Cal+PEG vs. InHay +Cal). However, the extent to which PEG supplementation reduced the protein-binding property of CT remains uncertain.

5 Conclusion and recommendations

In the present study, supplementation of a poor-quality basal diet with Calliandra resulted in reduced GIN infection, improved feed intake, improved liveweight gain and reduced CH_4 emission from sheep. However, these effects were partially offset by reduced crude protein and fibre digestibility. Small-holder livestock keepers in SSA may not be able to achieve high (40%) supplementation levels of forage from fodder trees because of competing interests from other food crops. Hence, lower supplementation levels may be optimal and more achievable in local farming situations. Calliandra crops, when well-managed, could potentially provide year-round green forage that helps control GIT parasite infection, improves the feeding value of the basal diet, hence lowering the cost of animal production. Furthermore, incorporating Calliandra can lead to a notable reduction in enteric CH_4 emissions, both in absolute (g/animal/day) and yield (g/kg DMI) basis.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by International Livestock Research Institute's Institutional Animal Care and Use Committee (IACUC, Reference number 2022-03). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

PM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. RE: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. IG: Conceptualization, Methodology, Supervision, Writing – review & editing. LM: Funding acquisition, Writing – review & editing. DM: Investigation, Writing – review & editing. JG: Investigation, Writing – review & editing, Data curation. SM: Conceptualization, Methodology, Supervision, Writing – review & editing. CP: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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