The Effect of Varying Levels of Purified Condensed Tannins on Performance, Blood Profiles, Meat Quality and Methane Emission by Male Bapedi Sheep Fed a Grass Hay and Sheep Pellet-Based Diet

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Abstract

This study determined the effect of purified condensed tannin inclusion levels in a diet on production, haematological indices, blood biochemical components, meat quality and methane emission by yearling indigenous male Bapedi sheep on a grass hay and sheep pellet-based diet in a 28-day trial. The diets contained similar ($P>0.05$) nutrients but with different ($P<0.05$) purified condensed tannin supplementation levels. A complete randomized design was used. Twenty-four yearling male Bapedi sheep were assigned to four dietary treatments having different purified condensed tannin levels of 0 ($\text{GH}_{80}\text{P}_{20}\text{PCT}_0$), 30 ($\text{GH}_{80}\text{P}_{20}\text{PCT}_{30}$), 40 ($\text{GH}_{80}\text{P}_{20}\text{PCT}_{40}$) and 50 ($\text{GH}_{80}\text{P}_{20}\text{PCT}_{50}$) g/kg DM. A quadratic type of equation was also used to determine condensed tannin supplementation levels for optimal performance and methane emission reduction by sheep. Supplementing diets with purified condensed tannins did not affect ($P>0.05$) intake, digestibility and performance of male Bapedi sheep. Supplementing diets with purified condensed tannins had no effect ($P>0.05$) on blood components of male Bapedi sheep. Bapedi sheep meat pH and sensory attributes were not influenced ($P>0.05$) by purified condensed tannin level in the diet. However, supplementing diets with purified condensed tannins decreased ($P<0.05$) methane emission by 51 to 60%. A 49.08g supplementation level with purified condensed tannins per kg DM diet was calculated, with the use of quadratic equations, to result in the lowest methane emission by male Bapedi sheep. Meat of male Bapedi rams on diets containing 30, 40 or 50g of purified condensed tannins per kg DM contained higher ($P<0.05$) antioxidant activities than meat from rams fed a feed without purified condensed tannins. These results indicate that purified condensed tannin supplementation levels used did not have adverse effects on Bapedi sheep. However, supplementation levels of 30, 40 or 50g of purified condensed tannins per kg DM diet reduced methane emission by 51 to 60%, and increased sheep meat antioxidant activity values. Supplementing diets with purified condensed tannins has potential to reduce methane production and emission by sheep. However, long-term studies are recommended to ascertain the present findings.

Introduction

Bapedi sheep, an indigenous South African sheep breed, are nutritionally, economically and culturally very important in South Africa (Matlebyane, 2005). It is important for milk, meat, hide, and skin (Snyman, 2014). However, methane (CH_4) produced by ruminants adversely contributes to climate change (Lassey, 2007; Kaufmann et al., 2006). About 80 million tons of methane is produced annually, where 47% of those emissions is contributed by agriculture and 39% of that is emitted by ruminants through the digestive processes (Eckard et al., 2010; Gerber et al., 2013). Eighty-five percent of methane produced by ruminants is from enteric fermentation, with cattle, buffalos and small ruminants contributing 77, 13 and 10%, respectively (Gerber et al., 2013). Methane emission results in a 12% loss of energy intake by ruminants (Johnson and Johnson, 1995). Tannins in a diet inhibit ruminal ammonia and methane production during microbial fermentation (Carulla et al., 2005). Low tannin intake levels reduce methane production and emission without any adverse effects (Makkar et al., 2007). Various studies indicate that dietary tannins improve meat quality (Ngambu et al., 2012, 2013; Bakare and Chimonyo, 2011). However,

Mapiye et al. (2010) and Mapiye et al. (2009) observed no differences in meat quality of Nguni cattle fed diets differing in tannin inclusion levels. Tannins are anti-nutritional factors and, hence, when taken in large amounts they can be toxic to the animal (Olafadehan et al., 2014). Such effects are manifested in haematological and biochemical indices (Taiwo and Anosa, 1995). However, data on purified condensed tannin supplementation levels for optimal methane emission reduction, meat quality and blood profiles of Bapedi sheep is not extensive and conclusive. This study, therefore, focused on the effect of supplementation level of purified condensed tannins in a diet on production, haematological indices, blood biochemical components, meat quality and methane emission by yearling male Bapedi sheep fed a diet based on grass hay and sheep pellets. Such information is useful in formulating strategies for improving sheep production and reducing their methane emission.

Methodology

Experimental design, treatments and data collection

This experiment was done at the University Experimental Farm (latitude 27.55°S and longitude 24.77°E). The area is semi-arid, and the vegetation is mainly of grass species with mixtures of shrubs and trees. The shrubs are mainly tanniniferous *Acacia* species (Shiringani, 2007). Twenty-four yearling male Bapedi sheep, weighing 25 ± 1.6 kg, were assigned to four dietary treatments with three replicates and each replicate having two sheep. A completely randomized design was used. The diets had different purified condensed tannin supplementation levels of 0 (GH₈₀P₂₀PCT₀), 30 (GH₈₀P₂₀PCT₃₀), 40 (GH₈₀P₂₀PCT₄₀) and 50 (GH₈₀P₂₀PCT₅₀) g/kg DM (Table 1). The experimental diets were isocaloric and isonitrogenous. The experiment lasted for 28 days (21-day preliminary and 7-day collection periods). The sheep were accommodated in well-ventilated individual metabolic pens (1.4 m x 2.0m) with separate compartments for faecal and urine collections. Feed was offered *ad libitum*, allowing a 15% refusal (Kaitho et al., 1996). The purified condensed tannins were purchased from Bondite Limited, South Africa. The animals were offered *ad libitum* water and a mineral lick (Table 2). An ethical clearance certificate (Certificate No: AREC/07/2019:PG) was obtained before the start of the experiment.

Daily diet intakes and weekly live weights were determined during the collection period (McDonald et al., 2011). All faeces were collected and apparent nutrient digestibility determined during the collection period (McDonald et al., 2011). Blood was collected from each Bapedi sheep jugular vein on the 21 and 28 days of the trial. A hand-held methane detector was used to measure methane emissions (Chagunda et al., 2009). Measurements were taken at 8.00hrs in the morning. Methane emitted was reported as parts per million-meter (ppm-m) (Chagunda et al., 2009).

All the sheep were slaughtered at the end of the experiment and their meat was subjected to pH and sensory attribute evaluations (Pavelková et al., 2013) to determine carcass characteristics. Sensory attributes of the meat were done according to the methods of Pavelková et al. (2013).

Table 1
Dietary treatments for the study

Experimental Code	Treatment Description
GH ₈₀ P ₂₀ PCT ₀	A mixture of 80% grass hay and 20% pellets without any purified condensed tannins.
GH ₈₀ P ₂₀ PCT ₃₀	A mixture of 80% grass hay and 20% pellets plus 30g of purified condensed tannins/kg DM feed.
GH ₈₀ P ₂₀ PCT ₄₀	A mixture of 80% grass hay and 20% pellets plus 40g of purified condensed tannins/kg DM feed.
GH ₈₀ P ₂₀ PCT ₅₀	A mixture of 80% grass hay and 20% pellets plus 50g of purified condensed tannins/kg DM feed.

Table 2
Nutrient composition of the mineral block

Nutrients	Quantity
Protein (g/kg DM)	210
Urea (g/kg DM)	20
Crude fibre (g/kg DM)	99
Calcium (g/kg DM)	20
Phosphorus (g/kg DM)	5
Moisture (g/kg)	130
Sulphur (g/kg DM)	8
Potassium (g/kg DM)	12
Copper (mg/kg DM)	15
Manganese (mg/kg DM)	100
Zinc (mg/kg DM)	120
Cobalt (mg/kg DM)	0.5
Iodine (mg/kg DM)	3
Iron (mg/kg DM)	75
Selenium (mg/kg DM)	0.5
Vitamin A (IU/kg)	88.
Energy (MJ/kg DM)	8.8
Source: Voermol Maxi Block, South Africa	

Chemical Analysis

AOAC (2012) procedures were used to determine nutrient composition of the diets. A bomb calorimeter was used to determine gross energy of diets and faeces (AOAC, 2012). Butanol-HCl method was used to determine condensed tannins (Makkar et al., 1995). The methods of Natt and Herrick (1952) and Mukkur and Bradley (1974) were used to determine total white blood cells and lymphocyte population, respectively. The procedures outlined by Dacie and Lewis (2001) were used to determine haematocrit and haemoglobin concentrations. Red blood cells and white blood cells were determined by Neubauer haemocytometer. The methods of Valley et al. (1980) were used to determine serum protein. Blood glucose was determined by enzymatic colorimetric test kit (Quimica Clinica Applicada, SA Kit). An atomic absorption spectrophotometer (Model 490, Gallenkamp and Co. Ltd., London) was used to estimate

mineral contents in feeds and blood. The methods of Reitman and Frankel (1957) and Roy (1970) were used to measure the activities of the enzymes. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method was used to estimate antioxidant activities in meat (Sgherri et al., 2012).

Data analysis

One-way analysis of variance procedures of SAS version 9.1.3 (SAS, 2008) were used. $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} = overall observation, μ = overall means, T_i = treatment effect and e_{ij} = residual error. Differences between treatments were separated by LSD test ($P < 0.05$; SAS 2008). The quadratic equation was used to determine purified condensed tannin level for optimal methane reduction by Bapedi sheep (SAS, 2008):

$$Y = a + b_1x + b_2x^2 + e$$

Where y = overall observation; a = intercept; b_1 and b_2 = coefficients of the quadratic equation; x = value of purified condensed tannin inclusion; $-b_1/2b_2$ = purified condensed tannin supplementation level for optimal response; and e = error.

Results

The nutrient contents of the diets were similar ($P > 0.05$) except for purified condensed tannins ($P < 0.05$) (Table 3). Supplementation with purified condensed tannins increased ($P < 0.05$) condensed tannin contents of the diets.

Supplementing diets with purified condensed tannins did not influence ($P > 0.05$) intake, digestibility and growth of yearling male Bapedi sheep (Table 4). However, supplementing diets with purified condensed tannins decreased ($P < 0.05$) methane emission by male Bapedi sheep. A 49.08g supplementation level with purified condensed tannins per kg DM diet was calculated, with the use of quadratic equations, to result in the lowest methane emission by male Bapedi sheep ($Y = 23.882 + -0.58883978x + 0.006066x^2$; $r^2 = 0.999$). The supplementation levels of 0, 30, 40 or 50g of purified condensed tannins per kg DM diet used in this study did not affect ($P > 0.05$) haematological indices, blood metabolites and electrolytes of yearling male Bapedi sheep (Table 5). Supplementation with 0, 30, 40 or 50g of purified condensed tannins per kg DM did not affect ($P > 0.05$) Bapedi sheep meat pH and sensory attributes (Table 6).

The effects of supplementing diets with purified condensed tannins on antioxidant activity in meat of male Bapedi ram are presented in Table 7. Meat from male Bapedi rams on diets containing 30, 40 or 50g of purified condensed tannins per kg DM had better ($P < 0.05$) antioxidant activities than those from rams on a feed without purified condensed tannins. However, meat samples of male Bapedi rams fed diets having 30, 40 or 50g of purified condensed tannins per kg DM had the same ($P > 0.05$) antioxidant activities.

Table 3
Nutrient composition of diets

Nutrient (%)	Diet [#] *			
	GH ₈₀ P ₂₀ PCT ₀	GH ₈₀ P ₂₀ PCT ₃₀	GH ₈₀ P ₂₀ PCT ₄₀	GH ₈₀ P ₂₀ PCT ₅₀
Dry matter (%)	95 ^a ± 0.53	94 ^a ± 0.70	95 ^a ± 0.55	95 ^a ± 0.57
Organic matter (%)	86 ^a ± 0.52	86 ^a ± 0.07	86 ^a ± 0.44	86 ^a ± 0.45
Crude protein (%)	12.5 ^a ± 0.65	13 ^a ± 0.58	13 ^a ± 0.53	13 ^a ± 0.51
NDF (%)	45 ^a ± 0.48	45 ^a ± 0.03	45 ^a ± 0.25	45 ^a ± 0.21
ADF (%)	35 ^a ± 0.23	35 ^a ± 0.44	35 ^a ± 0.32	35 ^a ± 0.38
Fat (%)	2.8 ^a ± 0.31	2.8 ^a ± 0.25	2.8 ^a ± 0.13	2.8 ^a ± 0.26
Energy (MJ/kg DM)	18.3 ^a ± 0.26	18.4 ^a ± 0.19	18.2 ^a ± 0.21	18.7 ^a ± 0.32
Condensed tannins [‡]	0 ^d ± 0.00	30 ^c ± 0.01	40 ^b ± 0.03	50 ^a ± 0.02
* : Values presented as mean ± standard error (SE).				
a, b, c, d : Means in the same row not sharing a common superscript are different (P<0.05)				
# : Treatments were supplementation with 0 (GH ₈₀ P ₂₀ PCT ₀) 30 (GH ₈₀ P ₂₀ PCT ₃₀), 40 (GH ₈₀ P ₂₀ PCT ₄₀) or 50 (GH ₈₀ P ₂₀ PCT ₅₀) g of purified condensed tannins per kg DM				

diet.

[‡] : 0, 30, 40 or 50g of condensed tannins per kg DM diet.

Table 4

Effect of supplementing diets with purified condensed tannins on intake, digestibility, methane emission and performance of male Bapedi rams

Diet ^{#*}				
Variable	GH ₈₀ P ₂₀ PCT ₀	GH ₈₀ P ₂₀ PCT ₃₀	GH ₈₀ P ₂₀ PCT ₄₀	GH ₈₀ P ₂₀ PCT ₅₀
DM Intake (g/sheep/day)				
Dry matter	265 ± 63.89	338 ± 40.30	368 ± 40.48	287 ± 40.95
Organic matter	230 ± 52.77	297 ± 34.95	320 ± 41.70	250 ± 34.01
Crude protein	18.9 ± 8.21	25.5 ± 3.30	30.1 ± 4.29	25.0 ± 3.60
NDF	161 ± 29.15	195 ± 22.97	214 ± 26.51	166 ± 22.40
ADF	109 ± 30.95	128 ± 19.96	151 ± 23.67	110 ± 25.85
Fat	5.2 ± 0.90	5.1 ± 0.58	5.1 ± 0.77	5.1 ± 0.55
Energy intake (MJ/day)	5.6 ± 0.71	6.0 ± 0.68	6.5 ± 0.72	5.1 ± 0.69
Intake (g/kgW ^{0.75})				
Dry matter	35 ± 17.52	53 ± 3.81	55 ± 8.67	46 ± 10.78
Organic matter	33 ± 14.48	49 ± 7.53	50 ± 8.47	43 ± 10.04
Crude protein	4.7 ± 2.07	7 ± 0.24	7 ± 1.36	6 ± 1.47
NDF	16 ± 7.24	26 ± 2.89	26 ± 3.29	21 ± 5.01
ADF	11 ± 4.17	17 ± 2.58	17 ± 2.83	14 ± 3.36
Fat	1.1 ± 0.811	1.1 ± 0.741	2.2 ± 0.571	1.2 ± 0.712
Energy intake (MJ/kg W ^{0.75})	0.91 ± 0.091	1.1 ± 0.064	1.1 ± 0.085	1.1 ± 0.174
Digestibility (decimal)				
Dry matter	0.6 ± 0.15	0.6 ± 0.07	0.6 ± 0.08	0.4 ± 0.28
Organic matter	0.6 ± 0.16	0.6 ± 0.07	0.6 ± 0.09	0.4 ± 0.27
Crude protein	0.4 ± 0.35	0.4 ± 0.12	0.4 ± 0.13	0.2 ± 0.48
NDF	0.5 ± 0.20	0.4 ± 0.09	0.6 ± 0.11	0.2 ± 0.38
ADF	0.3 ± 0.29	0.3 ± 0.12	0.4 ± 0.15	0.1 ± 0.53

* : Mean ± standard error (SE)

a, b : Means in the same row not sharing a common superscript are significantly different

	Diet ^{#*}			
Fat	0.7 ± 0.52	0.4 ± 0.20	0.4 ± 0.29	0.4 ± 0.92
Energy	0.5 ± 0.18	0.5 ± 0.08	0.6 ± 0.09	0.4 ± 0.34
Methane emission (ppm-m)	23.9 ^a ± 2.80	11.5 ^b ± 1.02	10.3 ^b ± 1.04	9.5 ^b ± 1.02
Live weight (g/sheep/day)				
Initial	25.0 ± 0.67	25.1 ± 0.58	24.9 ± 2.03	24.8 ± 2.67
Final	25.7 ± 2.31	25.8 ± 0.33	25.6 ± 4.36	25.5 ± 6.00
Wt. gain(g/sheep/day)	25 ± 0.33	25 ± 0.88	25 ± 0.33	25 ± 0.60
Feed conversion ratio	11 ± 2.33	13 ± 1.8	14 ± 2.8	12 ± 1.33
* : Mean ± standard error (SE)				
^{a, b} : Means in the same row not sharing a common superscript are significantly different				

(P<0.05).

[#] : Treatments were supplementation with 0 (GH₈₀P₂₀PCT₀) 30 (GH₈₀P₂₀PCT₃₀), 40 (GH₈₀P₂₀PCT₄₀) or 50 (GH₈₀P₂₀PCT₅₀) g of purified condensed tannins per kg DM diet

Table 5
Effects of supplementing diets with purified condensed tannins on blood components of Bapedi rams

Variable	Diet ^{#*}			
	GH ₈₀ P ₂₀ PCT ₀	GH ₈₀ P ₂₀ PCT ₃₀	GH ₈₀ P ₂₀ PCT ₄₀	GH ₈₀ P ₂₀ PCT ₅₀
Red blood cell (x10 ¹² /L)	1.7 ± 4.91	2.4 ± 0.86	2.0 ± 0.78	2.0 ± 0.33
Haemoglobin (g/dL)	9.4 ± 0.92	9.9 ± 0.93	9.7 ± 0.95	8.4 ± 0.92
Haematocrit (L/L)	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03
White blood cell (x10 ⁹ /L)	18.5 ± 10.6	19.8 ± 6.54	29.6 ± 7.72	17.5 ± 6.89
Metabolites (mmol/L)				
Urea	3.3 ± 0.62	3.4 ± 0.58	4.03 ± 0.69	2.5 ± 0.92
Glucose	1.7 ± 1.02	2.8 ± 0.30	2.89 ± 0.21	3.0 ± 0.19
Cholesterol	3.3 ± 0.84	2.8 ± 0.18	2.42 ± 0.13	2.5 ± 0.12
Proteins (g/L)				
Total protein	75.0 ± 2.82	71.3 ± 2.54	68.7 ± 2.50	70.0 ± 2.50
Albumin	24.5 ± 1.97	26.0 ± 1.97	25.0 ± 2.01	28.0 ± 2.00
Enzymes (IU/L)				
Alkaline phosphate	63.0 ± 30.98	85.0 ± 31.20	108 ± 29.52	84.0 ± 30.45
Alanine transaminase	16.5 ± 2.39	18.7 ± 1.83	17.0 ± 1.75	19.7 ± 1.94
Aspartate transaminase	65.0 ± 7.46	69.0 ± 5.26	69.3 ± 6.30	73.3 ± 5.83
Electrolytes (mmol/L)				
Sodium	143 ± 1.32	145 ± 1.42	143 ± 1.56	146 ± 1.33
Potassium	7.6 ± 0.61	6.7 ± 1.61	7.6 ± 0.49	7.1 ± 0.49
Chloride	110 ± 1.72	111 ± 1.52	114 ± 2.41	112 ± 1.32
Total calcium	2.2 ± 0.06	2.2 ± 0.06	2.2 ± 0.06	2.2 ± 0.06
Inorganic phosphate	1.8 ± 0.15	1.8 ± 0.14	2.0 ± 0.15	1.9 ± 0.16

* : Mean ± standard error (SE).

: Treatments were supplementation with 0 (GH₈₀P₂₀PCT₀) 30 (GH₈₀P₂₀PCT₃₀), 40

(GH₈₀P₂₀PCT₄₀) or 50 (GH₈₀P₂₀PCT₅₀) g of purified condensed tannins per kg DM

Variable	Diet ^{#*}			
	GH ₈₀ P ₂₀ PCT ₀	GH ₈₀ P ₂₀ PCT ₃₀	GH ₈₀ P ₂₀ PCT ₄₀	GH ₈₀ P ₂₀ PCT ₅₀
Magnesium	1.0 ± 0.06	1.0 ± 0.06	1.0 ± 0.06	1.1 ± 0.07
* : Mean ± standard error (SE).				
# : Treatments were supplementation with 0 (GH ₈₀ P ₂₀ PCT ₀) 30 (GH ₈₀ P ₂₀ PCT ₃₀), 40 (GH ₈₀ P ₂₀ PCT ₄₀) or 50 (GH ₈₀ P ₂₀ PCT ₅₀) g of purified condensed tannins per kg DM				

diet.

Table 6

Effect of supplementing diets with purified condensed tannins on Bapedi ram meat attributes

Variable	Diet [#]			
	GH ₈₀ P ₂₀ PCT ₀	GH ₈₀ P ₂₀ PCT ₃₀	GH ₈₀ P ₂₀ PCT ₄₀	GH ₈₀ P ₂₀ PCT ₅₀
pH				
Breast	5.9 ± 0.11	6.0 ± 0.01	6.0 ± 0.01	5.9 ± 0.21
Rump	5.7 ± 0.02	5.6 ± 0.11	5.7 ± 0.01	5.7 ± 0.01
Shank	5.4 ± 0.02	5.4 ± 0.01	5.4 ± 0.01	5.3 ± 0.11
Shoulder	5.6 ± 0.11	5.6 ± 0.10	5.8 ± 0.11	5.7 ± 0.09
Meat colour (rump)				
Lightness	49.3 ± 0.96	47.7 ± 0.85	48.0 ± 0.77	49.0 ± 0.82
Redness	10.3 ± 0.10	8.7 ± 0.10	15.3 ± 0.10	14.3 ± 0.10
Yellowness	5.7 ± 0.95	5.8 ± 0.81	5.7 ± 0.71	6.3 ± 0.90
Meat Attributes				
Tenderness	3.7 ± 1.53	4.3 ± 1.58	4.0 ± 1.30	3.3 ± 1.15
Juiciness	3.3 ± 0.58	3.0 ± 1.00	3.3 ± 0.58	3.3 ± 0.58
Flavour	3.6 ± 0.33	3.7 ± 0.33	3.7 ± 0.33	3.7 ± 0.33
Overall acceptability	4.0 ± 0.02	4.0 ± 0.08	4.0 ± 0.03	4.0 ± 0.03
Shear force	29.7 ± 4.04	32.5 ± 2.30	31.8 ± 1.42	29.9 ± 2.06
[‡] : Mean ± standard error (SE)				
[#] : Treatments were supplementation with 0 (GH ₈₀ P ₂₀ PCT ₀) 30 (GH ₈₀ P ₂₀ PCT ₃₀), 40 (GH ₈₀ P ₂₀ PCT ₄₀) or 50 (GH ₈₀ P ₂₀ PCT ₅₀) g of purified condensed tannins per kg DM				

diet.

Table 7
Antioxidant activities in meat extracts of male Bapedi rams using DPPH assay

Treatment*	Antioxidant activity [#]
GH ₈₀ P ₂₀ PCT ₀	20.6 ^b ±1.89
GH ₈₀ P ₂₀ PCT ₃₀	31.1 ^a ±2.99
GH ₈₀ P ₂₀ PCT ₄₀	36.1 ^a ±2.96
GH ₈₀ P ₂₀ PCT ₅₀	31.2 ^a ±2.98
a, b : Means in the same column not sharing a common superscript are significantly	

different (P<0.05).

* : Treatments were supplementation with 0 (GH₈₀P₂₀PCT₀) 30 (GH₈₀P₂₀PCT₃₀), 40 (GH₈₀P₂₀PCT₄₀) or 50 (GH₈₀P₂₀PCT₅₀) g of purified condensed tannins per kg DM diet.

: Mean ± standard error (SE).

Discussion

Diets contained similar protein and energy levels of 13% and 18MJ/kg DM, respectively. The diets met recommended nutrient requirements for slow-growing sheep (NRC, 2007). Diets had similar nutrient contents except purified condensed tannins ranging from 0 to 50g/kg DM. These diets were, therefore, appropriate for the determination of condensed tannin levels for optimal performance and reduced methane emission in yearling male Bapedi sheep. It was expected that the diets would have similar intake and digestibility values (McDonald et al., 2011) provided that condensed tannin supplementation did not have any adverse effects. Indeed, supplementing diets with purified condensed tannins did not affect intake, digestibility and weight gain of male Bapedi sheep. It is possible that the purified condensed tannin amounts were not too high to adversely affect intake, digestibility and weight gain of male Bapedi sheep. Al-Dobaib (2009) also reported that lambs fed diets having different levels of quebracho tannins had similar intakes and weight gains. However, other studies showed a decrease in intake and digestibility of nutrients when condensed tannins were incorporated into the ruminant diets (Makkar, 2007). The binding ability of tannins to microorganism cell walls adversely affects enzyme secretion and nutrient transportation within the organisms (McSweeney et al., 2001); thus, nutrient digestibility decreases because rumen microorganisms are not able to optimally ferment the diets. However, other studies found that condensed tannin inclusions in the diets had beneficial effects on intake and weight gain of ruminant animals (Min et al., 2003; Soltan et al., 2012).

Diet supplementation with purified condensed tannins reduced methane emission by Bapedi rams, by 51 to 60%. A 49.08g supplementation of purified condensed tannins per kg DM diet was calculated, with the use of quadratic equations, to optimize methane emission reduction by male Bapedi sheep. This indicates that condensed tannin levels used in the study were enough to inhibit methanogenesis (Makkar et al., 2007). Carulla et al. (2005) observed a 12% reduction in methane emission when sheep were fed diets having different levels of condensed tannins. The authors indicated that tannins reduced methane production by decreasing synthesis of acetate during digestion. Woodward et al. (2001) reported a 20 to 24% decrease in methane emission when sheep were fed diets containing condensed tannins. The present study indicates that purified condensed tannin supplementation levels of 30, 40 and 50g per kg DM diet resulted in similar methane emission levels by sheep; possibly meaning that the levels used were within the required effective amounts for inhibition of methanogens in sheep. Mbanzamihigo et al. (2002) observed similar methane emission amounts by sheep when fed diets with tanniniferous legume supplementation levels of 0.16, 0.35, 0.41 or 0.51g/kg DM.

Nutritional problems can be detected in the blood of animals (Ajao, 2013). In the present study, purified condensed tannin supplementation levels of 0, 30, 40 or 50g per kg DM did not affect blood parameters of male Bapedi sheep. Amounts of purified condensed tannins used induced no adverse effects in the animals. Haemoglobin values observed are normal for a health sheep (Daramola et al., 2005; Akinyemi et al., 2010; Akinrinmade and Akinrinde, 2012). The present results findings are like those of Dey et al. (2008) and Pathak et al. (2013). Purified condensed tannin supplementations did not affect haematocrit values of male Bapedi sheep, indicating that the animals were not anaemic (Purves et al., 2003; Ndlovu et al., 2007). Solaiman et al. (2010) and Brown et al. (2016) observed similar results in goats on diets differing in tannin amounts.

There were no significant variations in white blood cell (WBC) counts of Bapedi sheep on diets differing in purified condensed tannin supplementation levels, indicating that the animals were in good health (Ahamefule et al., 2008; Solaiman et al., 2010). Brown et al. (2016) observed similar results in Bapedi goats on diets differing in tannins.

Purified condensed tannin supplementation did not affect blood urea values of male Bapedi sheep. The blood urea values are within the range for a health sheep (Daramola et al., 2005; Akinyemi et al., 2010). Serum glucose values of male Bapedi sheep were not affected by dietary supplementation with purified condensed tannins, and they are within the normal range for a health sheep (Naumann et al., 2017; Pathak et al., 2013; Midaoui and de Champlain, 2005).

Blood cholesterol values were similar among Bapedi sheep fed diets with different levels of purified condensed tannins. These cholesterol levels, ranging from 2.42 to 3.3 mmol/L, were within the normal values for a healthy sheep (Naumann et al., 2017; Siri-Tarino et al., 2010). Olafadehan et al. (2014) observed similar cholesterol values when goats were on diets high in condensed tannins. Supplementation with purified condensed tannins did not affect blood protein indices of male Bapedi sheep. The blood protein indices were normal for a healthy sheep (Naumann et al., 2017; Jain, 1986).

Purified condensed tannin inclusion in the diet had no effect on serum enzyme levels of Bapedi rams, suggesting that the animals were in good health (Thapa and Walia, 2007). Dey et al. (2008) observed no effects of tanniniferous *Ficus infectoria* leaf meal supplementation on aspartate transaminase and alanine transaminase levels in lambs. Purified condensed tannin supplementation levels did not affect blood electrolytes and mineral values of Bapedi rams. The electrolytes and mineral values obtained were normal for healthy sheep (Naumann et al. (2017), possibly meaning that the amounts used did not affect mineral absorption in the gut of the animals (Naumann et al. (2017; Sowande et al., 2008).

Condensed tannin supplementation did not adversely affect male Bapedi meat pH, sensory attributes and shear force values. The amounts 0, 30, 40 or 50g of purified condensed tannins per kg DM diet did not affect the end products of digestion and hence no differences in meat attributes of the sheep were expected. Dentinho et al. (2020) observed similar results when lambs were fed diets having different tannin levels. Similarly, Brown and Ngámbi (2018) observed no differences in goat meat attributes when diets differing in *Vachelia karroo* leaf meals were fed to goats. However, Luzardo et al. (2019) and Luciano et al. (2009) observed that tanniniferous feeds improved meat colour of sheep. Gesteira et al. (2018) concluded that lamb meat tenderness decreased and shear force values increased as dietary tannin levels increased. Kobue-Lekalake et al. (2009) and Priolo et al. (2000) observed that meat of lambs on diets with lower tannin levels had better taste. The present study indicates that purified condensed tannin supplementation levels did not affect Bapedi sheep meat pH values which were within the range for a healthy sheep (Mostert, 2007). Priolo et al. (2002), also, observed that tannin supplementation had no effect on sheep meat pH values. However, other authors reported increased meat pH values when sheep were supplemented with purified condensed tannins (Ngambu et al., 2013).

Purified condensed tannin supplementation levels of 30, 40 or 50g/kg DM diet increased antioxidant activity values in ram meat. Sufficient uptake of dietary antioxidants has been observed as an effective method to prevent lipid oxidation and avoid oxidation of cells and tissues caused by excess free radicals and oxidative stress (Negukhula, 2010). High antioxidant activity values in meat increase the quality and shelf-life of meat by reducing lipid oxidation (Fernandez-Gines *et al.*, 2005; Karami et al., 2011; Juarez *et al.*, 2012; Kasapidou et al., 2012; Karre et al., 2013). Marume et al. (2012) found that Diet supplementation with tanniniferous *Acacia karroo* leaf meal improved goat meat sensory attributes (Marume et al., 2012). Velasco and Williams (2011) also reported that chicken meat sensory attributes can be improved by inclusion of antioxidants in the diets.

Conclusion

Supplementing diets with 0, 30, 40 or 50g of purified condensed tannins per kg DM did not affect intake, digestibility and growth of yearling Bapedi rams, possibly indicating that the amounts of tannins used had no adverse effects on the animals. This can be attributed to the fact that the diets were iso-energetic and iso-nitrogenous. The supplementation levels of 0, 30, 40 or 50g of purified condensed tannins per kg DM diet did not affect haematological parameters, blood metabolites and electrolytes, possibly indicating

that the levels used were not toxic to the rams. However, long term experiments are suggested to ascertain the present findings.

Supplementing diets with 30, 40 or 50g of purified condensed tannins per kg DM reduced methane emission by Bapedi rams by 51 to 60%. A 49.08g supplementation level with purified condensed tannins per kg DM diet was calculated to optimize methane emission reduction by Bapedi rams. These results indicate that condensed tannin levels used in the study were enough to inhibit methanogenesis and hence reduce methane production and emission. However, further long-term studies are suggested to ascertain the ruminal pathways. Purified condensed tannin supplementation levels of 0, 30, 40 or 50g of purified condensed tannins per kg DM did not adversely affect sheep meat pH and sensory attributes. However, dietary supplementation levels of 30, 40 or 50g of purified condensed tannins per kg DM increased antioxidant activity values in ram meat, possibly indicating improved meat shelf-life (Karre et al., 2013).

It is concluded that condensed tannin supplementation levels of 0, 30, 40 or 50g/kg DM diet did not affect diet digestibility, growth and Bapedi ram meat sensory attributes. However, condensed tannin supplementation levels used in this study increased antioxidant activity values in male Bapedi ram meat. Long term studies are suggested to ascertain the present results.

Declarations

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Conflicts of Interest: No conflict of interest.

Ethics approval: The study was ethically cleared (Certificate No: AREC/07/2019:PG).

Consent to participate: Not applicable.

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Code of availability: N/A

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