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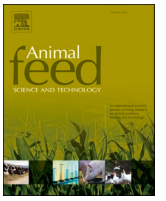

Effect of non-encapsulated and encapsulated mimosa (*Acacia mearnsii*) tannins on growth performance, nutrient digestibility, methane and rumen fermentation of South African mutton Merino ram lambs

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ABSTRACT

The use of mimosa tannins as feed additives in contemporary ruminant nutrition studies has gained wider acceptance, because of their potential to reduce enteric methane and enhance dietary protein utilization. However, tannin's astringency and quick dissolution decrease feed intake and digestibility. Microencapsulation technology could be adopted to neutralize the astringency and ensure controlled tannin solubility across ruminant digestive tract. The present study examined the influence of supplementing unencapsulated and encapsulated Mimosa (*Acacia tannin*) tannins on growth performance, digestibility, methane (CH_4) and rumen fermentation in South African mutton Merino sheep. A total of 40 weaned Merino ram lambs of 96 days old (34 −35 kg body weight, BW) were randomly allocated to one of the four dietary treatments in a randomized complete block design as follows: total mixed ration, (T1, TMR alone); TMR + Monensin at 75 mg/kg feed dry matter, DM (T2, Monensin); TMR + unencapsulated Mimosa tannin at 20 g/kg DM (T3, UMT) and TMR + encapsulated Mimosa tannin in sunflower oil at 20 g/kg DM equivalent (T4, EMT^S). The lambs were adapted to the diets over a period of 28 days thereafter, feed intake and weight changes were recorded for 77 days. The lambs were moved to metabolic crates and assessed for digestibility followed by methane measurement in open-circuit respiratory chambers. Finally, the animals were slaughtered, rumen fluids sampled and analysed for volatile fatty acids (VFAs) and ammonia nitrogen (NH_3-N). Inclusion of UMT and EMT^S did not affect ($P > 0.05$) feed intake and weight gain. Compared with the TMR alone, UMT and EMT^S increased intake of DM (T1, 1328 g/day vs T3, 1578.1 and T4, 1569 g/day; $P = 0.05$), DM adjusted to BW (T1, 69.3 g/kg BW vs T3, 83.8 and T4, 83.7 g/kg BW; $P = 0.04$), organic matter, OM (T1, 1254 g/kg vs T3, 1494 and T4, 1490 g/day; $P = 0.04$), neutral detergent fibre, NDF (T1, 344 g/day vs T3, 434 and T4, 416 g/day; $P = 0.01$) and acid detergent fibre, ADF (T1, 174 and T3, 241 g/day vs T4, 218 g/day; $P = 0.02$), compared with the control. However, only UMT decreased ($P = 0.01$) digestibility of DM (T3, 640 g/kg vs T1, 746 g/kg), OM (T3, 655 g/kg vs T1, 761 g/kg) and CP (T3, 734 g/kg vs T1, 829 g/kg). Furthermore, UMT increased ($P = 0.01$) faecal nitrogen (N) excretion (T3, 13.7 g/head/day vs T1, 8.03 g/head/day

Abbreviations: ADF, acid detergent fibre; NH_3-N, ammonia nitrogen; BW, body weight; CP, crude protein; DM, dry matter; EMT^S, encapsulated mimosa tannin in sunflower oil; CH_4, methane; N, nitrogen; OM, organic matter; TMR, total mixed ration; NDF, neutral detergent fibre; UMT, unencapsulated mimosa tannin; VFAs, volatile fatty acids.

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and T3, 266 g/kg N-intake vs T1, 171 g/kg N-intake). UMT and EMT^S reduced CH_4 in g/kg DM-intake (T3, 15.3 and T4, 14.8; vs T1, 18.9; P = 0.04). However, NH_3-N and total VFAs concentrations were not influenced (P > 0.05) by the inclusion of UMT and EMT^S. It was concluded that UMT and EMT^S could be utilized in mitigating enteric CH_4 while enhancing nutrient intake when supplemented at 20 g/kg DM of feed. Among the two, however, EMT^S reduced more CH_4 without affecting digestibility compared to equivalent proportion of UMT. Further studies should be conducted on the total tannin concentration of the encapsulated mimosa tannin in sunflower oil.

1. Introduction

Mitigation of enteric methane (CH_4) emission became a principal area of recent investigations in ruminant nutrition, because of the significant influence of CH_4 to global warming and loss of dietary energy. Methane is produced in the rumen by the bacteria known as *Methanogenic archaea*, using carbon dioxide, CO_2 and hydrogen gas, H_2 (Mcallister and Newbold, 2008). About 95% of enteric CH_4 is produced in the rumen which account for about 70 – 100 tera-gram of CH_4 yield annually (Gerber et al., 2013), and around 12% of animals' energy loss (Piñeiro-Vázquez et al., 2015). This led to the adoption of numerous dietary modifications to reduce methane emission by the ruminants which include improved forage quality, plant breeding and dietary additives such tannins, saponins, ionophores among others (Eckard et al., 2010). Monensin, the most extensively used ionophores which improve feed efficiency and with a methane mitigation potential, is a polyether antibiotic, which is orally fed as a sodium salt (Yang et al., 2007). However, in 2006, the European Union (EU) banned the use of antibiotics as feed additive due to its residual effects on human health (Millet and Maertens, 2011). Thus, utilization of plant secondary substances especially tannins gained more acceptance, because of their efficiency, safety and availability (Soltan et al., 2013).

Tannins are high molecular weight polyphenolic compounds classified into hydrolysable and condensed tannins based on their chemical structure and properties (Hassanpour et al., 2011). Nevertheless, condensed tannins are more extensively studied because of their abundance, anti-methanogenic activities and non-toxic to animals compared to hydrolysable tannins (Martin et al., 2010). *Acacia mearnsii* popularly known as Mimosa or Black wattle tannin is reported to be a rich source of both condensed and hydrolysable tannins (Bhatta et al., 2009), and is widely available in Southern Africa (Galatowitsch and Richardson, 2005). Numerous studies revealed that Mimosa tannin extracts were successfully utilized in manipulating rumen fermentation, methane emission and protein metabolism in ruminants, but with some adverse effects on intake and digestibility because of its astringency and instability across various pH of the gastrointestinal tract (Carulla et al., 2005; Grainger et al., 2009), especially when fed at higher dosage ($\geq$ 50 g/kg DM).

Hence, microencapsulation of Mimosa tannin using oily substances could mask the bitter taste thereby reducing CH_4 without adverse effects on intake and digestibility. Among the widely available lipids, sunflower oil is found in abundance and could serve as suitable encapsulant for tannin due to its low viscous nature and excellent emulsification (Flanagan and Singh, 2006) as well as its tasteless and desirable aroma (Bakry et al., 2016), in addition to its CH_4 abatement potential (Eckard et al., 2010). Recent in vitro studies have shown that Mimosa tannins encapsulated in either palm oil or sunflower oil reduced CH_4 yields (Adejoro et al., 2018), and slowed down quick tannin solubility in various pH simulating rumen, abomasum and small intestine (Adejoro et al., 2018; Ibrahim and Hassen, 2021). In addition, the use of encapsulated mimosa tannin as additive has been found to mask the tannins' astringency (Adejoro et al., 2020). However, to the best of our knowledge there is little or no data available on the long term effects of lipid-based encapsulated mimosa tannin on animal performance. The present study, therefore, aimed at evaluating the influence of unencapsulated and encapsulated mimosa tannin in sunflower oil on feed intake, growth performance, nutrient digestibility, methane and rumen fermentation of South African mutton Merino ram lambs.

2. Materials and methods

2.1. Animal ethics

The animal management procedure followed during this investigation was reviewed and approved by the animal ethics committee of the University of Pretoria with approval number: EC075–17. The committee acted in accordance with the recommendations specified in the South African National Standard 10,386 on the handling of animals for research purposes. This study was carried out at the small ruminant section of the University of Pretoria experimental farm (Pretoria, South Africa).

2.2. Preparation of experimental feed additives

The mimosa tannin used as feed additive in this study was obtained from UCL Company (Pty) Ltd. Dalton, South Africa. Before the experiment, the tannin powder was analysed in the laboratory to ascertain the concentrations of its total tannin (TT) following the procedure of Makkar et al. (1993), condensed tannin (CT) as described by Porter et al. (1986) and hydrolysable tannin (HT) according to the method of Singh et al. (2005). The tannin powder constituted of 677.6 g/kg DM of TT, 221.7 g/kg DM of CT and 463.8 g/kg DM of HT (Ibrahim and Hassen, 2022). The mimosa tannin was then encapsulated with sunflower oil using solid-in-oil-water method according to Ibrahim and Hassen (2021). Briefly, solid-in-oil portion was reconstituted by weighing 8.5 g of the tannin into a beaker containing 30 mL sunflower oil solution in dichloromethane (50 mg/mL), added with 0.5% (w/v) of Span80 and agitated for two

minutes using magnetic stirrer set at 400 revolutions per minute, rpm. While, the aqueous solution was formed by mixing 300 mL water and 1% (w/v) of Tween80 using an iron rod homogenizer (PRO400DS, Pro Scientific Inc., Oxford, CT 06478, USA) at 20,000 rpm for three minutes. The two solutions were added together and agitated for three hours using a magnetic stirrer set at 800 rpm to evaporate the dichloromethane. The encapsulated tannin was squeezed using cheese cloth, rinsed with water and freeze-dried for 5 days. The dried mimosa tannin encapsulated in sunflower oil was ground to powder using pestle and mortar, and refrigerated before use.

According to Ibrahim and Hassen (2021), compared to unencapsulated mimosa tannin, the scanning electron microscopy showed that the encapsulated tannins were smaller in size (~20–40 μm) and spherical in shape with encapsulation efficiency of 68%. In addition, encapsulated mimosa tannin had lower particle density (1.22 g/cm^3) compared to free tannin (1.44 g/cm^3). Furthermore, the proportion of tannins released by the unencapsulated tannin after 24 h in the rumen (94%), abomasum (92%) and small intestine (96%) simulated buffers were reduced to 24%, 21% and 19% for the encapsulated tannin in similar elution media and time.

2.3. Animals, experimental design and treatments

A total of 40 South African mutton Merino ram lambs of 96 days old (34–35 kg body weight) were used for the experiment. The lambs were grouped based on age and allocated to four dietary treatments in five blocks each with two lambs per cage in a randomised complete block design. The treatments were T1: Total mixed ration only, TMR (negative control); T2: TMR + Monensin @ 75 mg/kg DM of feed (positive control) based on manufacturers' recommendation; T3: TMR + Unencapsulated Mimosa tannin @ 20 g/kg DM (UMT) and T4: TMR + Encapsulated Mimosa tannin in sunflower oil @ 20 g/kg DM equivalent (EMT^S).

2.4. Dietary composition and growth performance trial

The diet adopted in this study was a total mixed ration formulated by a commercial feed company (AFGRIFEEDS Ltd, South Africa) to meet the growth and maintenance of growing lambs using Agricultural Modelling and Training System (AMTS) program which uses National Research Council, NRC, 2007 standard as shown in Table 1. The diet was formulated to meet an average daily gain (ADG) of around 250 g/head/day. To facilitate intake and limit selection, all diets were thoroughly blended after mixing all the ingredients using a vertical mixer.

The animal trial took place between 20th of August 2019–24 th January of 2020. The lambs were first adapted to the experimental diets for 4 weeks, during which the diets were gradually introduced to the lambs at 25%, 50%, 75% and 100% in 4 successive weeks, to replace the commercial pellets earlier fed to the animals. Following the adaptation period, the lambs were given their individual diets for a continuous period of eleven (11) weeks. The experimental diets were administered in the morning (07h00) and afternoon (1600) with a left-over allowance of 2%, while clean water was given ad libitum. During the growth trial, the average feed intake of the two lambs in each pen for the five blocks of four treatments were recorded on daily basis by subtracting the orts from the feed offered, while the individual lamb body weight was recorded every week using electric weighing scale. Feed conversion ratio was obtained as the fraction of dry matter intake to average daily gain.

Table 1
Ingredients and Chemical Composition of the Experimental Diets fed to South African mutton Merino Sheep.

Ingredient (g/kg)	Control	Monensin	UMT	EMT^S
Soybean meal	170	170	167	165
Yellow maize	280	280	275	272
Medicago sativa hay	200	200	196	194
Eragrostis curvula hay	227	227	223	221
Molasses	60.0	60.0	58.8	58.3
Wheat offal	50.0	50.0	49.0	48.6
Urea	8.00	7.99	7.84	7.77
Vitamin premix[a]	5.00	5.00	4.90	4.86
Monensin[b]	0.00	0.75	0.00	0.00
UMT[c]	0.00	0.00	19.6	0.00
EMT^{Sd}	0.00	0.00	0.00	28.2
Total volume	1000	1000	1000	1000
Chemical composition (g/kg)				
Dry matter	935	935	934	938
Organic matter	877	878	881	887
Crude Protein	217	215	201	207
Neutral Detergent Fibre	267	274	283	277
Acid Detergent Fibre	135	152	156	146

[a] Vitamin premix provided the following per kilogram diet: vit A, 18,000 iu; vit D, 3920 iu; vit E, 2.45 iu; Zn, 5.0 mg; Mn, 4.1 mg; Cu, 0.5 mg; Se, 0.2 mg; Mg, 28 mg; and Co, 0.3 mg.
[b] Monensin: @ 75 mg/kg DM of feed.
[c] UMT: unencapsulated Mimosa tannin @ 20 g/kg DM of feed.
[d] EMT^S: encapsulated Mimosa tannin in sunflower oil @ 20 g/kg DM of feed equivalent.

2.5. Nutrient digestibility and nitrogen balance trial

Immediately, after the growth performance experiment, a total of twenty (20) lambs (involving one representative animal from each pen for the 5 blocks of 4 dietary treatment group) were taken to separate metabolic cages and attached with faecal bags, while urine bottles were fitted to the mouth of urine pan underneath the cage. The lambs were adapted to the cages for a period of 7 days, thereafter total feed offered, orts, faeces and urine output were collected and recorded for 5 days. The feed, orts and faeces were sampled every day and kept at − 20 °C in the laboratory. The urine collected inside the bottles were sampled in plastic container already added with Sulphuric acid (10%, v/v) to prevent N-volatilization and then frozen. At the end of the 5-days sample collection, the individual ram diet, orts, faeces and urine were pooled across days and sub-sampled for chemical analysis. A representative sample of the feed, orts and faeces was measured and dried at 105 °C in the oven for 18 h thereafter, analysed for initial dry matter, while the remaining samples were dried at 55 °C for 48 h, milled to pass a 1 mm sieve, and analysed for chemical composition.

Method of the Association of Official and Analytical Chemists, AOAC (2000) was adopted for the analyses of dry matter (934.01), ash (942.05) and crude protein (968.06). The feeds, orts and faeces samples were evaluated for dry matter and ash by oven-drying at 105 °C and ashed at 550 °C in a furnace, while crude protein was determined using the Leco analyser (Leco TruMac nitrogen determinator Leco Corporation, St. Joseph, USA.). The nitrogen content of the urine was also analysed using the Leco analyser. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using ANKOM filter bag technique as described by Van Soest et al. (1991).

2.6. Methane quantification

At the end of the digestibility trial, lambs were moved to the small ruminant open-circuit respiratory chambers in 5 batches (4 animals per cycle) for methane evaluation following the procedure documented by Adejoro et al. (2020) with slight modification. Prior to the experiment, the whole room where methane chambers were installed was thoroughly cleaned and disinfected, the probes attached to the pipes were dusted to allow uninterrupted air flow, while each cage was cleaned and the floor was covered with mat to provide a conducive environment for the sheep. Methane recovery trials were carried out for each chamber by injecting a known concentration of standard methane (Methane Cylinder 8.1 kg N 3.5, AFROX LTD Germiston, GT, South Africa) to pre-calibrate the chambers a day before the commencement and the day after each cycle. Recovery percentages ranging from 76% to 96% were achieved, and used for correction.

During methane estimation, 4 lambs representing each dietary treatment (from the same block) were randomly assigned to an individual chamber and adapted for 24 h followed by methane measurement in the 4 successive days, during which time the animals were moved to the next cage every 24 h so that all the sheep had passed through the 4 chambers sequentially in order to reduce confounding effect associated with any variation in chambers and methane recovery. Experimental diets were offered to the lambs every morning and daily feed intake was recorded, while clean water was supplied ad libitum. Every morning, the chambers were cleaned, mat replaced and the animals locked for about 23 h, during which hot wire anemometers (fitted with automatic data loggers) were used to monitor the speed of the airflow within each cage. The gas emitted by the sheep and the ambient air in front of each chamber over the 23 h period were sucked using an 8-channel peristaltic pump (Masterflex 77292 −50 L/S, Cole-Palmer Instr., IL, USA), set at time dispose mode with 2-minutes on and 2-minutes off and then collected in 10 litres SKC gas sampling bags (SKC SamplePro$^{(R)}$ FlexFilm Sample Bag, Inc. Johannesburg, South Africa). The PVC tubes (4 mm × 6 mm) firmly attached to luer-locked syringes were connected to each bag and sampled the gas manually in 6 replicates. The gas samples for each batch were immediately taken to the gas chromatography (GC) room and injected manually into the gas chromatography (8610 C BTU Gas analyser GC System, SRI Instruments, Bad Honnef, Germany) fitted with a flame ionization detector. The methane peaks resulted from the gas sampled from 5 representative animals for each of the four dietary treatments were converted into methane concentration (mL) using standard curve generated with the Peak simple software.

2.7. Determination of rumen fermentation parameters

Following the methane estimation in the chamber, the lambs were taken to abattoir for slaughter. The sheep were rendered unconscious with the aid of an electrical stunner followed by immediate bleeding and evisceration using a sharp knife. The rumen was quickly removed and the content emptied and thoroughly mixed in a plastic bucket. Subsequently, 4 layers of cheesecloth was used to squeeze the rumen fluids into small plastic bucket. Syringes were used to sample 100 mL of rumen fluids into a bottle containing 20 mL of 25% Orthophosphoric acid (for volatile fatty acid, VFA determination), and 90 mL fluid sampled in another bottle containing 15 mL of 0.5 M Sulphuric acid (for ammonia nitrogen, NH_3-N analysis) as described by Adejoro et al. (2020). All the rumen fluid samples were kept in cooler boxes containing ice blocks and immediately transported to the laboratory and stored in the freezer at −20 °C before analysis.

The rumen fluid samples for VFA analysis were defrosted and centrifuged at 4500 rpm for 15 min and, thereafter sieved into GC tubes using Micropore filter (0.45 μm). Samples were injected into GC (Shimadzu GC-2010 Tracera; Shimadzu corp., Kyoto, Japan) connected with a 30 m Inert Cap Pure Wax column with Barrier Ionization Discharge (BID) detector (Webb, 1994). While, rumen fluids for NH_3-N determination were thawed, centrifuged and analysed using phenol-hypochlorite reagent spectrophotometry as described by Broderick and Kang (1980).

2.8. Statistical analysis

All statistical analyses were carried out using the general linear model procedure of SAS 9.4 (SAS Inst. Inc.; Cary, NC, USA). Average daily gain (ADG) of individual animal was obtained from the regression of the weekly body weight gains against time. The experimental design was randomized complete block design and the model used for data analysis was as follows:

$$y_{ij} = \mu + \text{Block} + A_i + \varepsilon_{ij}$$

where yij = observation j at different dietary additives (i; monensin, unencapsulated mimosa tannin or encapsulated mimosa tannin in sunflower oil); μ = overall mean; Block = effect of blocking (initial weight); Ai= effect of additives, and εij = random error with mean of 0 and variance σ2. Where significant differences existed, Tukey HSD was used for mean separation at $P \leq 0.05$.

3. Results

3.1. Growth performance

The influence of adding unencapsulated Mimosa tannins (UMT) and encapsulated Mimosa tannins in sunflower oil (EMT^S) on body weight gain and dry matter intake of South African mutton Merino ram lambs is summarised in Table 2. The results showed that inclusion of UMT and EMT^S did not affect ($P > 0.05$) individual lamb's final weight, total weight gain, dry matter intake (DMI), average daily gain (ADG) and feed conversion ratio (FCR) compared with total mixed ration alone and Monensin diet.

3.2. Nutrient intake and digestibility

Table 3 shows the effects of inclusion of UMT and EMT^S on nutrient intake and digestibility of South African mutton Merino ram lambs. The findings revealed that inclusion of UMT and EMT^S increased ($P = 0.05$) intake of feed dry matter, organic matter ($P = 0.04$), neutral detergent fibre ($P = 0.01$) and acid detergent fibre ($P = 0.02$) compared with control diet. However, intake of dietary protein was not influenced ($P > 0.05$) by the inclusion of both the non-encapsulated and encapsulated Mimosa tannin additives. Generally, addition of UMT and EMT^S at 20 g/kg raised the intake of dietary dry matter by 18.9% vs 18.2%, organic matter by 19.1% vs 18.8%, neutral detergent fibre by 26.3% vs 21.1%, and acid detergent fibre by 38.4% vs 25.1%, respectively.

With regards to nutrient digestibility, only UMT was found to reduce ($P = 0.01$) dry matter, organic matter and crude protein compared with the total mixed ration alone. In general, the digestibility of DM, OM and CP dropped by 14.2%, 14% and 11.4%, respectively, after inclusion of UMT at 20 g/kg of feed DM.

3.3. Nitrogen balance

The summary of nitrogen balance of South African mutton Merino sheep fed total mixed ration and added with either unencapsulated, encapsulated Mimosa tannins or Monensin is shown in Table 4. The results indicated that nitrogen intake, nitrogen excreted, urinary nitrogen and retained nitrogen were not affected ($P > 0.05$) by the inclusion of both the non-encapsulated and encapsulated Mimosa tannin additives. However, addition of UMT increased ($P = 0.01$) the excretions of faecal nitrogen (in g/head/day and g/kg N-intake) when compared with the control diet. Generally, addition of UMT raised nitrogen excretion in the faeces by 71.3% compared to lambs fed total mixed ration alone.

3.4. Methane quantification

Table 5 presents the effects of Monensin and Mimosa tannin additives on in vivo methane emission by South African mutton Merino sheep. The findings showed that both UMT and EMT^S reduced CH_4 in g/kg DM-intake ($P = 0.04$) compared to control diet. However,

Table 2
Growth performance of South African mutton Merino ram lambs fed total mixed ration with Monensin and Mimosa tannin additives.

	Dietary Treatments					
Parameters	Control	Monensin[a]	UMT[b]	EMT^{Sc}	SEM	*p* – values
Initial weight (kg)	34.6	34.8	34.4	35.4	1.88	0.56
Final weight (kg)	52.4	52.1	52.1	51.8	1.78	0.98
Total weight gain (kg)	17.7	17.3	17.5	16.5	0.67	0.67
Dry matter intake (g/day)	1752	1715	1743	1715	83.8	0.89
Average daily gain (g/day)	170	169	169	160	7.73	0.78
Feed conversion ratio	10.4	10.8	10.4	10.9	0.83	0.89

[a] Monensin: @ 75 mg/kg DM of feed.
[b] UMT: unencapsulated Mimosa tannin @ 20 g/kg DM of feed.
[c] EMT^S: encapsulated Mimosa tannin in sunflower oil @ 20 g/kg DM of feed equivalent.
SEM = Standard error of mean. Mean values are based on 2 animals per block and 5 blocks per treatment.

Table 3
Nutrient intake and apparent digestibility of South African mutton Merino ram lambs fed total mixed ration with Monensin and Mimosa tannin additives.

	Dietary Treatments					
Parameters	Control	Monensin[a]	UMT[b]	EMT[Sc]	SEM	p – values
Nutrient intake (g/day)						
DM	1328[b]	1329[b]	1578[a]	1569[a]	84.1	0.05
OM	1254[b]	1254[b]	1494[a]	1490[a]	79.2	0.04
CP	291	294	320	330	21.3	0.48
NDF	344[b]	327[b]	434[a]	416[a]	26.3	0.01
ADF	174[c]	181[bc]	241[a]	218[ab]	16.0	0.02
Apparent digestibility (g/kg)						
DM	746[a]	684[bc]	640[c]	706[ab]	16.1	0.01
OM	761[a]	700[bc]	655[c]	722[ab]	15.4	0.01
CP	829[a]	801[ab]	734[c]	793[ab]	13.0	0.01
NDF	511	303	378	462	53.8	0.10
ADF	429	314	333	407	65.0	0.58

[a] Monensin: @ 75 mg/kg DM of feed.
[b] UMT: unencapsulated Mimosa tannin @ 20 g/kg DM of feed.
[c] EMT[S]: encapsulated Mimosa tannin in sunflower oil @ 20 g/kg DM of feed equivalent.
Means with uncommon superscripts within a row differed significantly ($p \leq 0.05$). Mean values are based on one animal per block and 5 blocks per treatment. SEM = Standard error of mean; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre.

Table 4
Nitrogen balance of South African mutton Merino ram lambs fed total mixed ration with Monensin and Mimosa tannin additives.

	Dietary Treatments					
Parameters	Control	Monensin[a]	UMT[b]	EMT[Sc]	SEM	p – values
Nitrogen intake (g/head/day)	46.5	47.1	51.3	52.8	3.41	0.48
Nitrogen-excreted (g/head/day)	27. 6	27.8	36.4	30.5	2.80	0.11
Faecal nitrogen (g/head/day)	8.03[b]	9.47[b]	13.7[a]	10.7[b]	0.96	0.01
Urinary nitrogen (g/head/day)	19.5	18.1	22.7	19.7	2.48	0.60
Retained nitrogen (g/head/day)	19.0	19.7	14.9	22.4	2.90	0.36
Faecal nitrogen (g/kg N-intake)	171[b]	199[b]	266[a]	206[b]	13.0	0.01
Urinary nitrogen (g/kg N-intake)	421	399	449	368	48.3	0.66
Retained nitrogen (g/kg N-intake)	409	403	285	426	48.2	0.19

[a] Monensin: @ 75 mg/kg DM of feed.
[b] UMT: unencapsulated Mimosa tannin @ 20 g/kg DM of feed.
[c] EMT[S]: encapsulated Mimosa tannin in sunflower oil @ 20 g/kg DM of feed equivalent.
Means with different superscripts within a row differed significantly ($p \leq 0.05$). Mean values are based on one animal per block and five blocks per treatment. SEM = Standard error of mean; N = Nitrogen.

the effect of non-encapsulated and encapsulated Mimosa tannins additives were not significant with regards to CH_4 in g/kg NDF-intake, CH_4 in g/kg DM-digested and CH_4 in g/kg NDF-digested. Methane yield (in g/kg DM-intake) dropped by 19% and 22% for UMT and EMT[S]diets, respectively.

Table 5
Methane emissions of South African mutton Merino ram lambs fed total mixed ration with Monensin and Mimosa tannin additives.

	Treatments					
Parameters	Control	Monensin[a]	UMT[b]	EMT[Sc]	SEM	p – values
CH_4, g/kg DM-intake	18.9[a]	16.3[ab]	15.3[b]	14.8[b]	1.02	0.04
CH_4, g/kg NDF-intake	70.3	62.4	60.4	58.7	5.78	1.50
CH_4, g/kg DM-digested	34.0	31.4	37.8	32.4	1.96	0.12
CH_4, g/kg NDF-digested	139	155	169	135	27.6	0.60

[a] Monensin: @ 75 mg/kg DM of feed.
[b] UMT: unencapsulated Mimosa tannin @ 20 g/kg DM of feed.
[c] EMT[S]: encapsulated Mimosa tannin in sunflower oil @ 20 g/kg DM of feed equivalent.
Means with different superscripts within a row differed significantly ($p \leq 0.05$). Mean values are based on one animal per block and five blocks per treatment. SEM = Standard error of mean; CH_4 = Methane.

3.5. Rumen fermentation parameters

The influence of non-encapsulated and encapsulated Mimosa tannin on rumen fermentation parameters of South African mutton Merino ram lambs is presented in Table 6. The results revealed that all the additives had no effects ($P > 0.05$) on rumen ammonia nitrogen concentration. Similarly, total and individual volatile fatty acids were not affected ($P > 0.05$) by the inclusion of unencapsulated and encapsulated Mimosa tannins or Monensin.

4. Discussion

4.1. Growth performance

Sheep are widely reported to secrete salivary muco-protein rich in proline, which have a high capacity to bind with tannins (especially when fed at low concentration) and formed tannin-protein complexes; that are stable across the various pH ranges of the gastrointestinal tract, and thus, cancel the tannins' adverse effect on palatability, feed intake and digestibility (Austin et al., 1989; Hagerman and Butler, 1991; Narjisse et al., 1995; Foley et al., 1999). In concurrence with our finding, numerous studies have shown that inclusion of tannins below 50 g/kg DM of feed did not reduce voluntary feed intake and weight gain in sheep (Waghorn et al., 1994; Barry and McNabb, 1999; Hervas et al., 2003a; Frutos et al., 2004a). However, reduction in dry matter intake and weight gains were reported in sheep (Priolo et al., 2000; Carulla et al., 2005) and cows (Eckard et al., 2010), when tannins were added at higher (≥50 g/kg DM of feed) amount. The influence tannins' bitter taste on intake and digestibility is well known (Eckard et al., 2010; Martin et al., 2010), and strongly linked to tannin concentration (Frutos et al., 2004b; Piñeiro-Vázquez et al., 2015). Nevertheless, the degree to which it affects dry matter intake and daily gains in ruminants continue to differ (Animut et al., 2008).

4.2. Nutrient intake and digestibility

The increase in dry matter, organic matter, neutral detergent fibre and acid detergent fibre intake recorded in the current study for unencapsulated and encapsulated treatments could be attributed to increase in dietary intake as a result of neutralization of tannin's astringency owing to the sheep ability to secrete salivary proteins rich in proline (Frutos et al., 2004b). This result compares favourably with the findings of Adejoro et al. (2020) where unencapsulated Mimosa tannin additive did not affect DM, OM, CP, NDF and ADF intake of Merino lambs when added at 42 g/kg DM of feed. Zhang et al. (2019), also reported that addition of 30 g/kg DM of condensed tannins sources from bayberry and *Acacia mangium* as well as Valonia hydrolysable tannin did not reduce nutrient intake of lactating dairy cows. Similar trend was also recorded by Barry and McNabb (1999), when tannin rich *Lotus pedunculatus* additives were included in the diet of grazing sheep at 34 – 44 g/kg DM. In contrast to our findings, others have reported reduction in nutrient intake when Mimosa tannin extract were added above 20 g/kg DM of feed (Carulla et al., 2005; Grainger et al., 2009). According to Frutos et al. (2004a) and Piñeiro-Vázquez et al. (2015), the anti-nutritional effect of tannins is strongly related to their level of inclusion.

The reduction in digestibility revealed that unencapsulated Mimosa tannin acted more on the highly soluble protein and organic matter fraction than fibre portion of the diets due to its quick solubility in the rumen compared to the encapsulated tannin, of which the tannin dissolution was delayed by the lipid capsule (Adejoro et al., 2018; Ibrahim and Hassen, 2021). Numerous literature have indicated that tannins exhibit different affinity levels for dietary dry matter, fibre and protein (Makkar, 2003; Beauchemin et al., 2007; Piñeiro-Vázquez et al., 2015). In agreement with the present finding, Beauchemin et al. (2007), recorded a significant reduction in dietary protein degradation with no effect on NDF and ADF digestibility after inclusion of quebracho tannin in the diet of cattle at 20 g/kg DM. While, Carulla et al. (2005) reported substantial decrease in fibre and protein degradation on sheep added with mimosa tannin extract at 25 g/kg DM level. Similarly, Adejoro et al. (2020) also observed a significant reduction in DM, OM, CP, NDF and ADF digestibility in South African mutton Merino sheep after adding mimosa tannin at 42 g/kg DM of feed.

Table 6
Rumen characteristics of South African mutton Merino ram lambs fed total mixed ration with Monensin and Mimosa tannin additives.

	Treatments					
Parameters	Control	Monensin	UMT	EMT^{S}	SEM	p – values
Ammonia nitrogen (mg/dL)	28.6	32.4	27.6	26.4	2.46	0.31
TVFA (mmol/L)	120	116	116	122	13.0	0.98
Acetate (mol/100 mol)	60.7	59.2	58.9	59.8	1.13	0.59
Propionate (mol/100 mol)	20.4	23.0	23.4	21.7	1.45	0.23
Isobutyrate	2.04	1.77	1.60	1.86	0.24	0.64
Butyrate (mol/100 mol)	11. 7	11.0	11.2	11.5	0.55	0.84
Isovalerate	3.49	3.10	3.35	3.50	0.36	0.87
Valerate (mol/100 mol)	1.73	1.69	1.62	1.69	0.07	0.74
Acetate: propionate ratio	3.00	2.65	2.60	2.79	0.21	0.29

[1]Monensin: @ 75 mg/kg DM of feed.
[2]UMT: unencapsulated Mimosa tannin @ 20 g/kg DM of feed.
[3]EMT^{S}: encapsulated Mimosa tannin in sunflower oil @ 20 g/kg DM of feed equivalent.
SEM = Standard error of mean. Mean values are based on one animal per block and five blocks per treatment.

4.3. Nitrogen balance

The increased in faecal nitrogen excretion obtained for non-encapsulated diet could be traced to higher protein intake (320.4 g/day) of the UMT group compared to 290.8 g/day for control group as recorded in the current study. Adejoro et al. (2020), reported that mimosa tannin reduced soluble protein degradation in the rumen, facilitated nitrogen flow to the lower gut and increased faecal-N excretion as against urine-N. The results of this study are also consistent with the earlier reports that tannins did not influence nitrogen retention in cattle and sheep (Tiemann et al., 2008; Stewart et al., 2019; Adejoro et al., 2020). However, Grainger et al. (2009) observed an enhanced nitrogen utilization with Mimosa tannin addition in the diet of dairy cows, attributed to the improvement in protein flow to the duodenum and shift in nitrogen excretion from urine to faeces. Moreover, the review by Waghorn (2008), showed that absorption of bypassed protein depends on the degree of tannin-protein binding and amount of nutrients required by the animals. Aboagye et al. (2018), also noted that the nitrogen content of the faeces which is more stable is not easily lost to the environment and thus, more environmentally friendly. However, urinary-N is largely urea and therefore more rapidly released to the surface as source of atmospheric nitrous oxide and nitrate contamination in water bodies (Eckard et al., 2010).

4.4. Methane

The observed decrease in enteric methane emission showed that the impact of Mimosa tannin is likely related to suppression of dry matter and fibre degradation than directly affecting methanogens (Lawal, 2022). The present result compares favourably with that of Carulla et al. (2005), who reported around 13% decrease in CH_4 at 25 g/kg DM inclusion of Mimosa tannin extract in sheep. Tan et al. (2011) also reported CH_4 reduction (–33%) at 20 g/kg DM of *L. leucocephala* extracts. It has been well documented that tannins modulate enteric CH_4 emission through reduction in feed intake and digestibility or toxicity to *Methanogenic archaea* (Animut et al., 2008; Jayanegara et al., 2012; Hristov et al., 2013). However, some studies revealed that inclusion of tannins at 20 g/kg DM did not reduce methane emission in sheep (Hervás et al., 2003b; Adejoro et al., 2020). These variations could be attributed to differences in the tannin sources, structure and their biological activity.

4.5. Rumen fermentation parameters

Several studies reported little or no impact of tannins on rumen fermentation parameters when added at lower (20 g/kg DM) concentration (Hervas et al., 2003a; Carulla et al., 2005; Getachew et al., 2008; Hassanat and Benchaar, 2013). In agreement with our finding, Carulla et al. (2005); Aboagye et al. (2018); Adejoro et al. (2020) did not observe a significant effect of tannin on TVFA. In contrast, when Mimosa tannins were added at $\geq$ 50 g/kg DM there were significant decreases in the proportions of total volatile fatty acids, acetate, butyrate, valerate and branched-chain VFA (Hassanat and Benchaar, 2013). Similarly, reduction in the concentrations of NH_3-N and TVFAs have been found due to the establishment of tannin–protein and tannin–carbohydrate bonds that are not easily digestible (Makkar, 2005; Martınez et al., 2006) or tannin's toxicity to rumen microorganisms (Bento et al., 2005; Bhatta et al., 2009) as well as delay in cellulolytic activity and longer retention times (Priolo et al., 2000). Moreover, higher inclusions of tannin are found to reduced acetic acid production by inhibiting the activities of acetate forming bacteria (Castro-Montoya et al., 2011), while, enhancing the concentrations of propionic acid giving rise to reduction in acetate to propionate ratio (Beauchemin et al., 2007). Similarly, addition of adequate proportion of tannins in the diets of ruminants have been reported to reduce iso-butyric acid and iso-valeric acid productions (Singh et al., 2005; Getachew et al., 2008; Bhatta et al., 2009). According to Hassanat and Benchaar (2013), these branched-chain VFAs are produced from the breakdown of the carbon skeleton of amino acids by rumen microorganisms.

5. Conclusion

Based on the results of this study it was concluded that both non-encapsulated Mimosa tannin and encapsulated Mimosa tannin in sunflower oil as additives could be used in modulating rumen fermentation, reduce enteric methane emission as well as facilitate dietary protein bypass without adverse effect on dry matter intake and animal weight gain when added at 20 g/kg DM of feed. Among the two tannins studied, the encapsulated Mimosa tannin reduced more methane emitted by South African mutton Merino sheep without affecting nutrient digestibility compared to similar inclusion level of unencapsulated Mimosa tannin. Further studies should be conducted on the total tannin concentration of the encapsulated mimosa tannin in sunflower oil.

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CRediT authorship contribution statement

Shehu Lurwanu Ibrahim: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Abubeker Hassen:** Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing – review & editing, Project administration, Resources, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that there is no competing interest in this work.

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