Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect

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Highlights

- Calcium nitrate effectively substituted urea in the diets of growing Merino lambs.
- Lambs fed with nitrate had superior average daily gain and reduced methane emission.
- Tannin supplementation reduced digestibility and average daily weight gain.
- Tannin did not reduce methane emission.
- No interaction effect was observed between tannin and nitrate.

Abstract

This study investigated the effect of urea or calcium nitrate with or without the inclusion of Acacia tannin extract on dry matter intake, nutrient digestibility, growth performance, and methane emission in growing lambs. Forty South African Mutton Merino lambs (95 days old, average body weight of 34.7kg) were blocked by weight and sex and randomly allocated to four groups and fed diets supplemented with: urea (control); nitrate; urea+tannin; and nitrate+tannin. The concentration of urea and nitrate source in the diet was 10g/kg and 32g/kg, respectively, while the tannin was an extract of Acacia mearnsii added at 42g/kg DM. Lambs were gradually adapted to the diets for 21 days after which growth performance was monitored for 60 days. At the end of the experimental period, methane was measured in open-circuit respiratory chambers, and digestibility was carried out inside metabolic cages. Dry matter and other nutrient intakes of lambs were not influenced by NPN source or the inclusion of tannin extract in the diet with the exception of the crude protein intake, which was higher ($P \leq 0.05$) in lambs receiving urea-containing diets. Tannin inclusion reduced ($P < 0.05$) digestibility of dry matter, crude protein, neutral detergent fibre and acid detergent fibre of diets but did not affect methane emission of lambs. Tannin inclusion shifted N-excretion from urinary-N to faecal-N although overall N-retention was not affected. Tannin inclusion reduced acetate and increased propionate proportion in the urea-containing diet ($P < 0.01$), whereas, it had no such effect in the nitrate-containing diet. Higher ($P \leq 0.05$) average daily gain and lower methane emission ($P < 0.01$) were recorded in lambs fed the nitrate-containing diets. In contrast, the inclusion of tannin reduced ($P < 0.05$) the average daily gain without any reduction in methane production. This study demonstrated that nitrate could be used as a source of non-protein nitrogen with the additional benefit of reducing enteric methane emission and improving the average daily gain of lambs. In contrast, tannin extract did not show any positive effect to justify its use under the dietary conditions of this study.

Keywords: Enteric methane; Growth perfomance; Nitrate; Non-protein nitrogen; Rumen fermentation; Tannin

Abbreviations: ADF, acid detergent fibre; ADG, average daily gain; CH_4, methane; CP, crude protein; CT, condensed tannin; DM, dry matter; N, nitrogen; NDF, neutral detergent fibre; NPN, non-protein nitrogen; OM, organic matter; SEM, standard error of means; VFA, volatile fatty acid.

1. Introduction

Urea is widely used as a non-protein nitrogen source in ruminant diets in both smallholder and commercial farming systems across the tropics (Adejoro and Hassen, 2018; Newbold et al., 2014). Nitrate salts are a potential alternative to urea, because they are not only able to meet the ammonia nitrogen needs of rumen microbes but they also serve as alternative hydrogen sinks in the rumen, thus competing with methanogens and resulting in a considerable reduction in methane production (Adejoro and

Hassen, 2018; Nolan et al., 2010; Van Zijderveld et al., 2011). Diets rich in rumen degradable protein or supplemented with NPN could induce a high concentration of ammonia in rumen and blood. High blood urea is capable of altering hepatic metabolism by increasing ureagenesis and glucose metabolism in the liver and other tissues (Taylor-Edwards et al., 2009), with the resultant effect on performance and health of animals (Chumpawadee et al., 2006).

Tannins are becoming important dietary additives based on the extensive evaluation of their beneficial properties (Naumann et al., 2013; Stewart et al., 2019). Higher dietary bypass protein, which can be achieved with tannin supplementation (Archimède et al., 2016; Grainger et al., 2009), could lower the metabolic burden associated with detoxification of ammonia triggered by non-protein nitrogen (NPN) supplementation (Adejoro et al., 2019a, 2019b; Cériac et al., 2019). Aside from the increased bypass protein available to the animal in the intestine, condensed tannin (CT) extracts have been reported to have enteric methane reducing and antiparasitic effects (Hassen et al., 2016; Naumann et al., 2014). Nevertheless, feeding ruminants with a high dietary concentration of tannin may lead to reduced feed intake (Eckard et al., 2010).

The activities of rumen microbes that play important roles in rumen function could be differentially influenced by the presence of nitrate and tannins (Molina-Botero et al., 2019; Pal et al., 2015). The antibacterial effects of tannins may be modulated by the presence of nitrate, with both, being capable of exerting a toxic effect on rumen microbes (Pal et al., 2014). Furthermore, nitrate could competitively scavenge for reducing equivalents within the rumen ecosystem. The concurrent use of tannin with urea or nitrate would potentially alleviate the toxic effect of high rumen ammonia, thus improving nitrogen efficiency, but may also reduce enteric methane emission. It is hypothesised that NPN source has a significant effect on nitrogen metabolism, rumen fermentation, animal performance and enteric methane production as a result of dietary tannin inclusion. The objectives of this study, therefore, was to assess the effect of replacing urea with nitrate, with or without CT extract supplementation on dry matter intake, nutrient digestibility, growth performance and methane emission in growing Merino lambs.

2. Materials and methods

Animal management protocol was approved by the University of Pretoria animal ethics committee with approval number EC061-14. The procedures complied with the guidelines stipulated in the South African National Standard 10,386 on the care and use of animals for scientific purposes. The experiment was conducted at the small stock unit of the University of Pretoria experimental farm (Pretoria, South Africa).

2.1. Animal, experimental design and adaptation

The experiment was carried out from July- December 2017 as a randomised complete block design. The Power procedure of SAS software 9.4 (SAS Inst. Inc.; Cary, NC, USA) was used to predict the number of animals required. A total of 40 lambs of 95 days old were used for the experiment. Lambs were grouped for sex and body weight and subsequently allocated to four dietary treatments at the start of the study. The treatments were 1) urea-containing diet 2) urea-containing diet + tannin, 3) nitrate-containing diet and 4) nitrate-containing diet + tannin. The total mixed ration contained 10 g/kg feed grade urea or 32 g/kg calcium nitrate salt while *Acacia mearnsii* tannin extract obtained from UCL Company (Pty) Ltd. Dalton, South Africa was included at 42 g/kg feed DM. From laboratory analysis using the procedures of Makkar et al. (1993) and Porter et al. (1986), the extract contained total phenol and total tannin concentration of 65.8 g/100 g and 58.5 g/100 g (as tannic acid equivalent) respectively while condensed tannin concentration was 30.5 g/100 g (as leucocyanidin equivalent). The quantity of dietary extract supplemented therefore corresponded to 17 g CT/ kg feed as leucocyanidin equivalent.

2.2. Diets and feeding

The diet consisted of roughage to concentrate ratio of 43:57 based on eragrostis and lucerne hay as the roughage fraction (Table 1). Sunflower meal and ground maize were the main concentrate fraction while urea or calcium nitrate as main NPN sources. The proportion of total nitrogen from NPN was 31 %. Experimental diets were mixed weekly as a total mixed ration, using a vertical mixer. A gradual introduction of lambs to the diets as recommended by Nolan et al. (2010) was done over an initial 21 day period. During the adaptation, animals were allocated 30 %, 60 % and 100 % of the experimental diets in three consecutive weeks, to replace the commercial pellets previously consumed by the lambs. After the 21-day adaptation period, animals were fed their respective experimental diets for a continuous period of 60 days. Diets were offered *ad libitum* in two equal portions at 0700 and 1600 h daily with a refuse allowance of 10 %, and freshwater was available at all times. During the experimental period, the average feed consumed by two lambs within a pen was monitored daily, while the individual body weight of lambs was measured weekly.

2.3. Nutrient digestibility, nitrogen balance evaluation

At the end of the growth trial period, twenty males comprising five lambs per treatment were placed in individual metabolic cages and fitted with faecal bags for total and separate collection of faeces and urine. Adaptation to the cages was done for 7 days, followed by 5 days of the complete collection of faeces, feed refusal and urine output while feed consumption was also monitored. Samples of feed offered, refusals and faeces were collected daily and frozen at −20 °C. Urine excretion was collected inside the aluminium pan under the metabolic cage and through the funnel-shaped end, emptied into plastic bottles containing sulphuric

Table 1
Ingredients and composition of experimental diets.

Ingredient (g/kg DM)	Urea-containing diet		Nitrate-containing diet	
	-tannin	+ tannin	-tannin	+ tannin
Sunflower meal	168	161	171	164
Fine milled maize	276	264	282	270
Wheat bran	49	47	34	33
Molasses	59	57	51	49
Lucerne meal	197	189	190	182
Eragrostis hay	232	222	231	221
Urea	10.0	10.0	0	0
Coarse salt	5	5	5	5
Premix[a]	4	4	4	4
Nitrate Source[b]	0	0	32	31
Tannin extract[c]	0	42	0	42
Chemical composition				
DM, g/kg	912	909	910	910
OM, g/kg DM	930	934	920	923
CP, g/kg DM	204	209	189	184
NDF, g/kg DM	334	364	377	354
ADF, g/kg DM	196.1	218.9	215.3	195.4

[a] supplied in g/kg the following: 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] $5Ca(NO3)_2.NH_4.NO_3.10H_20$; 155 g/kg N, 75 % NO_3 in DM.
[c] Contains 350 g/kg condensed tannin (leucocyanidin equivalent).

acid (10 %, v/v) to prevent N-volatilization and from this, aliquots were frozen for analysis. At the end of the collection period, samples of each animal's diet, refusals, faeces and urine were pooled across days and sub-sampled for analysis. A portion of feed, orts and faeces was weighed, and oven-dried at 105 °C for 18 h for dry matter determination (AOAC, 2000), while a second portion was dried at 55 °C for 48 h, milled to pass a 1 mm sieve, and stored for chemical analysis.

Animals had only access to the orts from the previous day feed before being taken to the abattoir, which was 30 min from the farm. Lambs were slaughtered by electrical stunning followed by exsanguination, and the rumen was separated soon thereafter. The entire rumen content for each lamb was emptied into a plastic bucket and mixed thoroughly and samples were strained through 4 layers of cheesecloth. From a sample, rumen pH was measured immediately with a portable pH meter (HANNA HI-8424, Charlton Scientific, Oxfordshire, UK). A sample was preserved with 25 % orthophosphoric acid (for analysis of volatile fatty acid, VFA), and another sample was preserved in 0.5 M sulphuric acid (for ammonia-N analysis). All samples were thereafter transported to the lab in cooler boxes until frozen at −20 °C.

2.4. Methane emission measurement

Following the digestibility trial, lambs were kept in the open-circuit respiratory chamber for in vivo methane measurement and methane was estimated as described by Storm et al. (2012). Each chamber was pre-calibrated with methane gas of known concentration and a recovery percentage was determined both at the beginning and the end of each cycle. These recovery percentages (78 %–107 %) was used as a correction factor to adjust the total volume of methane. To minimise the effect of wide variation associated with methane recovery, and across-chambers, animal effects, lambs within each block were rotated across the four chambers during the methane sampling periods.

During each cycle of methane measurement, the sheep were adapted to the chamber for 1 day before methane output measurement in the four subsequent days. In every cycle, four animals coming from the same block were included, i.e. one from each treatment group was placed in each chamber and every day, the lamb was rotated to a different chamber until all the animals had passed through the 4 different chambers. Animals were fed once daily and daily feed consumption was monitored to determine the intensity of methane produced while water was provided *ad-lib*. The chambers were cleaned and thereafter kept closed for about 22.5 h daily during which airflow speed was recorded automatically by the hot wire anemometers fitted with automatic data loggers. Samples of air flowing out from each chamber and the ambient air in front of each chamber over the 22.5 h were collected in deflatable Teflon balloons, using an 8-channel peristaltic pump (Masterflex 77292−50 L/S, Cole-Palmer Instr., IL, USA) at 5 min intervals for each chamber. From each balloon, 5 different samples of gas collected were analysed with a gas chromatography fitted with a flame ionization detector (8610C BTU Gas analyser GC System, SRI Instruments, Bad Honnef, Germany). The GC was equipped with a solenoid column packed with silica gel and a flame ionisation detector and regularly calibrated with analytical grade 100, 250, 500 and 1000 ppm methane in nitrogen gas (Portagas, Pasadena TX 77503, USA.). Gas samples were injected manually into the GC using a syringe and methane peaks were converted into concentration using standard curve generated with the Peak simple software.

2.5. Chemical analysis

Samples of feed offered, orts and faeces were analysed according to AOAC (2000) for dry matter (DM; ID 934.01), ash (ID 942.05) and crude protein (ID 968.06) using the Leco analyser (Leco TruMac N determinator Leco Corporation, St. Joseph, USA.). Urine-N was also analysed using the Leco analyser which had been appropriately maintained for the high sulphuric acid preservative in the urine samples. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) was determined according to the method of Van Soest et al. (1991). Heat stable alpha-amylase and sodium sulphite were incorporated in the NDF assay following the ANKOM filter bag technique. Acid detergent fibre (ADF) was also determined using the ANKOM filter bag technique, and both NDF and ADF expressed exclusive of residual ash.

Rumen fluid samples were analysed for pH (model PB-10/c, Sartorius, Germany), while samples for VFA analysis were thawed and centrifuged at 4500 rpm for 15 min at 4 °C and subsequently filtered using 0.45 μm microspore filter into a 2 ml GC vials. Samples were injected into gas chromatography (Shimadzu GC-2010 Tracera; Shimadzu corp., Kyoto, Japan) with Barrier Ionization Discharge (BID) detector and fitted with a 30 m Inert Cap Pure Wax column (df = 0.25 μm, I.D. = 0.25 mm). The operational conditions of the column include: carrier gas flow, 6.1 mL/min; initial temperature of 100 °C, injection port temperature of 250 °C, detector temperature of 280 °C; Helium gas flow at detector was 50 mL/min. Calibration standards were prepared using analytical grade acetic acid, propionic acid, isobutyric acid, isovaleric acid and valeric acid, purchased from Merck Millipore (Massachusetts 01803, USA). Six standard solutions were analysed before sample analysis, and the concentration of each VFA was calculated based on the peak areas generated with Lab solutions software. Ammonia nitrogen was analysed by using the phenol-hypochlorite reagent spectrophotometric procedures according to Broderick and Kang (1980).

2.6. Statistical analysis

Weekly body weight measurements were used to regress individual average daily gain (ADG) and feed consumption was averaged within each pen. The statistical model used included a block effect and treatment effect, which was further partitioned into the effects of NPN source and tannin inclusion, and interaction between NPN source and tannin inclusion. The model used for analysis was as follows:

$$y_{ijk} = \mu + \text{Block} + A_i + Bj + (AB)_{ij} + \varepsilon ijk$$

where y_{ijk} = observation k at different nitrogen source (i; urea or nitrate) and level j of tannin inclusion (j; no-tannin, with tannin); μ = overall mean; Block = effect of blocking (initial weight × sex); A_i = the effect of nitrogen source; Bj = the effect of tannin extract inclusion; $(AB)_{ij}$ = the effect of the interaction of nitrogen source with or without tannin inclusion, and εijk = random error with mean of 0 and variance σ^2. Data were analysed as a 2 × 2 factorial treatment combination in a randomised complete block design with replicates. Statistical analyses were performed using the General linear model procedure of SAS 9.4 (SAS Inst. Inc.; Cary, NC, USA). Where significant interaction was observed, the slice statement was used to compare the simple effects of tannin inclusion within each nitrogen source. Results are reported as the least significant means and standard error of means. Significant differences were declared when $P \leq 0.05$ and a tendency for significance at $0.05 < P < 0.10$.

3. Results

3.1. Effect of NPN source and tannin inclusion on intake, nutrient digestibility and nitrogen balance in growing merino lambs

There was no interaction effect between nitrogen source and tannin inclusion on DM, CP, NDF and ADF intake. Equally, nitrogen source did not affect DM, OM, NDF and ADF intake of lambs (Table 2). However, lambs on urea-containing diets consumed more CP (in g per head per day) compared to lambs consuming nitrate-containing diets ($P < 0.05$). Tannin inclusion did not affect DM, OM, CP, NDF, and ADF intake of lambs. Similar to nutrient intake, no significant interaction effect was observed between nitrogen source and tannin inclusion, on nutrient digestibility. While DM, OM, CP, and ADF digestibility was not affected by nitrogen source, NDF digestibility was affected ($P < 0.05$), with nitrate-containing diets showing higher NDF digestibility when compared to urea-containing diets. In contrast, the inclusion of Acacia tannin extract (ATE) in the diets reduced DM, OM, CP, NDF and ADF digestibility ($P < 0.01$).

There was no significant interaction between nitrogen source and tannin inclusion on nitrogen intake, nitrogen excretion and nitrogen retention in the experimental lambs (Table 3). The higher crude protein content of the urea-containing diet resulted in increased N-intake and N-excretion by the lambs. In the urea containing diet, while urine-N excretion (g/head/d) was higher ($P < 0.01$), only a tendency for increased faecal-N excretion (g/head/d) was observed ($P = 0.07$). However, the proportion of faecal-N and urine-N from total N-intake was not affected by the NPN source. Total retained-N expressed in g/head/d or g/kg N-intake was equally not affected by NPN source.

Tannin inclusion did not show any effect on N-intake and total N-excretion. However, while faecal-N excretion (g/head/d) was not affected by tannin inclusion, the faecal-N proportion of N-intake was increased by tannin inclusion ($P < 0.01$). In contrast, tannin inclusion reduced ($P < 0.05$) urinary-N excretion, with a tendency also for a reduced urinary-N proportion of N-in-

Table 2
Intake and apparent nutrient digestibility of lambs fed urea or nitrate-containing diets with or without Acacia tannin extract.

Parameters	Urea-containing diet		Nitrate-containing diet			[2]P-values		
	- tannin	+ tannin	- tannin	+ tannin	SEM[1]	N	T	N*T
Nutrient intake								
DM, g/d	1595	1484	1489	1419	57.7	0.46	0.43	0.86
DM, g/kg $BW^{0.75}$	86.5	80.2	75.6	76.9	2.96	0.27	0.69	0.55
OM, g/d	1481	1381	1370	1309	53.9	0.43	0.49	0.87
CP, g/d	335	330	282	266	13.19	0.05	0.37	0.88
NDF, g/d	499	489	565	486	23.98	0.50	0.34	0.47
ADF, g/d	296	298	321	265	14.04	0.89	0.33	0.28
Apparent nutrient digestibility, g/kg								
DM	673	598	654	626	8.7	0.73	<0.01	0.09
OM	683	604	670	638	9.0	0.42	<0.01	0.09
CP	799	745	789	740	7.8	0.50	<0.01	0.82
NDF	388	294	459	374	18.6	0.01	<0.01	0.85
ADF	358	235	405	234	21.9	0.45	<0.01	0.41

[1] SEM, standard error of mean.
[2] N, effect of nitrogen source; T, effect of tannin inclusion; N*T, interaction effect of nitrogen source and tannin inclusion.

Table 3
Nitrogen balance in growing lambs fed urea or nitrate-containing diets with or without Acacia tannin extract.

Parameter	Urea-containing diet		Nitrate-containing diet		[1]SEM	[2]P-values		
	- tannin	+ tannin	- tannin	+ tannin		N	T	N*T
Nitrogen (N)-intake, g/head/d	53.6	52.8	45.1	42.5	2.11	0.01	0.63	0.78
N-intake, g/kg $BW^{0.75}$/d	2.90	2.85	2.29	2.30	0.11	<0.01	0.93	0.86
N-excretion, g/head/d	39.2	37.6	32.6	30.4	1.23	<0.01	0.35	0.88
N-excretion, g/kg $BW^{0.75}$/d	2.13	2.05	1.66	1.66	0.08	<0.01	0.78	0.77
Faecal-N, g/head/d	11.4	13.6	9.5	11.1	0.65	0.07	0.12	0.82
Urinary-N, g/head/d	27.8	24.1	23.1	19.2	0.93	<0.01	0.01	0.96
Retained-N, g/head/d	14.3	15.2	12.4	12.1	1.12	0.21	0.88	0.76
Faecal-N, g/kg N-intake	212	255	211	260	7.31	0.85	<0.01	0.80
Urinary-N, g/kg N-intake	524	472	516	459	18.87	0.72	0.08	0.93
Retained-N, g/kg N-intake	264	273	273	282	16.09	0.76	0.74	0.99

[1] SEM, standard error of mean.
[2] N, effect of nitrogen source; T, effect of tannin inclusion; N*T, interaction effect of nitrogen source and tannin inclusion.

take (P = 0.08). Tannin inclusion did not influence overall N-retention or the proportion of N-intake that is retained. The proportion of dietary nitrogen retained ranged between 273–302 g/kg N-intake across the treatments.

3.2. Effect of NPN source and tannin inclusion on rumen fermentation characteristics in growing merino lambs

Lambs fed the urea-containing diets had a higher concentration of total VFA compared to lambs fed the nitrate-containing diets ($P \leq 0.05$) (Table 4). Tannin inclusion did not reveal any effect on ruminal pH and total VFA concentration in the lambs but re-

Table 4
Ruminal characteristics of growing lambs fed a urea or nitrate-containing diets supplemented with or without Acacia tannin extract.

[1]Parameter	Urea-containing diet		Nitrate-containing diet		[2]SEM	[3]P-values		
	- tannin	+ tannin	- tannin	+ tannin		N	T	N*T
pH	6.11	5.96	6.22	6.02	0.06	0.47	0.16	0.85
Ammonia, mg/dL	29.7	22.9	26.8	18.9	1.80	0.21	0.01	0.82
TVFA, mmol/L	117	130	111	112	2.97	0.05	0.23	0.26
VFA molar proportion, mol/100 mol								
Acetate	58.4	51.5	54.2	55.7	0.71	0.86	0.02	<0.01
Propionate	20.5	23.3	22.5	23.2	0.39	0.07	<0.01	0.05
Butyrate	15.5	18.8	17.5	15.8	0.37	0.40	0.19	<0.01
Branched-chain VFA	3.66	3.96	3.80	3.30	0.10	0.19	0.54	0.04
Valerate	1.95	2.39	1.98	2.00	0.06	0.02	<0.01	0.01
Acetate: propionate	3.03	2.25	2.46	2.43	0.09	0.18	0.01	0.01

[1] TVFA, Total volatile fatty acid; Branched chain VFA, Iso-butyrate + Iso-valerate.
[2] SEM, standard error of means.
[3] N, effect of nitrogen source; T, effect of tannin inclusion; N*T, interaction effect of nitrogen source and tannin inclusion.

duced rumen ammonia concentration ($P < 0.05$). The molar proportions of the respective VFAs revealed that there was a strong interaction between nitrogen source and tannin inclusion on acetate, propionate, butyrate, valerate, and branched-chain VFA proportion, as well as on the A/P ratio ($P < 0.05$). When tannin inclusion was compared within each nitrogen source, tannin reduced acetate proportion and increased propionate proportion in the urea-containing diet ($P < 0.01$), whereas, it had no such effect in the nitrate-containing diet. While tannin inclusion resulted in increased butyrate proportion in the urea containing diet, it reduced butyrate in the nitrate-containing diet ($P < 0.01$). In the urea-containing diet, valerate proportion was increased ($P < 0.01$) by tannin inclusion unlike in the nitrate-containing diet where tannin inclusion did not affect valerate proportion. Tannin inclusion did not affect the proportion of branched-chain VFAs in both urea-containing diet and the nitrate-containing diet. However, A/P ratio was reduced ($P < 0.01$) by tannin inclusion in the urea-containing diets, whereas, it had no such effect in the nitrate-containing diet.

3.3. Effect of NPN source and tannin inclusion on growth performance and enteric methane emission in growing merino lambs

The nitrate-containing diet improved ($P \leq 0.05$) average daily gain (ADG) by 20 % as compared to lambs fed the urea-containing diet (149 vs 182 g/head/d) while tannin inclusion reduced ADG ($P < 0.05$) (Table 5). Nitrogen source did not have any effect on feed conversion ratio but total weight gain showed a tendency to be higher in lambs consuming the nitrate-containing diets ($P = 0.09$). Tannin inclusion reduced the efficiency of feed utilization as shown by reduced ADG, total weight gain and increased feed conversion ratio ($P < 0.05$).

Animals on nitrate-containing diets produced 20 % less methane (g/d) compared to animals on the urea-containing diets ($P < 0.05$) (Table 6). Equally, in terms of methane intensity (g/kg of DM-intake and g/kg NDF-intake), nitrate reduced methane production by 17 % and 21 %, respectively. However, tannin inclusion did not significantly reduce methane production with only a marginal decrease of 4 % (g/d), and 7 % when expressed in g/kg DM-intake. When methane emission is related to ADG, methane production in lambs consuming urea-containing diets was 0.24 g/g weight gain and 0.16 g/g weight gain in lambs consuming nitrate-containing diets. In contrast, lambs consuming feed without tannin had methane emission of 0.18 g/g weight gain and 0.22 g/g weight gain in lambs consuming feed with tannin.

4. Discussion

In this study, both urea and nitrate-containing diets, the proportion of total nitrogen from NPN was 31 % and this was to ensure sufficient rumen nitrogen despite the potential binding of dietary proteins by tannin. Lower CP intake in animals consuming the nitrate-containing diets is due to lower CP concentration of diets (186 g/kg vs 207 g/kg) which was due to the slightly lower nitrogen concentration of the calcium nitrate salt. Despite this, the lack of differences in nutrient digestibility associated with NPN source observed in this study agrees with the report of Olijhoek et al. (2016). The astringency properties of CTs are well documented (Bhatta et al., 2002; Eckard et al., 2010). However, the extent to which it affects dry matter and other nutrient intakes in sheep and other ru-

Table 5
Body weight, average daily gain (ADG), and dry matter intake of lambs during growth trial, fed urea or nitrate-containing diets supplemented with or without Acacia tannin extract (n = 40).

Parameter	Urea-containing diet		Nitrate-containing diet		[1]SEM	[2]P-values		
	- tannin	+ tannin	- tannin	+ tannin		N	T	N*T
Dry matter intake, g/d	1121	1074	1194	1086	22.7	0.32	0.09	0.47
Initial body weight, kg	34.7	34.5	34.7	34.9	0.63	0.84	0.98	0.87
Final body weight, kg	47.0	43.8	49.3	45.7	0.98	0.25	0.09	0.92
Total weight gain, kg	12.8	9.87	15.2	11.5	0.72	0.09	0.02	0.73
Average daily gain, g/d	173	125	197	168	9.39	0.05	0.03	0.58
Feed conversion ratio	5.8	7.3	4.9	6.5	0.38	0.27	0.04	0.93

[1] SEM, standard error of mean.
[2] N, effect of nitrogen source; T, effect of tannin inclusion; N*T, interaction effect of nitrogen source and tannin inclusion.

Table 6
Methane emissions of growing lambs fed a urea or nitrate-containing diets supplemented with or without Acacia tannin extract in methane chambers.

[1]Parameter	Urea-containing diet		Nitrate-containing diet		[2]SEM	[3]P-values		
	- tannin	+ tannin	- tannin	+ tannin		N	T	N*T
Methane, g/d	34.6	34.2	28.8	26.4	1.04	< 0.01	0.35	0.51
Methane, g/kg $BW^{0.75}$/d	1.86	1.85	1.44	1.43	0.07	< 0.01	0.96	0.97
Methane, g/kg DMI	24.1	23.0	20.4	18.6	0.88	0.02	0.35	0.83
Methane, g/kg NDF-intake	72.3	63.2	54.0	52.5	2.75	< 0.01	0.23	0.39

[1] DMI, dry matter intake; NDF, neutral detergent fibre.
[2] SEM, standard error of mean.
[3] N, effect of nitrogen source; T, effect of tannin inclusion; N*T, interaction effect of nitrogen source and tannin inclusion.

minants is variable (Animut et al., 2008; Bhatta et al., 2002). Furthermore, crude *Acacia mearnsii* tannin contains some amount of hydrolysable tannin and other non-tannin phenolics which may influence its astringency and protein binding activity (Adejoro et al., 2019a, 2019b). While the prolonged consumption of the ATE did not reduce feed intake, the reduction in nutrient digestibility was significant and consistent with previous reports (Adejoro et al., 2019a, 2019b; Kamra et al., 2012; Molina-Botero et al., 2019) on the impact of CTs on rumen fermentation and nutrient degradability. Beauchemin et al. (2007) observed a significant reduction in crude protein digestibility whereas, no reduction in NDF and ADF digestibility was observed in cattle consuming quebracho CT up to 20 g/kg feed DM. Carulla et al. (2005) found a significant reduction in CP, NDF and ADF in animals consuming *Acacia mearnsii* CT at 25 g/kg DM. Condensed tannins are known to have varying affinity levels for feed protein, microbial protein and fibre (Beauchemin et al., 2007; Makkar, 2003).

Nitrogen balance was positive in all the treatments, and differences in N-retention was not affected by nitrogen source. This agrees with the report of Olijhoek et al. (2016) who did not observe any differences in N-retention as a result of urea or nitrate inclusion. The higher faecal N loss in urea-containing diet is attributable to higher N-intake. This is reflected in the faecal-N proportion of total N-intake, which was not significantly affected by the NPN source. Higher dietary nitrogen, particularly in the form of NPN, may increase urinary-N excretion (Minnee et al., 2018). Overall nitrogen retention was not affected by tannin inclusion in this study. While some studies (Grainger et al., 2009; Hristov et al., 2013) have reported improved utilization of N with dietary tannin inclusion as a result of protein bypass, Tiemann et al. (2008) as well as Stewart et al. (2019) did not observe such improvement. How well bypass protein is subsequently digested and absorbed in the hindgut may vary widely depending on the extent of protein binding and animal nutrient requirements among others (Waghorn, 2008). However, the result of this study agrees with previous reports that feeding tanniferous forages or tannin extracts could decrease rumen soluble protein degradation, increase protein flow to the small intestine and shift N-loss from urine to faeces (Adejoro et al., 2019a, 2019b; Stewart et al., 2019). The binding of tannin to faecal protein would ensure that there is a prolonged dissociation of the protein-tannin complex in the faeces before its nitrogen content is lost (Aboagye et al., 2018). This has been noted to ameliorate the intensity of nitrogen loss, unlike urinary-N which is a more volatile source of nitrate pollution in water bodies, and atmospheric nitrous oxide emission (Eckard et al., 2010). While nitrogen loss, irrespective of its form remains a concern in terms of the environmental footprint of livestock production, faecal-N loss at the expense of urinary-N loss is, therefore, more environmentally friendly.

The reduction in ammonia concentration is due to reduced protein degradation in the rumen as a result of tannin binding to dietary protein (Adejoro et al., 2018; Carulla et al., 2005; Waghorn, 2008). Equally, previous studies (Aboagye et al., 2018; Carulla et al., 2005) did not observe any effect of tannin on TVFA and the current study is in concurrence. However, reduced TVFA due to reduced cellulolytic activity and longer retention times have been reported (Priolo et al., 2013). Reduction in acetate proportion due to tannin consumption is related to a higher inhibitory effect of tannins on acetate forming bacteria, either by inhibiting them directly or by inhibiting the production of hydrogen (Castro-Montoya et al., 2011). This, coupled with increased propionate results in reduced A/P ratio (Beauchemin et al., 2007). This trend of reduced acetate and increased propionate was observable in animals consuming the urea-containing diets. This was, however, not significant in the nitrate-containing diets. The potential direct shift from propiogenesis due to competitive nitrate scavenging for reduction equivalents may have played a crucial role in this regard as reported in previous studies (Latham et al., 2016; Van Zijderveld et al., 2011). Reduced A/P ratio due to reduced acetate and higher propionate could be associated with reduced fibre digestion (Carulla et al., 2005) and this was observable in lambs consuming the urea-containing diets where NDF digestibility was lower compared to the nitrate-containing diets. Within the urea-containing diets, the effect of tannin inclusion in reducing A/P ratio also corresponded with reduced fibre digestion. Branched-chain VFAs were not affected by tannin in both urea and nitrate-containing diets. These VFAs are produced from the breakdown of amino acid skeletons by rumen microbes (Hassanat and Benchaar, 2013). The high concentration of NPN and fibre in the diets may have ensured that the nitrogen needs of the microbes are met and therefore minimising the breakdown of amino acid skeletons.

Animals on nitrate-containing diets had a higher average daily gain (ADG) compared to those on urea-containing diets even though dry matter intake (DMI) and feed conversion ratio (FCR) were not different. Similar to the current study, Olijhoek et al. (2016) did not observe any differences in dry matter intake when nitrate substituted urea in the diet of dairy cows, although reduced DMI associated with nitrate consumption has been related to the suppressive effect of nitrite on NDF digestibility or reduced palatability of nitrate-containing diets (Newbold et al., 2014). Equally, the consumption of nitrate with tannin-containing leaves did not reduce feed intake in sheep (Pal et al., 2015). Guo et al. (2009) had predicted that a more efficient microbial protein synthesis is likely to occur when nitrate replaces urea in ruminant diets. Equally, the potential energy loss in methane may have been shifted into energy for higher weight gain in lambs consuming the nitrate-containing diets as noted by previous studies (Hristov et al., 2013; Molina-Botero et al., 2019). The significant reduction in enteric methane production associated with nitrate diets confirms earlier findings reported in nitrate-fed sheep and cattle (Guyader et al., 2016; Nolan et al., 2010; Olijhoek et al., 2016). In these studies, nitrate supplementation reduced methane (g/d) by 23 % in sheep (Nolan et al., 2010) and by 16 % in dairy cows (Van Zijderveld et al., 2011). The inhibition of methane production can be attributed to a direct toxic effect of nitrate and nitrite on methanogens (Guyader et al., 2014; Latham et al., 2016). Furthermore, nitrate-induced anti-methanogenesis is related to a more favourable use of available hydrogen in nitrate reduction to ammonia and competing with the process of CO_2 reduction to methane.

The variability in methane response to tannin supplementation can be adduced, partly to the level of tannin inclusion (García et al., 2017). Dietary CT inclusion up to 20–50 g/kg of dry matter may be effective against methanogenesis (Jayanegara et al., 2012; Patra and Saxena, 2011), the inclusion level in this trial was 42 g ATE/kg feed DM. The complex characteristics of CTs like the extent of condensation, heterogeneity of functional groups and degree of polymerization are linked to the biological activity of tannins (Naumann et al., 2013). As noted by Beauchemin et al. (2007) a reduced digestibility of nutrient and especially CP, without a reduc-

tion in methane may indicate that the ATE has a greater affinity for feed protein than either microbial protein or microbial enzymes. In this study, methane production in relation to lamb growth reveals that tannin inclusion, rather than reduce methane per unit of weight gain, ~~it~~ increased it, whereas, nitrate reduced methane production per unit of weight gain in the lambs.

5. Conclusion

The use of calcium nitrate as replacement of urea in the diet of South African Mutton Merino lambs consistently reduced methane emissions and increased lamb growth performance. In contrast, the addition of ATE to the diet did not improve total–N retention or suppress methane but tended to reduce the proportion of urinary-N excretion.

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CRediT authorship contribution statement

Festus Adeyemi Adejoro: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Abubeker Hassen:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Abiodun Mayowa Akanmu:** Data curation, Writing - review & editing, Visualization. **Diego P. Morgavi:** Writing - review & editing, Visualization.

Declaration of Competing Interest

The authors declare that no competing interest has interfered with the conduct of the study and the result as presented.

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