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# Effects of dietary supplementation with *Acacia mearnsii* tannin extract on carcass characteristics and meat quality of lambs

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## Abstract

**Background** Plant extracts are used as possible methane mitigants and to replace antibiotic feed supplements previously used prophylactically to improve the adaptation of lambs in intensive feeding systems. This study investigated the effects of *Acacia mearnsii* tannin extract used as anti-methanogenic feed additives on carcass and meat quality of lambs.

**Methods** Forty Dohne Merino lambs, with an initial mass between 23.90 kg and 37.40 kg, were first stratified according to their initial body weight and thereafter one of the eight lambs with similar body weight were randomly assigned to four treatment groups (n = 10 lambs/treatment). The lambs were fed ad libitum with a basal total mixed ration. Four experimental diets were formulated: TMR diet (control); TMR diet with Rumensin® at a dosage of 75 mg/kg of DM feed (Monensin; Positive control); TMR diet with raw condensed Acacia tannin at 20 g/kg of DM feed (Crude tannin); TMR with addition of encapsulated condensed Acacia tannin at 20 g/kg of DM feed (Encapsulated condensed tannins). The lambs were slaughtered at a live mass of about 50 kg after a 19 week trial period.

**Results** Dietary tannin additives did not affect the carcass composition or colour aspects of lamb meat. However, there were minor changes in fatty acid profiles, particularly in the intramuscular adipose tissue. The addition of encapsulated tannin extract resulted in a higher proportion of C18:2n6t (0.15% ± 0.03 *vs.* 0.19% ± 0.03; $p < 0.05$), C18.3n3 (0.24% ± 0.04 *vs.* 0.29% ± 0.04; $p < 0.05$), C22:6n3 (0.02 ± 0.01 *vs.* 0.05 ± 0.03; $p < 0.05$), in comparison to monensin. This improvement in fatty acid profiles is presumably beneficial for human health, but it could affect the sensory quality of meat.

**Conclusion** Our results suggest that *Acacia mearnsii* tannin extracts can be included as anti-methanogenic feed additives in lamb diets without compromising product quality.

**Keywords** Plant extracts, Fatty acids, Anti-methanogenic feed additives, Condensed tannins, Colour stability, Meat quality

## Background

Ionophores such as monensin are widely used in the livestock industry to improve feed efficiency and reduce enteric methane emissions (Almeida et al. 2021 and Martin et al. 2010). However, these antibiotic feed supplements are relatively expensive, and their efficacy may decrease after long-term use (Almeida et al. 2021). Moreover, there is a growing concern about the use of antibiotic feed supplements such as monensin in livestock

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which increase the risk of antibiotic resistance in both animals and humans. Thus, many plant additives have been studied as possible methane mitigants to replace antibiotic feed supplements (Akanmu et al. 2020).

Tannin-containing plants are being used as inexpensive and safe approaches to replace antibiotic feed supplements and as methane mitigating agents (Kuralkar and Kuralkar 2021). *Acacia mearnsii* (Mimosa or Black wattle tannin) is a rich source of both condensed and hydrolysable tannins (Bhatta et al. 2009) and it is widely distributed in Southern Africa. Feeding tannin-rich plants such as *Acacia mearnsii* can have positive effects on the animals through their antioxidant, antimicrobial, antiparasitic, antimutagenic, and anti-inflammatory properties (Kuralkar and Kuralkar 2021).

Research revealed that tannins can be successfully used to manipulate rumen fermentation and reduce rumen methane production, possibly, through their antimicrobial effect on rumen microbes (Ibrahim and Hassen 2022). However, feeding strategies that alter rumen fermentation patterns can affect rumen biohydrogenation which in turn affects the deposition and composition of fat and subsequent carcass and meat quality (Amin and Mao 2021). Several studies have been conducted to evaluate the effects of dietary supplementation with tannin extracts and tannin-rich plants on carcass characteristics, physicochemical quality, sensory properties, and fatty acid composition of lamb meat. However, the results are still not consistent. For example, Fernandes et al. (2021) reported that natural tannins from *Mimosa tenuiflora* could be used in sheep diets to improve carcass fat deposition, physicochemical and sensory properties, and fatty profiles of lamb meat. Other studies have shown that the dietary inclusion of tannins in lamb diets does not have beneficial effects on carcass composition, meat quality, and fatty acid composition of lambs (Dentinho et al. 2020, Guerreiro et al. 2020 and Biondi, et al. 2019). In contrast, Buccioni et al. (2015) reported a slight increase in saturated fatty acids at the expense of monounsaturated fatty acids in milk (and presumably meat) from ewes fed diets supplemented with chestnut tannin extract, which might be detrimental to human health.

Before implementing any new technology into commercial practice, it is important to investigate its effect on the entire production cycle including the potential adverse effects on product composition and quality. In this study, we investigated the effect of *Acacia mearnsii* tannin extracts as a feed additive on the carcass and meat quality of lambs. We tested the effect of tannin extracts both in crude form and encapsulated in oil. Encapsulating condensed tannin extracts is a promising technique for improving their bitterness and astringent sensation (Ibrahim and Hassen 2022), which might broaden their use in the livestock industry. We hypothesise that supplementation of lamb diets with *Acacia mearnsii* tannin extracts does not have negative effects on the carcass composition, visual quality, and meat fatty acid composition of lambs.

## Materials and methods

The study is part of a project on enteric methane mitigating strategies through the addition of *Acacia mearnsii* tannin extracts to high forage-based diets using sheep as the model animal. The preparation of the *Acacia mearnsii* tannin feed additives used in the present study is described by Ibrahim and Hassen (2022).

### Management of experimental animals

A total of 40 Dohne Merino weaned ram lambs (approximately 96 days old) with an average mass of 31.93 ± 3.92 kg were used in a completely randomised block design. The lambs were stratified according to their initial mass ranging from 23.90 kg to 37.40 kg. Eight lambs were taken at a time from each block and from these the first 4 lambs with similar body mass were randomly allocated to one of the four pen (2 lambs per pen) within a block that received one of the four experimental dietary treatmens. This was repeated for the remaining lambs within a block in order to house 2 lambs per oen. A completely randomised block design was used to assign the lambs to a total of 20 pens. There were four dietary treatments and thus each dietary treatment diet had 10 lambs assigned to it as replicates. The pens were in covered house measuring 3.2 × 2.2 m, with five pens of two lambs per treatment. The pens were considered experimental units, and the two sheep in each pen were the observational units. The lambs were kept at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa.

A total mixed ration (TMR) was formulated by a commercial feed company (Afgri Feeds Ltd, South Africa) using Agricultural Modelling and Training System (AMTS) programme which uses the National Research Council (2007) standard. The TMR used was formulated to support an average daily gain (ADG) of approximately 250 g/head/day following the recommendations of the Agricultural Research Council of South Africa (1980). The formulation and chemical composition of the TMR is presented in Table 1.

All experimental animals received the same total mixed ration (TMR) during the experimental period. The following experimental treatments were formed:

- TMR only (Control);

**Table 1** Formulation and chemical composition on DM basis of the total mixed ration

| **Ingredient** | **Composition (%)** |
|---|---|
| Yellow maize | 28.0 |
| Eragrostis curvula hay | 22.2 |
| Alfalfa hay | 20.0 |
| Soybean meal | 17.0 |
| Molasses | 6.0 |
| Wheat | 5.0 |
| Urea | 0.8 |
| Vitamin premix | 0.5 |
| ***Parameter*** | ***Chemical composition*** |
| Dry matter (%) | 89.7 |
| CP (%) | 17.2 |
| Ash (%) | 6.50 |
| Starch (g/kg | 64.9 |
| NDF(g/kg) | 340 |
| ADF (g/kg) | 242 |
| Lignin (g/kg) | 24.6 |
| ME (MJ/kg) | 9.1 |

- TMR with the addition of Rumensin (containing the active ingredient Monensin) at 75 mg/kg of feed (Monensin treatment, Positive control);
- TMR with the addition of raw condensed Acacia tannin at 20 g/kg of feed (Crude tannin treatment);
- TMR with the addition of encapsulated condensed Acacia tannin at 20 g/kg of feed (Encapsulated condensed tannin treatment)

The lambs from all treatment groups were fed ad libitum and clean water was also available ad libitum. Feed intake was calculated by subtracting the amount of refused feed from the feed offered the day before. A random sample was collected from the amount of feed offered each day as a retention sample for feed analysis later. The initial body mass of the animals was recorded for three consecutive days before the start of the experiment and thereafter at seven-day intervals before the morning feeding until the end of the experimental period. The final mass of the animals was also recorded for three consecutive days before the morning feeding. The lambs were reared over a 19 week trial period to a slaughter mass of about 50 kg (7–8 months old). The data on adaptation period, feed intake, nutrient digestibility, ADG, and methane measurement were documented by Ibrahim and Hassen (2022). The lambs were slaughtered when they reached a marketable mass of 50 kg and the meat samples collected were used for the current study.

### Slaughter and sampling procedure

The lambs were slaughtered according to standard abattoir procedures. Carcasses were classified using the South African Carcass Classification System for beef, sheep, and goat carcasses (Webb 2015). This carcass classification system classifies carcasses based on their physical and compositional attributes, which include age (age categories: A, AB, B, and C), carcass fatness (carcass fat codes: 1 to 6), carcass conformation (carcass conformation codes: 1 to 5) and damage (1 to 3) (Webb 2015). The carcasses were then chilled at 4 °C for 24 h.

After 24 h in the chilling room, the carcasses were transferred to the laboratory for dissection under refrigerated conditions. A three-rib sample was cut from the 8th, 9th, and 10th lumbar vertebrae on the left side of each carcass, the ventral extremity of the sample being on a line drawn from the pubic symphysis to the middle of the first rib to obtain an estimate whole carcass composition (Casey et al. 1988). The three-rib cut samples were vacuum-packed and stored in the freezer at − 20 °C until further analysis. Subcutaneous fat and intramuscular fat samples of approximately 5 g each were dissected from *Longissimus* muscle and stored in polythene bags at − 20 °C for fatty acid analysis (Casey et al. 1988).

### Carcass composition and meat colour analysis

The three-rib cut samples were left to bloom at room temperature and then dissected into individual rib cuts

for colour analysis. Meat colour analysis was done using the Konica Minolta CM-600d colour measuring spectrophotometer. The colour readings (L*, a* and b*) were taken from the same side of each *Longissimus* muscle from each sample in triplicate. Hue angle (H*) was calculated as follows:

$$H^* = \tan^{-1}\left(b^*/a^*\right) \times \left(180/\pi\right)$$

The meat samples were individually vacuum packed and stored at 4 °C for 7 days re-analysed for colour to determine the treatment effect on colour stability. The three-rib samples were dissected into meat, fat and bone to obtain an estimate of the total carcass composition (Casey et al. 1988).

### Ether extract

Ether extract of the *Longissimus* muscle samples was determined with the method used by the Association of Official Analytical Chemists (AOAC 2000). The method involved boiling about 1 g of freeze-dried meat samples in petroleum ether for two hours and then oven drying until all the petroleum ether had evaporated. Thereafter, the samples were weighed and expressed as a percentage of the whole sample.

### Meat fatty acid analysis

The lipid extraction procedure and determination of fatty acid methyl esters were described by Webb and Casey (1995). The lipid extraction method by Ways and Hanahan, (1964) was used with some modifications of the chloroform: methanol (2: 1, v/v) proportion in the method (Folch et al. 1957). Butylated hydroxytoluene (2.6 DI-tertBUTYL-P-CRESOL) was included as antioxidant. Methyl esters of the fatty acid component of the neutral triglycerides were prepared according to the NaOH/methanol method (AOAC 1975). These esters were separated on a polar phase SP2330 column (2 m X 3 mm, packed with Silar 1OC coated on Gas Chrom Q) fitted to a Varian 3700 gas chromatograph with a flame ionization detector as previously described by Webb et al. (1994). Profiles of the cis–trans fatty acids from subcutaneous adipose tissue were obtained from fat samples that were treated with n-Hexane at 35 °C for 24 h, after which the fatty acids were esterified according to the method of Van Wijngaarden (1967). The cis–trans fatty acids isomers were then separated on a SP2560 fused silica capillary column (100 mX 0.2 mm) fitted to a Varian 3700 gas chromatograph (Webb et al. 1994). Standards for the fatty acids were obtained from Nu-Chek-Prep. Inc. (Elysim, MI, USA). Fatty acids were expressed in both normalised (molar proportion) and gravimetric (milligrams per gram of fresh tissue) formats (Huerta-Leidenz et al. 1993).

### Statistical analysis of data

Data was recorded in Microsoft Excel and checked for errors. The data on carcass characteristics, meat colour, and meat fatty acids were first tested for normality and homoscedasticity with the Shapiro–Wilk and Levene's tests, respectively. Statistical analysis was performed using the General Linear Model (GLM) ANOVA procedure in Genstat, and the model included the treatment effect. Differences were considered significant at $P < 0.05$ and a tendency for significance at $0.05 < P < 0.10$. A Tukey 95% confidence intervals test was performed to determine the differences between treatment means.

## Results

### Carcass characteristics

The results of the carcass characteristics of the control lambs and those fed diets supplemented with *Acacia mearnsii* tannin extracts are presented in Table 2. In terms of tissue composition, estimated by the three-rib cut, the average meat, fat, and bone proportions were 44.1%, 40.4%, and 15.5%, respectively. The average

**Table 2** The effects of supplementing the diet with *Acacia mearnsii* tannin extracts on carcass composition of South Africa Dohne Merino ram lambs

| Parameter | Control | Monensin[a] | CT[b] | ECT[c] | SEM[d] | *p*-value |
|---|---|---|---|---|---|---|
| Slaughter mass (kg) | 52.4 | 52.1 | 52.6 | 51.8 | 1.78 | 0.98 |
| Fat % | 41.5 | 40.4 | 39.7 | 40.0 | 1.47 | 0.79 |
| Bone % | 14.8 | 15.8 | 14.8 | 16.5 | 0.61 | 0.09 |
| Muscle % | 43.8 | 43.8 | 45.5 | 43.5 | 1.36 | 0.73 |
| IMF[e]% | 22.2 | 23.1 | 23.1 | 20.5 | 0.97 | 0.09 |

[a] Monensin: @ 75 mg/kg DM of feed

[b] CT: Crude Tannin @ 20 g/kg DM of feed

[c] ECT: Encapsulated tannin @ 20 g/kg DM of feed

[d] SEM: Standard error of means

[e] IMF: Intramuscular fat

intramuscular fat percentage was 22.2%. There were no significant differences ($P > 0.05$) in slaughter mass, the proportions of meat, fat, and bone and intramuscular fat percentage across the four treatment groups.

### Meat colour and colour stability

The results of meat colour and colour stability of the control lambs and those fed diets supplemented with *Acacia mearnsii* tannin extracts are presented in Table 3.

Regardless of dietary treatment meat lightness (L*) values did not change ($P > 0.05$) with increasing duration of storage. However, the redness (a*) values decreased ($P < 0.01$) over the 7-day storage period, across all treatment groups. Treatment effects were significant on the yellowness (b*) values, both on day 1 ($P = 0.047$) and day 7 ($P = 0.01$) of storage. Monensin treatment group had higher b* values than encapsulated condensed tannin and control treatment groups on day 1 and day 7 of storage, respectively. A similar trend was observed for hue angle (H*) values. Across all four treatments, the yellowness (b*) values did not change significantly over the 7 day storage period, although the b* values tended ($P = 0.08$) to decrease rapidly in the control treatment. The hue angle (H*) values increased (shifted from red to yellow) over storage time in all treatment groups ($P < 0.01$), except in the control group ($P > 0.05$).

### Meat fatty acid profiles

The molar proportions of fatty acids in the subcutaneous and intramuscular fat tissues of lambs are presented in Tables 4, 5, respectively. Saturated fatty acids (SFA) comprised about half the total fatty acids. There was no treatment effect on the proportion of any SFA in subcutaneous adipose tissue. However, dietary treatment affected the proportion of C21:0 ($p = 0.03$) in the intramuscular fat tissue, but the means were not significantly different.

The molar proportion of monounsaturated fatty acids (MUFA) accounted for about 42.2 to 46.5% of total fatty acids. The inclusion of tannin extract did not affect the proportion of MUFA in both subcutaneous and intramuscular fat depots.

Total polyunsaturated fatty acids (PUFA) accounted for about 2 to 5% of total fatty acids. Dietary tannin additives did not affect the proportions of PUFA in subcutaneous fat tissue, but minor changes were observed in

**Table 3** The effects of supplementing the diet with *Acacia mearnsii* tannin extracts on colour coordinates of the *Longisssimus* muscle of South African Dohne Merino ram lambs on day 1 and day 7 of storage at 4 °C

| Colour coordinate | Treatment | Day 1 | Day 7 | % Difference | SEM[a] | *p*-value |
|---|---|---|---|---|---|---|
| L* | Control | 38.3 | 38.1 | 0.45 | 0.44 | 0.83 |
| | Monensin[b] | 39.4 | 39.0 | 0.97 | 0.59 | 0.67 |
| | CT[c] | 37.8 | 38.1 | 0.62 | 0.53 | 0.88 |
| | ECT[d] | 38.0 | 38.4 | 0.94 | 0.72 | 0.75 |
| | *p*-value | 0.16 | 0.04 | | | |
| a* | Control | 15.0 | 12.6 | 16.3 | 0.48 | 0.005 |
| | Monensin | 15.0 | 12.3 | 18.9 | 0.40 | 0.003 |
| | CT | 15.6 | 12.5 | 19.7 | 0.56 | 0.002 |
| | ECT | 15.0 | 12.2 | 18.0 | 0.54 | 0.005 |
| | *p*-value | 0.52 | 0.56 | | | |
| b* | Control | 7.10[e,f] | 5.91[f] | 16.8 | 0.48 | 0.08 |
| | Monensin | 7.64[e] | 7.37[e] | 4.94 | 0.44 | 0.57 |
| | CT | 7.31[e,f] | 6.75[e,f] | 7.65 | 0.41 | 0.38 |
| | ECT | 6.47[f] | 6.36[e,f] | 1.70 | 0.53 | 0.89 |
| | *p*-value | 0.046 | 0.01 | | | |
| H* | Control | 25.2[e,f] | 26.0[f] | 3.05 | 1.30 | 0.42 |
| | Monensin | 27.0[e] | 30.1[e] | 11.4 | 1.29 | 0.006 |
| | CT | 25.0[e,f] | 28.7[e,f] | 14.9 | 1.87 | 0.004 |
| | ECT | 22.8[b] | 28.2[e,f] | 23.6 | 1.90 | 0.002 |
| | *p*-value | 0.008 | 0.05 | | | |

[a] SEM: Standard error of means

[b] Monensin: @ 75 mg/kg DM of feed

[c] CT: Crude Tannin@ 20 g/kg DM of feed

[d] ECT: Encapsulated tannin @ 20 g/kg DM of feed equivalent

[e,f] Means in the same column with different subscript letters differ ($p < 0.05$)

**Table 4** The effects of supplementing diet with *Acacia mearnsii* tannin extracts on subcutaneous fatty acid profiles (w/w%) of South Africa Dohne Merino ram lambs

| Fatty acids | Treatment | | | | SEM[d] | *p*-value |
|---|---|---|---|---|---|---|
| | Control | Monensin[a] | CT[b] | ECT[c] | | |
| C10:0 | 0.10 | 0.09 | 0.11 | 0.11 | 0.01 | 0.45 |
| C12:0 | 0.09 | 0.08 | 0.13 | 0.09 | 0.02 | 0.22 |
| C14:0 | 2.81 | 2.71 | 3.06 | 2.88 | 0.18 | 0.73 |
| C16:0 | 25.2 | 26.1 | 25.5 | 24.9 | 0.54 | 0.23 |
| C17:0 | 2.28 | 2.37 | 2.27 | 1.95 | 0.23 | 0.78 |
| C18:0 | 23.3 | 23.6 | 22.5 | 22.8 | 0.39 | 0.99 |
| C20:0 | 0.10 | 0.10 | 0.10 | 0.10 | 0.01 | 0.99 |
| C21:0 | 0.38 | 0.28 | 0.33 | 0.39 | 0.04 | 0.21 |
| C22:0 | 0.05 | 0.05 | 0.04 | 0.05 | 0.01 | 0.37 |
| Total SFA[e] | 54.4 | 55.4 | 54.0 | 53.3 | 0.76 | 0.94 |
| C14:1 | 0.07 | 0.06 | 0.08 | 0.07 | 0.01 | 0.81 |
| C16:1 | 1.36 | 1.31 | 1.27 | 1.26 | 0.12 | 0.87 |
| C18:1n9t | 2.94 | 3.07 | 2.89 | 3.33 | 0.21 | 0.35 |
| C18:1n9c | 38.6 | 37.7 | 38.6 | 39.2 | 1.51 | 0.95 |
| C20:1 | 0.04 | 0.05 | 0.06 | 0.06 | 0.01 | 0.41 |
| Total MUFA[f] | 43.0 | 42.2 | 42.9 | 43.9 | 0.75 | 0.95 |
| C18:2n6t | 0.17 | 0.15 | 0.16 | 0.19 | 0.02 | 0.14 |
| C18:2n6c | 2.11 | 1.96 | 2.50 | 2.27 | 0.14 | 0.09 |
| C18:3n3 | 0.26 | 0.24 | 0.31 | 0.27 | 0.02 | 0.11 |
| C20:2 | 0.03 | 0.04 | 0.04 | 0.04 | 0.01 | 0.25 |
| C20:4n6 | 0.06 | 0.06 | 0.07 | 0.06 | 0.01 | 0.96 |
| Total PUFA[g] | 2.64 | 2.45 | 3.07 | 2.83 | 0.09 | 0.25 |

[a] Monensin: @ 75 mg/kg DM of feed

[b] CT: Crude Tannin @ 20 g/kg DM of feed

[c] ECT: Encapsulated tannin @ 20 g/kg DM of feed

[d] SEM: Standard error of means

[e] SFA: Saturated fatty acids

[f] MUFA: Monounsaturated fatty acids

[g] PUFA: Polyunsaturated fatty acids

the intramuscular fat depot. Higher proportions of linoleic acid (C18.2n6t; omega-6), alpha-linoleic (C18:3n3; omega-3), and docosahexaenoic acid (C22:6n3; omega-3) were deposited in intramuscular fat tissue of lambs fed diets containing encapsulated condensed tannin compared to those in the monensin treatment group.

## Discussion

In this study, we tested the effect of *Acacia mearnsii* tannin extracts as a feed additive on the carcass characteristics, meat quality and meat fatty acid profiles of lambs.

The target slaughter mass of 50 kg was higher than the recommended 43 kg for ideal carcass quality that conforms to consumer preference (Van der Merwe et al. 2020). Nevertheless, most of the lambs in this study were in the A3 class (no permanent incisors with a fat score of 3) and they still possess a high commercial value, according to the current market trends. In terms of tissue composition, our results agree with previous studies which have reported that the addition of natural tannin extracts in lamb diets does not affect tissue composition (Fernandes et al. 2021).

Evaluating the ratio of meat to fat in the saleable meat portions, as predicted by three rib-cut samples, lambs in this study presented a relatively higher degree of carcass fatness with meat: fat ratio of 1.09:1 compared to 1.36:1, previously reported for Dohne Merino lambs under similar conditions (Van der Merwe et al. 2020). This difference is mainly attributable to the variation in the age at slaughter of the rams. The Dohne Merino is an intermediate-maturing breed and the lambs deposit fat at an earlier age than late-maturing breeds (Cloete et al. 2012). Future studies and feedlots should consider slaughtering such lamb breeds when they reach live

**Table 5** The effects of supplementing with *Acacia mearnsii* tannin extracts on intramuscular fatty acid profile (w/w%) of South Africa Dohne Merino ram lambs

| Fatty acids | Treatment | | | | SEM[d] | *p*-value |
|---|---|---|---|---|---|---|
| | Control | Monensin[a] | CT[b] | ECT[c] | | |
| C10:0 | 0.11 | 0.12 | 0.12 | 0.12 | 0.01 | 0.59 |
| C12:0 | 0.10 | 0.10 | 0.15 | 0.10 | 0.02 | 0.06 |
| C14:0 | 2.20 | 2.40 | 2.58 | 2.36 | 0.11 | 0.18 |
| C16:0 | 27.2 | 28.5 | 28.0 | 27.9 | 0.46 | 0.13 |
| C17:0 | 1.07 | 0.99 | 0.99 | 0.92 | 0.03 | 0.21 |
| C18:0 | 17.7 | 17.7 | 16.4 | 17.6 | 0.56 | 0.62 |
| C20:0 | 0.07 | 0.08 | 0.07 | 0.07 | 0.01 | 0.39 |
| C21:0 | 0.30 | 0.28 | 0.35 | 0.35 | 0.02 | 0.03 |
| C22:0 | 0.18 | 0.17 | 0.16 | 0.20 | 0.02 | 0.12 |
| C24:0 | 0.03 | 0.03 | 0.03 | 0.03 | 0.01 | 0.52 |
| Total SFA[e] | 48.8 | 50.3 | 48.8 | 49.7 | 0.29 | 0.34 |
| C14:1 | 0.06 | 0.06 | 0.07 | 0.06 | 0.01 | 0.49 |
| C16:1 | 1.37 | 1.52 | 1.36 | 1.49 | 0.08 | 0.55 |
| C18:1n9c | 45.1 | 43.9 | 45.0 | 43.6 | 0.63 | 0.29 |
| C20:1 | 0.05 | 0.04 | 0.05 | 0.05 | 0.01 | 0.38 |
| C24:1 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.77 |
| Total MUFA[f] | 46.5 | 45.5 | 46.5 | 45.3 | 0.34 | 0.57 |
| C18:2n6t | 0.17[h,i] | 0.15[b] | 0.18[h,i] | 0.19[h] | 0.01 | 0.04 |
| C18:2n6c | 3.07 | 2.77 | 3.21 | 3.41 | 0.22 | 0.21 |
| C18:3n3 | 0.28[i,j] | 0.24[c] | 0.32[h] | 0.29[h,i] | 0.02 | < .001 |
| C18:3n6 | 0.03 | 0.02 | 0.03 | 0.03 | 0.00 | 0.21 |
| C20:2 | 0.05 | 0.05 | 0.05 | 0.05 | 0.00 | 0.98 |
| C20:3n6 | 0.07 | 0.06 | 0.06 | 0.08 | 0.01 | 0.25 |
| C204n6 | 0.90 | 0.76 | 0.77 | 0.97 | 0.09 | 0.38 |
| C20:5n3 | 0.03 | 0.04 | 0.05 | 0.04 | 0.01 | 0.09 |
| C22:6n3 | 0.04[h,i] | 0.02[i] | 0.03[h,i] | 0.05[h] | 0.01 | 0.04 |
| Total PUFA[g] | 4.63 | 4.13 | 4.70 | 5.10 | 0.19 | 0.71 |

[a] Monensin: @ 75 mg/kg DM of feed

[b] CT: Crude Tannin @ 20 g/kg DM of feed

[c] ECT: Encapsulated tannin @ 20 g/kg DM of feed

[d] SEM: Standard error of means

[e] SFA: Saturated fatty acids

[f] MUFA: Monounsaturated fatty acids

[g] PUFA: Polyunsaturated fatty acids

[h,i] Means in the same row with different subscript letters differ ($p < 0.05$)

mass of approximately 40–45 kg, to minimise the costs of production.

In terms of carcass conformation by combining the yields of meat and fat and expressing it relative to that of bone, it seems that lambs in this study had better conformation than previously reported for Dohne Merino lambs (Van der Merwe et al. 2020) and would presumably yield a higher portion of saleable meat. However, Ngo et al. (2016) argue that cold carcass mass is the best predictor of lamb carcass yield and of the overall value of a carcass.

The amount of intramuscular fat has a positive influence on the eating quality of meat as increasing levels in meat are associated with increased flavour, tenderness and juiciness (Realini et al. 2021). In the present study, the IMF percentages of lambs were not significantly different across the four dietary treatment groups, suggesting that the addition of natural tannin extracts in lamb diets does not compromise the sensory eating quality of lamb meat.

The results of meat colour and colour stability of lambs show that meat lightness did not change with increasing

duration of storage. This contrasts with previous studies that have reported darker meat (lower L*) in tannin-fed sheep over storage time (Luciano et al. 2009). However, the studies are not fully comparable since Luciano et al. (2009) measured colour changes in minced meat samples over 14 days while we used the meat chops and a shorter storage period.

Colour is the single most important quality attribute affecting consumer purchasing decisions of red meats and consumers prefer brick red lamb colour (Kennedy et al. 2004). Changes in redness (a*) and yellowness (b*) values over time describe meat colour deterioration from red to brown and reflect the myoglobin concentration and its redox state in meat (Mancini and Hunt 2005). In the present study, the redness (a*) values decreased over the 7 day storage period, across all treatment groups. This change in fresh meat colour is due to the oxidation of myoglobin (bright red colour) to metmyoglobin (brown colour) over time. Other studies have reported that dietary tannin supplements can reduce oxidative deterioration in red meat samples through their antioxidant properties (Luciano et al. 2011), but this was not observed in the present study. These differences can be due to many factors, such as the type of tannins and the dosage used (Biondi et al. 2019).

Monensin treatment group had higher b* values than encapsulated condensed tannin and control treatment groups on day 1 and day 7 of storage, respectively. The higher b* coordinate values suggest that monensin treatment can cause the meat to turn from red to brown faster than the other treatments.

Hue angle is a good descriptor of meat browning resulting from the decreases in a* relative to b* (Lee et al. 2005). It has been previously reported that polyphenols such as tannins can maintain meat colour for a longer period (Luciano et al. 2011; 2009). However, in the present study, we did not find any co-beneficial effect of dietary tannin additives on the colour and colour stability of lamb meat.

In the present study, SFA comprised about half the total fatty acids, which is typical of lambs kept on high-forage diets (Bhatt et al. 2022). The SFA are generally considered unhealthy because they can potentially increase LDL cholesterol, increasing the risk of cardiovascular diseases (Briggs et al. 2017). However, studies have shown that it is only lauric acid (12:0), myristic acid (C14:0), and palmitic acid (C16:0) that are associated with an increased risk of hypercholesterolemia (Hunter et al. 2010). Similar to the findings of Bhatt et al. (2022), tannin supplementation did not alter the SFA proportions in both subcutaneous and intramuscular fat depots. These results are important considering that SFA are the main fatty acids in lambs. Since there were no changes in the concentration of lauric acid, myristic acid and palmitic acid, which by implication means *Acacia mearensii* tannin extract used as anti-methanogenic feed additives do not cause increased risk to human health. Our results also suggest that tannin extract feed additives do not affect the technological quality of lamb meat, as SFA particularly stearic acid (C18:0) contribute to the firmness of ruminant fat tissue (Wood et al. 2004).

The molar proportion of MUFA were about 42.2 to 46.5% of total fatty acids, and the major MUFA was oleic acid (C18:1n9c), as previously reported for lambs (Biondi et al. 2019 and Realini et al. 2021). Generally, oleic acid is perceived as beneficial in terms of human health and may contribute to several health benefits observed in people who consume diets enriched with oleic acid (Lopez-Huertaz 2010).

In this study, the inclusion of tannin extract did not affect the proportion of MUFA in both subcutaneous and intramuscular fat depots. This contrasts with previous studies which have reported an unfavorable decrease in MUFA with tannin supplementation, particularly oleic acid (C18:1n9c) (Biondi et al. 2019 and Jacondino et al. 2022), which can lower blood cholesterol levels. The lack of changes in the proportions of MUFA, observed in this study, is important considering that these fatty acids are beneficial for human health.

Total PUFA were about 2 to 5% of total fatty acids and conjugated linoleic acid (C18:2n6c) was the main PUFA in both fat depots, as previously reported for lamb meat (Biondi et al. 2019). Tannins have been reported to inhibit the rumen biohydrogenation process, where dietary PUFA are converted SFA, especially stearic acid (Vasta et al. 2019 and Makmur et al. 2022). This effect enhances the accumulation of unsaturated fatty acids in the adipose tissue of ruminants, which is beneficial for human health.

In the present study, higher proportions of linoleic acid (C18.2n6t; omega-6), alpha-linoleic (C18:3n3; omega-3), and docosahexaenoic acid (C22:6n3; omega-3) were deposited in intramuscular fat tissue of lambs fed diets containing encapsulated condensed tannin compared to those in the monensin treatment group. Indeed, polyphenols slowed down rumen kinetics fermentation with the consequent accumulation of PUFAs (Calabrò et al. 2009).

Polyunsaturated fatty acids (omega-3 and omega-6) are generally regarded as beneficial for human health (Scollan et al. 2006). In this context high importance is attributed to the omega 6- omega 3 ratio (Cavaliere et al. 2018). Higher proportions of n–3 and n-6 fatty acids observed in this study in the encapsulated tannin group over monensin can be considered a co-benefit to the methane mitigating effects of dietary tannin additives. However, increasing n–3 fatty acids in meat can increase oxidative

degradation and the production of volatiles associated with the development of off-flavours, thus decreasing the overall consumer acceptability of meat (Francisco et al. 2015). Further research should establish if the inclusion of *Acacia mearensii* tannin extracts in lamb diets would affect the sensory quality and consumer acceptability of the product.

## Conclusions

Dietary inclusion of *Acacia mearnsii* tannin extract as a feed additive did not affect carcass composition, intramuscular fat percentage, colour, and colour stability of lamb meat. However, small differences were observed in meat fatty acid profiles, particularly in the intramuscular tissue with tannin supplementation. The intramuscular fat of lambs fed with diets containing encapsulated condensed tannin extracts resulted in higher proportions of omega-3 and omega-6 fatty acids compared to the monensin treatment group, which can be considered beneficial for human health.

Practical implication: *Acacia mearnsii* tannin extracts can be safely used as an inexpensive anti-methanogenic feed additives to possibly reduce the environmental impact of the livestock industry, without compromising product quality.

#### Abbreviations

| | |
|---|---|
| ADG | Average daily gain |
| AMTS | Agricultural Modelling and Training System |
| CT | Crude tannin |
| DM | Dry matter |
| ECT | Encapsulated tannin |
| GLM | General linear model |
| IMF | Intramuscular fat |
| MUFA | Monounsaturated fatty acid |
| PUFA | Polyunsaturated fatty acids |
| SEM | Standard error of means |
| SFA | Saturated fatty acid |
| TMR | Total mixed ration |

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#### Author contributions

Conceptualisation, AH and ECW; methodology, ECW; software, AH; validation, ECW and AH; formal analysis, ECW; investigation, MV; resources, AH; data curation, AH; writing—original draft preparation, MV and PP; writing—review and editing, PP, ECW and AH; visualization, AH; supervision, AH and ECW; project administration, AH; funding acquisition, AH.

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#### Availability of data and materials

Data supporting the reported results are available upon request.

## Declarations

#### Ethics approval and consent to participate

The animal study protocol was approved by the Animal Ethics Committee of the University of Pretoria (NAS201/2020 06 October 2020).

#### Consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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## Publisher's Note