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The effect of dietary non-protein nitrogen content on the meat quality of finishing lambs

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Abstract

The effect of increasing the non-protein nitrogen content of low-fibre finishing diets on the meat quality of South African Mutton Merino wether lambs was investigated. Four similar dietary treatments were formulated with different non-protein nitrogen contents (16.6 g/kg, 28.3 g/kg, 40 g/kg, and 51.7 g/kg) on a dry matter basis. The study was conducted over 71 days. Muscle lipid content was higher on the 40 g/kg compared to 16.6 g/kg and 28.3 g/kg non-protein nitrogen diets. Myristic acid and total saturated fatty acid content of lamb muscle tissue was higher, whereas the total unsaturated fatty acid content of the same tissue was lower following a high dietary non-protein nitrogen content. A high non-protein nitrogen diet (51.7 g/kg) reduced conjugated linoleic acid content of adipose tissue, compared to 28.3 and 40 g/kg non-protein nitrogen treatments. Malonaldehyde content of fresh lamb meat was higher on the 28.3 g/kg compared to the 16.6 g/kg non-protein nitrogen diet. The malonaldehyde content of the treatment containing 40 g/kg non-protein nitrogen was higher than the 51.7 g/kg when stored for 90 days. Therefore, the non-protein nitrogen content of low-fibre lamb finishing diets does not affect mutton fatty acid composition and quality parameters. Protein quality and its effect on ruminant meat quality requires more attention.

Keywords: Fatty acid, mutton, shear force, soybean meal, urea
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Introduction

Elucidating protein metabolism in ruminants gives a clearer understanding of the complex system that separates nitrogen for microbes from that of post ruminal amino acid supply to the host (Owens *et al*., 2014). Crude protein, which is the simplest form, is provided to ruminants and can be divided into degradable and undegradable intake protein (Bohnert *et al*., 2002). Nutrients found in the blood of a ruminant depend on microbial fermentation, which plays a remarkable role in changing and producing substances to be absorbed and used by the animal. Degradable intake protein is broken down within the rumen by microorganisms, whereas undegradable intake protein escapes rumen fermentation and ends up in the small intestine for enzymatic digestion and absorption (Bohnert *et al*., 2002). Non-protein nitrogen (NPN) compounds are not proteins but can be converted into proteins by rumen microbes (Zurak *et al*., 2023) and include urea, uric acid, and ammonia (Tadele & Amha, 2015). NPN remains relevant in the field of ruminant nutrition (Zhu *et al*., 2022). Ammonia is not only the most important source of nitrogen for rumen bacterial growth (Bohnert *et al*., 2002) but is the preferred nitrogen source of cellulolytic bacteria (NRC, 2007). Hoover (1986) notes that urea as sole nitrogen source is not sufficient because microorganisms lack specific amino acids.

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Ruminant products contain high amounts of saturated fatty acids (SFAs) as a result of ruminal biohydrogenation (BH) (Harfoot & Hazlewood, 1997). A high intake of SFAs is associated with increased risk of lifestyle-related diseases such as increased blood cholesterol levels and atherosclerotic growth (Junkuszew *et al*., 2020). Thus, to improve human nutrition, feeding strategies for ruminants aim to reduce SFAs and increase polyunsaturated fatty acids (PUFA). Fatty acid hydrogenation contributes to the accumulation of conjugated linoleic acid (CLA) isomers in ruminant products (Fievez *et al*., 2007) and contains anticarcinogenic properties (Mcguire & Mcguire, 1999) and improves immune response (Jenkins *et al*., 2008). The negative connotation to SFAs should be re-visited since only stearic acid (C18:0) has an atherogenic affect when consumed excessively (Chilliard *et al*., 2006).

Dietary nitrogen concentration affects the rates of lipolysis and hydrogenation (Gerson *et al*., 1983). Lipolysis is more extensive when nitrogen is increased in high starch diets (Dewhurst *et al*., 2006). With sufficient nitrogen, the relative rates are such that unsaturated fatty acids (UFA) accumulate in the rumen (Gerson *et al*., 1983). Additionally, some microorganisms require, or are stimulated by, different forms of nitrogen (Polan, 1992). Hydrogenating bacteria consist mainly of cellulolytic organisms. Cellulolytic bacteria such as *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens,* and *Ruminococcus albus* are actively involved in the biohydrogenation (BH) of fatty acids (FA) (Harfoot & Hazlewood, 1997). *Butyrivibrio fibrisolvens* has proteolytic activity and is actively involved in the BH of FAs (Harfoot & Hazlewood, 1997). Therefore, any nutrient favouring the growth of bacteria responsible for ruminal BH, like ammonia (Steward *et al*., 1997) from NPN sources, can affect the free UFAs in the rumen. Hence, the research focusses on this concept.

Over a century of ruminant nutrition research has been devoted to understanding ruminant nitrogen metabolism and studying processes and practices that can increase nitrogen utilization efficiency (Hristov *et al*., 2019). However, the research on the nitrogen content of ruminant diets affecting FA composition only accounts for rumen contents (*in vitro*), blood plasma, liver, muscle, and perirenal fat in sheep (Gerson *et al*., 1982, 1983). No literature regarding the effect of finishing diet NPN concentrations on the FA composition of ruminant products was found. It is possible that free ammonia (from NPN sources) in the rumen can stimulate the growth of BH microbes and ultimately affect lamb meat FA composition and quality. The aim of this study was therefore to determine the effect of increasing dietary NPN content within a standard low-fibre finishing diet on meat quality of South African Mutton Merino (SAMM) lambs.

Materials and Methods

All procedures conducted during this study were approved by the Interfaculty Animal Ethics Committee for Animal Experimentation at the University of the Free State (Animal Experiment No. UFS-AED2016/0038).

A production study was conducted with 60 SAMM wether lambs over 71 days. All lambs with an initial live weight of 25.5 ± 2.6 kg (mean ± SD) were randomly allocated to four dietary treatments culminating in a randomised trial design with n = 15 lambs per treatment (n = 1 lamb per experimental unit). The animals received a standard health and vaccination programme prior to the study: they were injected with an antiparasitic remedy, dosed for tapeworm and inoculated against pulpy kidney, malignant oedema, blackquarter, tetanus, pasteurellosis, as well as pneumonic and septicaemic pasteurellosis. Animals were housed in pens (n = 1 lamb/pen; 1.404 m²) on elevated, wooden, slatted floors in a naturally-ventilated building.

Four finishing diets with a similar nutrient composition (155 g crude protein/kg dry matter (DM) and 180 g neutral detergent fibre (NDF)/kg DM) were formulated, with each containing increments in NPN content (Table 1). The NDF content was kept as low as possible because a low-fibre diet results in a low ruminal pH, which inhibits the growth of the main BH cellulolytic bacteria (Martin & Jenkins, 2002). The protein source for the incremental NPN content was feed grade urea. Soybean meal and prime gluten meal were used to ensure a similar degradable and undegradable intake protein content, respectively. Maize protein is relatively high in undegradable intake protein (Swanson *et al*., 2004). The bypass protein potential of soybean meal is low (Cheeke, 2005) and is an example of a protein source containing high degradable intake protein content (NRC, 2007) with a high percentage of nitrogen present as true protein (±90%) (Kellems & Church, 2010). Urea is 100% degradable within the rumen (Soto-Navarro *et al*., 2003).

Table 1 The physical and chemical composition of the four experimental diets with increments in non-protein nitrogen (NPN) content

	Treatment diets[1]			
	CON	**NPN1**	**NPN2**	**NPN3**
Physical composition (g/kg as is):				
Maize meal	578	582	586	590
Citrus pulp	64,8	92,8	121	150
Soybean oil	29,8	29,9	29,9	30,0
Maize germ oil	1,40	0,900	0,500	-
Soybean hulls	164	158	152	146
Prime gluten meal	-	11,5	23,0	34,5
Soybean meal	143	101	58,9	15,9
Urea	-	4,70	9,40	14,10
Limestone	1,50	1,00	0,500	-
Monocalcium phosphate	-	0,700	1,30	2,00
Calcium chloride	10,0	10,0	10,0	10,0
Salt	5,00	5,00	5,00	5,00
Premix	2,50	2,50	2,50	2,50
Total	**1000**	**1000**	**1000**	**1000**
Nutrient composition (g/kg dry matter):				
Dry matter	902	905	901	901
Organic matter	954	953	956	953
Crude protein	138	150	143	161
Degradable intake protein[2]	87.7	90.1	92.5	94.9
Undegradable intake protein[3]	62.3	60.4	58.5	56.6
Non-protein nitrogen[4]	**16.6**	**28.3**	**40.0**	**51.7**
Non-structural carbohydrate[5]	598	588	578	592
Neutral-detergent fibre	187	181	200	174
Acid-detergent fibre	127	119	124	121
Ether extract	60.7	59.6	55.0	60.8
Ash	46.3	47.3	44.5	46.8
Calcium	9.10	9.40	10.50	10.80
Phosphorus	3.00	2.80	2.80	2.70

[1]Treatment diets containing NPN content per dry matter (DM): CON (control) = 16.6 g/kg; NPN1 = 28.3 g/kg; NPN2 = 40 g/kg; NPN3 = 51.7 g/kg. [2]Degradable ingested protein calculated from NRC (1996). [3]Undegradable ingested protein calculated from NRC (2007). [4]Non-protein nitrogen calculated from NRC (1996). [5]Non-structural carbohydrate content calculated from Van Soest *et al*. (1991)

Treatments were described according to NPN content in ascending order as control (CON: 16.6 g/kg), NPN1 (28.3 g/kg), NPN2 (40 g/kg), and NPN3 (51.7 g/kg). All other chemical parameters were formulated to be similar. Treatment diets were fed to the animals in a processed mash form.

The FA composition of the four experimental diets is presented in Table 2. Only the major FAs contained in the feed, as well as those deemed important to ruminant meat, were reported in this study. Because the prime gluten 60 inclusion increased with decreasing soybean meal inclusion (from CON to NPN3), maize germ oil was added by difference and calculated according to soybean oil inclusion. This ensured comparable FA composition between the diets and similar lipid content. Soybean oil was used in this study to increase the UFA content of all diets. Representative feed samples were taken at the start (day 0) of the production study and stored at -18 °C until the FA profile was determined.

Table 2 Lipid content and fatty acid composition of finishing diets fed to wether lambs

	Treatment diets[1]			
	CON	**NPN1**	**NPN2**	**NPN3**
Proximate analysis:				
Feed lipid content (g/kg DM)	60.7	59.6	55	60.8
Fatty acid[#] (% of total fatty acids)				
Myristic (C14:0)	0.0755	0.0780	0.0820	0.0835
Palmitic (C16:0)	13.1	13.2	13.4	13.2
Stearic (C18:0)	4.86	4.83	4.96	5.01
Palmitoleic (C16:1c9)	0.0655	0.0690	0.0710	0.0730
Oleic (C18:1c9; n-9)	21.8	21.4	21.4	21.4
Vaccenic (C18:1t11)	2.39	2.38	2.41	2.42
Linoleic (C18:2c9,12; n-6)	51.9	52.1	51.7	51.8
α-Linolenic (C18:3c9,12,15; n-3)	5.09	5.21	5.17	5.33
Total saturated fatty acids (SFA)	18.6	18.6	19.0	18.8
Total monounsaturated fatty acids (MUFA)	24.2	23.8	23.9	23.9
Total polyunsaturated fatty acids (PUFA)	57.2	57.6	57.1	57.3
Total omega-6 fatty acids (n-6)	52.1	52.3	51.9	52.0
Total omega-3 fatty acids (n-3)	5.09	5.21	5.17	5.33
n-6:n-3	10.3	10.1	10.0	9.76
PUFA:SFA	3.08	3.09	3.00	3.05
Arachidonic acid (C20:4c5,8,11,14; n-6)	-	-	-	-
Eicosopentaenoic acid (C20:5c5,8,11,14,17; n-3)	-	-	-	-
Docosapentaenoic acid (C22:5c7,10,13,16,19; n-3)	-	-	-	-
Docosahexaenoic acid (C22:6c4,7,10,13,16,19; n-3)	-	-	-	-

[1]Treatment diets containing non-protein nitrogen (NPN) content per dry matter (DM): CON (control) = 16.6 g/kg; NPN1 = 28.3 g/kg; NPN2 = 40 g/kg; NPN3 = 51.7 g/kg

No feed additives or rumen modifiers, which may have affected the rumen environment, were included in the diets. A synthetic antioxidant containing a combination of butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin and trisodium citrate was, however, included in all diets to prevent FA oxidation.

At the onset of the study (day 0), the lambs underwent a standard adaptation period. Lucerne hay was provided *ad libitum* and each respective treatment diet was fed to the lambs with incremental increases of 100 g/day/animal for 10 days. The animals were fed twice daily, at 08h00 and at 16h00. Feed intake was recorded on a weekly basis. Fresh, clean water was freely available to all animals.

At the end of the production study, all lambs (49.1 ± 4.4 kg; mean ± SD) were slaughtered at a commercial abattoir. Three loin chops (the left 9th to the 11th rib) from each carcass were evaluated for meat quality.

Total lipid from *longissimus* muscle and subcutaneous fat samples were quantitatively extracted according to the method of Folch *et al*. (1957). Chloroform and methanol were used in a ratio of 2:1. Total extractable intramuscular fat was determined gravimetrically from the extracted fat and expressed as percentage fat (w/w) per 100 g tissue. An antioxidant (butylated hydroxytoluene) was added at a concentration of 0.001% to the chloroform:methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum. The extracts were dried overnight in a vacuum oven at 50 °C using phosphorus pentoxide as a moisture adsorbent.

The extracted fat from the feed, muscle, and subcutaneous fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at -20 °C, pending FA analyses. A lipid aliquot (20 mg) of feed, muscle, and subcutaneous lipid were converted to methyl esters by base-catalysed transesterification with sodium methoxide (0.5 M solution in anhydrous methanol) for 2 h at 30 °C to avoid CLA isomerisation, as proposed by Kramer *et al*. (2002) and Alfaia *et al*. (2007). Fatty acid methyl esters (FAME) from the feed, muscle, and subcutaneous lipid were quantified using a Varian 430 flame ionization gas chromatograph with a fused silica capillary column (Chrompack CPSIL 88; 100 m length; 0.25 mm ID; 0.2 µm film thickness).

Analysis was performed using an initial isothermic period (40 °C for 2 min). Thereafter, temperature was increased at a rate of 4 °C/min to 230 °C. Finally, an isothermic period of 230 °C was held for 10 min. Fatty acid methyl esters in n-hexane (1 µl) were injected into the column using a Varian CP-8400 Autosampler. The injection port and detector were both maintained at 250 °C. Hydrogen, at

45 psi, functioned as the carrier gas, whereas nitrogen was used as the makeup gas. Galaxy Chromatography Data System Software recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Conjugated linoleic acid standards were obtained from Matreya Inc. (Pleasant Gap, Unites States of America). These standards included: C18:2c9,t11 and C18:2t10,c12 isomers. All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa).

Fatty acids were expressed as the proportion of each individual FA to the total of all FAs present in the sample. The following were also calculated: total SFAs, total monounsaturated fatty acids (MUFA), total PUFAs, total omega-3 (n-3), and total omega-6 (n-6) FAs. Total fatty acid data were used to calculate the following ratios: PUFA/SFA, n-6/n-3, and Δ^9 desaturase index (C18:1c9/C18:0). Atherogenicity index was calculated as (Chilliard *et al*., 2003):

$$\text{Atherogenicity index } (C12{:}0 + 4 \times C14{:}0 + C16{:}0)/(\text{MUFA} + \text{PUFA}) \quad (1)$$

One loin chop from each lamb carcass was individually placed in a polystyrene tray containing an absorbent pad, wrapped with oxygen-permeable polyvinyl chloride (PVC) meat stretch wrap, and stored for 8 d (evaluated every second day) at 4 °C under fluorescent light for fresh meat stability studies. Another loin chop was vacuum sealed and stored for 90 d at -18 °C in the dark for frozen storage stability evaluation.

Muscle colour (L*, a* and b*-values) was subsequently determined in triplicate using the refrigerated (4 °C) loin chop over a period of 8 d using a Minolta chromometer. Chroma or saturation index, which is related to colour intensity of meat, and hue angle were calculated according to the formulae of Ripoll *et al.* (2011):

$$\text{Chroma index} = (a^{*2} + b^{*2}) \times 0.5 \text{ for muscle} \quad (2)$$

$$\text{Hue angle} = \tan\text{-}1(b^*/a^*) \quad (3)$$

Thiobarbituric acid reactive substance (TBARS) was used to determine the malonaldehyde content/kg meat. A 5-g sample of lean meat was removed from the middle of each loin chop on day zero, day eight (refrigerated at 4 °C), and day 90 (frozen storage at -18 °C), and the aqueous acid extraction method of Raharjo *et al*. (1992) was applied.

Meat tenderness was determined using the *longissimus muscle* (left 11th to 13th rib sample), which was vacuum sealed and frozen at -20 °C. Frozen samples were thawed at 4 °C for 18 h before preparation. Thawed cuts were prepared according to an oven-broiling method using direct heat (AMSA, 1978). An electric oven was set on “broil” 10 min prior to preparation (260 °C). Steaks were placed on an oven pan on a rack to allow meat juices to drain during cooking and placed in the pre-heated oven, 9 cm below the heat source. The cuts were cooked to an internal temperature of 35 °C, then turned over and finished to 70 °C. Cuts were cooled at room temperature (20–25 °C) for at least 3 h before measuring shear force. Four cylindrical samples (12.5-mm core diameter) of each sample were cored parallel to the grain of the meat and sheared perpendicular to the fibre direction using a Warner Bratzler shear force device mounted on a Universal Instron apparatus (cross head speed = 200 mm/min; one shear in the centre of each core). The reported value in kg represents the average of the peak force measurements of each sample.

The data was analysed as a completely randomized design using the General Linear Model (GLM) procedures of the Statistical Analysis System (SAS) program (SAS, 1999). Means were compared using the LSMEANS/DIFF with treatment as fixed effects. For post hoc analysis, Tukey’s honestly significant difference (HSD) test was used to identify significant differences between treatment means and significance was declared at the 5% probability level. The description of the model used for ANOVA was:

$$Yij = \mu + ti + \varepsilon ij \quad (4)$$

where Yij is the individual observations (dependent variable) of the i-th treatment (independent variable) and the j-th random error, μ is the general effect, ti is the effect of the i-th treatment, and εij is the random variation or experimental error.

The i-th treatment effect (dietary NPN content) during this study was defined and presented on a DM basis:

i_1 = 16.6 g/kg (CON), i_2 = 28.3 g/kg (NPN1), i_3 = 40 g/kg (NPN2), i_4 = 51.7 g/kg (NPN3)

Results and Discussion

The daily dietary DM intake (g DM per animal) was not affected (P >0.05) by NPN addition for CON (1306 ± 139), NPN1 (1285 ± 114), NPN2 (1227 ± 130), and NPN3 (1234 ± 144) (Einkamerer *et al.*, submitted for publication). Hence, the FA composition of treatment diets (total FA intake) was discounted as a possible cause for any substantial effects regarding meat FA composition. The effect of NPN content in low-fibre finishing diets on the lipid content and FA composition of wether lamb muscle tissue is presented in Table 3. A change in feed can influence total FA intake and consequently, the FA composition of ruminants and products (Dang Van *et al*., 2011). The amount (Beam *et al*., 2000; Scollan *et al*., 2003; Santos-Silva *et al*., 2004; Gómez-Cortés *et al*., 2009; Hur *et al*., 2017) and composition (Vatansever *et al*., 2000; Ivan *et al*., 2001; Chilliard & Ferlay, 2004; Cooper *et al*., 2004; Castro *et al*., 2009; Booyens *et al*., 2012; Lerch *et al*., 2012; Du Toit *et al*., 2013) of dietary lipid consumed influences the FA composition of rumen content, as well as ruminant products. If FAs are naturally protected from hydrogenation, like maize lipids due to the lower rumen degradability of maize protein and starch compared to other cereals (Bas & Morand-Fehr, 2000), it may aid the provision of intact FAs within the small intestine. An increased rumen outflow of oils (increased intake) may also lessen the effects of BH (Demeyer & Doreau, 1999) and therefore reduce exposure of lipids to lipases and BH in the rumen (Dewhurst *et al*., 2006).

The NPN content influenced the lipid content of lamb *longissimus dorsi* muscle tissue (Table 3). The lipid content was higher with an NPN content of 4.00% (treatment NPN2) than the CON (NPN 1.66%) and NPN1 (NPN 2.83%). There might have been a hormonal effect relating to dietary protein content and fat accretion/mobilisation in the lambs. However, Pittroff *et al*. (2006) found that protein: energy ratios could not indicate whether the diet given to sheep resulted in a change of internal energy reserves. The authors found that leptin, which indicated higher circulating content, of lambs fed straw and duodenal available protein (150 g/d fish meal) did not respond with an accelerated rate of fat mobilization or maintained protein mass due to available nitrogen, compared to lambs fed only straw. Thus, dietary protein provision has an apparent hormonal effect on lamb fat content. No literature could be found on dietary NPN content and lamb muscle lipid tissue content. More research is required in this regard.

The NPN content of low-fibre finishing diets had a limited effect on the FA composition of lamb muscle tissue. The myristic acid content of lamb muscle was, however, higher (P = 0.0365) on treatment NPN3 compared to treatments CON and NPN1. Total muscle SFA content of treatment NPN3 was also higher than treatment NPN1. The fatty acid profile of lamb becomes more saturated as the animal ages (Ye *et al*., 2020). The contents of MUFA and SFA both increase faster in the carcass with increasing fatness compared to the PUFA content (De Smet *et al*., 2004). These marked effects in muscle SFA content in the current study are therefore comparable to its lipid content. In contrast, increasing the NPN content of diets to 5.17% of DM (NPN3) decreased (P = 0.0214) the total UFA content of the same tissue compared to treatment NPN1 only. A negative association between meat PUFA and total lipid contents has been reported in ovine studies where PUFA decreases as animals age due to the relative increase of neutral lipids in tissue (Ye *et al*., 2020).

Dietary lipid amounts, blends thereof (Bessa *et al*., 2007), as well as full-fat whole seeds (Bauman *et al*., 1999; Chilliard & Ferlay, 2004; Lestingi *et al*., 2015) can influence the FA composition of animal products. Dietary plant protein feed ingredients, which increases protein content, may therefore influence the FA composition of adipose tissues and muscles of lambs, probably because of the high content of specific total FAs (Bas & Morand-Fehr, 2000). These authors found that the addition of cotton seed meal with a high FA content brought about a substantial rise in stearic and linoleic acid percentages in lamb fat deposits. Additionally, fish meal in lamb diets increased the oleic acid percentage in fat tissues (Bas & Morand-Fehr, 2000). Extruded linseed also had beneficial effects on FA composition of dairy bulls' meat (Dawson *et al*., 2010). The effect of varying dietary lipids was controlled in the current study by providing diets with similar FA compositions (Table 2). A possible hormonal effect from dietary protein content and fat accretion/mobilisation of lambs was reported (Pittroff *et al*., 2006), which was related to total protein consumption and not protein quality *per se*. No literature is available on diet protein quality and its effect on lamb meat FA composition and this requires more attention.

Table 3 The effect of non-protein nitrogen (NPN) content in finishing diets on the lipid content and fatty acid (FA) composition of South African Mutton Merino lamb muscle tissue (mean ± SD)

	Treatment diets[1]					
	CON	**NPN1**	**NPN2**	**NPN3**	***P*-Value**	**CV[#] (%)**
Muscle lipid content (g/kg DM)	33.1^{bc} ± 5.20	32.5^{c} ± 4.40	40.4^{a} ± 8.30	39.8^{ab} ± 8.80	0.0021	19.1
Fatty acid[#] (% of total fatty acids):						
Myristic (C14:0)	2.31^{b} ± 0.313	2.32^{b} ± 0.432	2.59^{ab} ± 0.460	2.67^{a} ± 0.438	0.0365	16.8
Palmitic (C16:0)	28.1 ± 1.38	27.8 ± 1.50	28.9 ± 2.10	29.3 ± 2.01	0.0916	6.22
Stearic (C18:0)	13.4 ± 0.646	13.5 ± 1.06	13.5 ± 1.88	14.0 ± 1.31	0.6164	9.58
Palmitoleic (C16:1c9)	1.55 ± 0.167	1.56 ± 0.231	1.72 ± 0.317	1.60 ± 0.316	0.3014	16.5
Oleic (C18:1c9; n-9)	34.6 ± 3.43	35.3 ± 2.08	36.1 ± 2.19	34.7 ± 1.63	0.3047	6.90
Vaccenic (C18:1t11)	3.28 ± 0.532	2.98 ± 1.05	2.35 ± 1.56	2.24 ± 1.55	0.0750	46.0
Linoleic (C18:2c9,12; n-6)	10.9 ± 2.82	10.6 ± 2.02	9.28 ± 2.08	9.42 ± 2.18	0.1487	22.9
α-Linolenic (C18:3c9,12,15; n-3)	0.612 ± 0.0740	0.580 ± 0.0930	0.580 ± 0.0963	0.566 ± 0.0969	0.5516	15.5
Conjugated linoleic acid (C18:2c9,t11; n-6)	0.274 ± 0.0718	0.280 ± 0.0771	0.322 ± 0.112	0.252 ± 0.125	0.2805	35.2
Total saturated fatty acids (SFA)	45.4^{ab} ± 1.48	45.2^{b} ± 2.02	46.6^{ab} ± 2.55	47.8^{a} ± 3.32	0.0215	5.28
Total monounsaturated fatty acids	40.5 ± 3.54	40.9 ± 2.55	41.2 ± 2.31	39.6 ± 2.33	0.3883	6.74
Total polyunsaturated fatty acids (PUFA)	14.1 ± 3.48	13.8 ± 2.29	12.2 ± 2.55	12.6 ± 2.37	0.1754	20.6
Total unsaturated fatty acids	54.5^{ab} ± 1.50	54.8^{a} ± 2.03	53.4^{ab} ± 2.55	52.2^{b} ± 3.32	0.0214	4.55
Total omega-6 fatty acids (n-6)	13.0 ± 3.33	12.9 ± 2.15	11.3 ± 2.45	11.7 ± 2.24	0.1775	21.1
Total omega-3 fatty acids (n-3)	1.02 ± 0.196	0.974 ± 0.160	0.921 ± 0.132	0.907 ± 0.245	0.3501	19.7
n-6:n-3	12.8 ± 2.46	13.3 ± 1.21	12.2 ± 1.80	14.3 ± 7.61	0.5576	31.5
PUFA:SFA	0.310 ± 0.0790	0.307 ± 0.0560	0.264 ± 0.0628	0.268 ± 0.0643	0.1063	23.0
Δ^9 desaturase index	2.58 ± 0.273	2.62 ± 0.188	2.73 ± 0.386	2.50 ± 0.292	0.2252	11.3
Atherogenicity index	0.564^{ab} ± 0.0441	0.555^{b} ± 0.0535	0.598^{ab} ± 0.0722	0.623^{a} ± 0.0865	0.0244	11.3
Arachidonic (C20:4c5,8,11,14; n-6)	1.79 ± 0.563	1.86 ± 0.320	1.54 ± 0.496	1.56 ± 0.521	0.1952	28.7
Eicosopentaenoic acid (C20:5c5,8,11,14,17; n-3)	0.153 ± 0.0636	0.148 ± 0.0552	0.132 ± 0.0525	0.135 ± 0.0789	0.7681	44.7
Docosapentaenoic acid (C22:5c7,10,13,16,19; n-3)	0.270 ± 0.0759	0.246 ± 0.0557	0.209 ± 0.0652	0.206 ± 0.0951	0.0617	32.0
Docosahexaenoic acid (C22:6c4,7,10,13,16,19; n-3)	-	-	-	-	-	-

[a,b,c] Mean values with different superscripts in the same row differ significantly (P <0.05); CV = coefficient of variation (%)

[1]Treatment diets containing NPN per dry matter (DM): CON (control) = 1.66%, NPN1 = 2.83%, NPN2 = 4.00%, NPN3 = 5.17%

Proteolytic isolates are recognised as *Ruminobacter amylophilus*, *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, *B. alactacidigens*, *Selenomonas ruminantium,* and *Streptococcus bovis* (Dehority, 2003). Cellulolytic bacteria are actively involved in the BH of FAs (Harfoot & Hazlewood, 1997). *Butyrivibrio fibrisolvens* contains cellulolytic (French *et al*., 2000), proteolytic, and BH activities (Harfoot & Hazlewood, 1997). French *et al*. (2000) stated that the growth and activity of *B. fibrisolvens* was influenced by rumen conditions, which could subsequently determine the amount of CLA available for absorption from the gastrointestinal tract. Hence, it is plausible that the growth of *B. fibrisolvens* was stimulated by an increased NPN content, which could have affected the total SFA and total UFA content of lamb meat. Different *B. fibrisolvens* strains prefer to utilize either ammonia, or mixtures of amino acids (Dehority, 2003). Therefore, the correct bacterial strain needs to be identified. Further research is required in this regard as microorganism composition and rumen pH were not measured in the current study, which was a limitation of this study.

A significant effect on the atherogenicity index of lamb intramuscular lipid tissue was noted due to dietary treatment. This was primarily ascribed to the marked effect of a high NPN content (NPN3) on the same muscle's myristic acid content. Atherogenicity and thrombogenicity indices should both present smaller values than 1.0 and 0.5 to assure a protective potential for coronary artery health (Murariu *et al*., 2023). The atherogenicity index of muscle tissue can be reduced if the UFA content is increased (Carvalho *et al*., 2014). The contrary seems to occur with an increased SFA content. Manso *et al*. (2009) confirmed that a lack of influence on the intramuscular fat atherogenicity index was related to a lack of effect on the SFA and MUFA content. Palmitic acid concentration is important because it is harmful to human health by increasing low-density lipoprotein (LDL) levels in the blood (Carvalho *et al*., 2014). The marked effect on the atherogenicity index of lamb intramuscular lipid tissue in the current study is therefore justified due to the treatment effect on lamb muscle myristic acid, total SFA, and UFA content. Dietary treatment had no effect on the longer-chain FA (C20-plus) composition of lamb muscle tissue.

The effect of increasing NPN content in low-fibre finishing diets on the lipid content and FA composition of wether lamb subcutaneous lipid tissue is presented in Table 4. It is evident that dietary NPN content had less of an effect on the FA composition of lamb subcutaneous lipid tissue compared to that of the muscle tissue (Table 3). Even though differences in FA tissue response (adipose versus muscle tissue) are not uncommon (Oka *et al*., 2002), this result was not expected as the FA composition of muscle tissue is frequently less influenced by the diet than the adipose tissue (Scollan *et al*., 2006). This is because most of the muscle FAs are located in phospholipids and cellular membranes. Cell membrane FA composition often changes little despite large differences in dietary lipid composition (Jimenez *et al*., 2020). At least 90% of the lipids contained in adipose tissue are triacylglycerol or neutral lipids, whereas those within muscle tissue are predominantly phospholipids (Wood *et al*., 2008). This same deposit site effect was also acknowledged by Ladeira *et al.* (2014). Dietary NPN content did however affect the CLA (C18:c9,t11) content of lamb subcutaneous lipid tissue; treatment NPN3 was lower than treatments NPN1 and NPN2. It is important to note that dietary treatments within the current study contained no trace of any CLA isomers (Table 2).

Table 4 The effect of non-protein nitrogen (NPN) content in finishing diets on the lipid content and subcutaneous fatty acid (FA) composition of South African Mutton Merino lamb meat (mean ± SD)

	Treatment diets[1]					
	CON	**NPN1**	**NPN2**	**NPN3**	***P*-Value**	**CV[#] (%)**
Adipose lipid content (g/kg DM)	831 ± 17.1	826 ± 25.8	827 ± 29.9	832 ± 14.2	0.8690	2.73
Fatty acid (% of total fatty acids):						
Myristic (C14:0)	3.38 ± 0.456	3.33 ± 0.3631	3.39 ± 0.661	3.59 ± 0.628	0.5824	15.8
Palmitic (C16:0)	30.1 ± 2.10	29.0 ± 1.43	29.1 ± 1.95	30.0 ± 1.33	0.1951	5.86
Stearic (C18:0)	18.3 ± 1.75	18.6 ± 1.84	17.5 ± 2.94	17.6 ± 2.26	0.4643	12.5
Palmitoleic (C16:1c9)	0.932 ± 0.120	0.891 ± 0.137	0.989 ± 0.186	0.966 ± 0.206	0.4053	17.6
Oleic (C18:1c9; n-9)	30.1 ± 2.32	31.2 ± 1.46	32.1 ± 2.57	30.7 ± 3.04	0.1478	7.80
Vaccenic (C18:1t11)	2.78 ± 0.415	2.70 ± 0.275	2.48 ± 0.818	2.87 ± 0.414	0.2025	19.3
Linoleic (C18:2c9,12; n-6)	8.12 ± 2.04	7.55 ± 2.14	7.51 ± 1.75	7.71 ± 1.28	0.7937	23.8
α-Linolenic (C18:3c9,12,15; n-3)	0.758 ± 0.137	0.719 ± 0.181	0.703 ± 0.159	0.702 ± 0.0948	0.6974	20.4
Conjugated linoleic acid (C18:2c9,t11; n-6)	0.335^{ab} ± 0.0654	0.385^{a} ± 0.114	0.387^{a} ± 0.132	0.275^{b} ± 0.116	0.0218	31.7
Total saturated fatty acids (SFA)	55.6 ± 2.74	55.2 ± 2.19	54.4 ± 3.29	55.4 ± 3.14	0.6625	5.21
Total monounsaturated fatty acids	35.0 ± 2.85	36.0 ± 1.65	36.9 ± 2.98	35.8 ± 3.66	0.3526	8.02
Total polyunsaturated fatty acids (PUFA)	9.41 ± 2.23	8.80 ± 2.32	8.75 ± 1.94	8.83 ± 1.37	0.7841	22.3
Total unsaturated fatty acids	44.4 ± 2.74	44.8 ± 2.19	45.6 ± 3.29	44.6 ± 3.13	0.6630	6.40
Total omega-6 fatty acids (n-6)	8.65 ± 2.10	8.09 ± 2.14	8.04 ± 1.80	8.13 ± 1.28	0.7899	22.6
Total omega-3 fatty acids (n-3)	0.758 ± 0.137	0.719 ± 0.182	0.703 ± 0.159	0.702 ± 0.0948	0.6974	20.4
n-6:n-3	11.3 ± 0.917	11.2 ± 0.609	11.5 ± 0.836	11.6 ± 0.434	0.6109	6.35
PUFA:SFA	0.170 ± 0.0450	0.161 ± 0.0480	0.162 ± 0.0421	0.160 ± 0.0252	0.8970	25.1
Δ^9 desaturase index	1.66 ± 0.203	1.69 ± 0.174	1.90 ± 0.415	1.79 ± 0.405	0.1689	18.1
Atherogenicity index	0.761 ± 0.0939	0.727 ± 0.0606	0.722 ± 0.0941	0.763 ± 0.0913	0.4077	11.6
Arachidonic acid (C20:4c5,8,11,14; n-6)	0.0489 ± 0.0289	0.0380 ± 0.0224	0.0377 ± 0.0302	0.0509 ± 0.0326	0.4599	66.1
Eicosopentaenoic acid (C20:5c5,8,11,14,17; n-3)	-	-	-	-	-	-
Docosapentaenoic acid (C22:5c7,10,13,16,19; n-3)	-	-	-	-	-	-
Docosahexaenoic acid (C22:6c4,7,10,13,16,19; n-3)	-	-	-	-	-	-

[a,b,c] Mean values with different superscripts in the same row differ significantly (P <0.05); CV = coefficient of variation (%)

[1]Treatment diets containing NPN content per dry matter (DM): CON (control) = 1.66%, NPN1 = 2.83%, NPN2 = 4.00%, NPN3 = 5.17%

Conjugated linoleic acids are a mixture of geometric and positional isomers of conjugated dienoic derivatives (Hur *et al.*, 2017). An increased lipid CLA content could be connected to two possible causes: firstly, an incomplete BH affected by rumen pH with both a decreased (Qiu *et al*., 2004; Xu *et al*., 2014) and increased pH (Hur *et al*., 2017) and secondly, due to the activity of the Δ^9 desaturase enzyme (CoA-desaturase) in body tissues (Ribeiro *et al*., 2020). The majority of CLA (c9,t11) is produced in animal tissues (Ishlak *et al*., 2015) and the activity of Δ^9 desaturase is greatest in adipose tissue of growing ruminants (Kucuk *et al*., 2008). The second cause was ruled out as a possible tissue effect on the lipid CLA (c9,t11) content in the current study because the Δ^9 desaturase index was unaffected by dietary treatment.

Similar to lamb muscle tissue, dietary treatment had no marked effect on the longer-chain FA composition of lamb adipose tissue. Wood *et al*. (2003) reported that the long chain (C20 to C22) n-3 PUFAs were not detectable in beef and lamb neutral lipids (triacylglycerol). This was similar to the current study with special reference to eicosopentaenoic, docosapentaenoic, and docosahexaenoic acids.

There is a paucity of research regarding proteins and their effect on ruminant meat FA composition. The available research concentrated on total nitrogen content (Gerson *et al*., 1982, 1983) and not quality *per se*. Gerson *et al*. (1982) studied the composition of rumen volatile and unesterified FAs and the FA composition of blood plasma, liver, muscle, and perirenal fat of Romney wethers on diets with varying proportions of nitrogen but similar digestible organic matter. In similar research, the rates of lipolysis and hydrogenation was determined *in vitro* using sheep rumen digesta after high-starch diets (30% to 50%) also containing increasing proportions of nitrogen (0.72%, 1.22%, 1.72%, 2.47%, and 3.72%) (Gerson *et al*., 1983). Rates of lipolysis and hydrogenation were lowered by low dietary nitrogen content (Gerson *et al.*, 1983), and in diets containing approximately 1% nitrogen (DM basis), the relative rates were such that UFAs accumulated in the rumen.

There was a linear increase in the rate of lipolysis as nitrogen content increased *in vitro* (Gerson *et al*., 1983). The rate of *in vitro* BH increased after a 1.2% nitrogen concentration and tended to decrease above 2.5% nitrogen (Gerson *et al*., 1983). Gerson *et al*. (1983) concluded that microbial composition may have been a prime factor influencing the rates of lipolysis and hydrogenation. However, no conclusive evidence of a change in microbial species composition during incubation was presented. The authors confirmed that previous experience indicated that animals had to be kept for at least two weeks before microbial adjustment within the rumen was complete. This data refers to an *in vitro* study representing two highly-degradable intake protein sources with increased nitrogen content. It was, however, established that the addition of finely ground casein (true protein source) to the incubate was without affect (Gerson *et al*., 1983), probably due to the incubation period. Similar microbial populations and total bacterial count were recorded in sheep fed purified diets containing urea and casein (Oltjen, 1969). Calcium caseinate (900 g crude protein /kg DM) is referred to as a true protein source, which is 100% degradable in the rumen (Nolte & Ferreira, 2005).

Gerson *et al*. (1982) increased the amount of dietary nitrogen (0.54%, 1.10%, 1.42%, and 3.39%) in dietary DM fed to wethers for 50 d with similar degradable organic matter intake and recorded crude protein content of 3.38%, 6.88%, 8.88% and 21.19%, respectively. The percentage nitrogen is multiplied by 6.25 (McDonald *et al*., 2011) for the current discussion and comparisons. Gerson *et al*. (1982) explained that the proportion of nitrogen in wether diets rather than nitrogen intake, determined the amount and composition of plasma and tissue lipid, as well as the extent of BH in the rumen. Decreased BH in sheep fed 1.10% and 1.42% nitrogen (low nitrogen content) was noted in rumen contents. Increased concentrations of oleic and linoleic acids in the rumen were reflected in the plasma, muscle, and perirenal fat composition of the same sheep (Gerson *et al*., 1982). Similar results on BH were reported by Gerson *et al*. (1983). Tissue lipids were most unsaturated when nitrogen constituted approximately 1.40% (8.75% crude protein on a DM basis) of the diet (Gerson *et al*., 1982). The very low nitrogen treatment (0.54%), as well as highest (3.39%) in their research, resulted in higher lipolysis and BH, as judged by high saturation of rumen and plasma FAs. Gerson *et al*. (1982) found the effect of the low nitrogen treatment surprising because the opposite result was expected due to a depletion of the microbial population in the rumen. A higher dietary crude protein content fed to lambs again resulted in lower tissue palmitic and stearic acid contents, whereas the palmitoleic, oleic, and α-linolenic acid contents increased (Bas & Morand-Fehr, 2000). When the diet is deficient in protein or the protein resists degradation, the growth of rumen organisms will be slow (NRC, 2007). It appears that a more likely explanation for the results is a possible modification of the rumen bacterial population related to the 1.10% and 1.42% nitrogen treatments. The nitrogen content of these diets probably did not favour

microorganisms involved in the completion of the hydrogenation process, where BH seemed to be affected by alternate rumen nitrogen contents (Gerson *et al*., 1982). The authors acknowledge that a number of possible causes for this change in rumen BH may be considered (Gerson *et al*., 1982).

Lastly, and in contrast, dietary crude protein content in differing roughage:concentrate diets had no influence on varying the concentration of microbial cell matter FA composition (Bas *et al*., 2003). Even though the crude protein content of treatment NPN3 was slightly higher than the other treatments (Table 1), the possible cause and its effect on the FA composition of ruminant products and rumen digesta is rejected in the current study due to the overall lack of effect on lamb meat FA composition.

There is a possibility that urea could affect rumen pH (Oltjen, 1969; Soto-Navarro *et al*., 2003). Its increased inclusion in the current treatment diets could have affected rumen pH. Hence, FA metabolism in a low pH results in decreased rumen BH and lipolysis (Jenkins, 1993). Lower CLA (c9,t11) is observed at low rumen pH conditions (Choi *et al.*, 2005). High starch digestibility (AlZahal *et al*., 2008) and low dietary fibre content (Gerson *et al*., 1985) reduce the rate of lipolysis and BH and are connected to rumen pH. The statement regarding urea and its effect on rumen pH is referred to with caution as small fluctuations in rumen pH were noted (Kropp *et al*., 1977), whereas Soto-Navarro *et al*. (2003) referred to inconsistent results of urea on rumen pH. Some rumen pH values were even reported to be similar when urea and soybean protein isolate were fed to animals (Oltjen, 1969). The diets in the current study were also formulated on a low NDF content and rumen pH was not measured. Thus, information on rumen pH was not collected and more research is required in this regard.

Omega-3 and n-6 FAs are involved in human cardiovascular health. Dietary manipulation to alter lipid composition of small ruminants to favour human health have been investigated extensively (NRC, 2007; Ishlak *et al*., 2015). Fatty acid ratios are also considered important for human health. These include the PUFA:SFA ratio (0.45; Demirel *et al*., 2006) and n-6:n-3 ratio (4.00; Lestingi *et al*., 2015). The lower the n-6:n-3 ratio, the more beneficial it is for human health (Nong *et al*., 2020). Research has shown that the n-6:n-3 FA ratio is 15/1 to 16.7/1 in the typical Western diet, leading to increased incidences of lifestyle-related diseases. A lower n-6:n-3 FA ratio can reduce the risk of cardiovascular disease, cancer, and respiratory disease (Junkuszew *et al*., 2020). Dietary NPN content had no effect on any FA ratios within this study.

There is no literature available where protein quality (particularly NPN) was used as an independent variable to test for its effect on the FA composition of ruminant meat. Only literature regarding the quantity of nitrogen was found and reported in this study. It is recommended that more research should be conducted in this regard.

Because meat colour (Yagoubi *et al*., 2018), taste, and nutritional value are important quality attributes, any change in FA composition can influence quality aspects (Webb & O'Neill, 2008). The effects of increasing NPN content in low-fibre finishing diets on the colour stability and tenderness of wether lamb muscle tissue are presented in Table 5. Dietary NPN content of low-fibre finishing diets had no influence (P >0.05) on wether lamb muscle colour stability and tenderness (Table 5). This result was not expected as dietary treatment had a substantial effect on the total UFA composition of lamb muscle tissue (Table 3). The deterioration of red meat colour is triggered by a higher UFA content (Vatansever *et al*., 2000) but depends particularly on the content of PUFAs (Buckley *et al*., 1995). A meta-analysis showed that a high PUFA in pork decreased lightness (L*) because PUFA supplementation led to myoglobin oxidation and decreased the ratio of oxygen myoglobin to reduce lightness (Wang *et al*., 2021). Red meat colour is an important consumer sensory attribute (Abdalla Filho *et al*., 2017). The oxidation of lipids and pigment in meat are closely related, with the increase in one resulting in a similar increase in the other (Carvalho *et al*., 2014). Thus, the oxidation of myoglobin during storage leads to changes in meat colour (Hajji *et al*., 2016; Yagoubi *et al*., 2018).

Table 5 The effects of non-protein nitrogen (NPN) content in finishing diets on the colour stability and tenderness of South African Mutton Merino lamb muscle tissue (mean ± SD)

	Treatment diets[1]					
	CON	**NPN1**	**NPN2**	**NPN3**	***P*-Value**	**CV (%)**
Day 0:						
L*	40.3 ± 2.69	40.1 ± 2.11	40.3 ± 1.51	40.4 ± 1.78	0.9929	5.14
a*	17.0 ± 1.86	17.2 ± 1.77	17.2 ± 1.14	17.0 ± 1.06	0.9580	8.76
b*	6.31 ± 1.26	6.48 ± 1.11	6.39 ± 0.838	6.26 ± 0.779	0.9362	16.0
Chroma	18.2 ± 2.15	18.4 ± 2.00	18.4 ± 1.35	18.1 ± 1.18	0.9540	9.40
Hue angle (degree)	20.1 ± 2.18	20.5 ± 1.93	20.3 ± 1.42	20.2 ± 1.88	0.9514	9.25
Day 2 (refrigerated storage at 4°C):						
L*	48.2 ± 2.35	48.5 ± 1.50	47.6 ± 1.96	48.5 ± 1.58	0.4703	3.90
a*	12.6 ± 0.925	12.3 ± 0.910	12.1 ± 0.819	12.4 ± 0.538	0.4894	6.58
b*	7.66 ± 1.04	7.67 ± 1.12	7.89 ± 0.786	7.86 ± 0.731	0.8585	12.0
Chroma	14.7 ± 1.20	14.5 ± 1.21	14.5 ± 0.866	14.7 ± 0.717	0.8555	6.98
Hue angle (degree)	31.2 ± 2.98	31.8 ± 3.27	32.9 ± 3.00	32.2 ± 2.20	0.4351	9.02
Day 4 (refrigerated storage at 4°C):						
L*	47.1 ± 3.47	47.2 ± 1.89	47.0 ± 1.65	47.3 ± 1.85	0.9823	4.95
a*	12.0 ± 1.07	11.7 ± 1.09	11.3 ± 0.868	11.9 ± 0.696	0.1584	8.08
b*	7.97 ± 1.05	7.93 ± 1.26	8.07 ± 0.964	8.07 ± 0.786	0.9761	12.9
Chroma	14.4 ± 1.19	14.1 ± 1.41	13.9 ± 1.01	14.4 ± 0.886	0.5415	8.03
Hue angle (degree)	33.5 ± 3.75	34.1 ± 3.80	35.5 ± 3.47	34.1 ± 2.28	0.4197	9.87
Day 6 (refrigerated storage at 4°C):						
L*	48.1 ± 3.59	47.9 ± 2.15	47.5 ± 1.77	48.5 ± 2.01	0.7247	5.17
a*	11.4 ± 1.26	11.0 ± 1.36	10.8 ± 1.04	11.5 ± 0.737	0.3327	10.0
b*	8.01 ± 0.963	7.96 ± 1.37	8.43 ± 0.744	8.12 ± 0.725	0.5662	12.1
Chroma	14.0 ± 1.07	13.7 ± 1.51	13.8 ± 0.842	14.1 ± 0.832	0.6964	7.92
Hue angle (degree)	35.2 ± 4.94	35.7 ± 5.32	38.0 ± 4.07	35.1 ± 2.57	0.2475	12.1
Day 8 (refrigerated storage at 4°C):						
L*	47.7 ± 3.59	47.5 ± 2.60	46.7 ± 2.26	47.7 ± 1.81	0.6962	5.58
a*	10.6 ± 1.69	10.3 ± 2.16	9.88 ± 1.39	10.9 ± 0.971	0.3683	15.5
b*	8.29 ± 1.00	8.29 ± 1.47	8.59 ± 0.884	8.41 ± 0.946	0.8613	13.1
Chroma	13.5 ± 1.24	13.3 ± 2.12	13.2 ± 1.00	13.8 ± 1.13	0.6730	10.7
Hue angle (degree)	38.4 ± 6.62	39.0 ± 6.83	41.3 ± 5.74	37.6 ± 3.27	0.3446	14.8
Tenderness:						
Warner–Bratzler shear force (kg)	2.27 ± 0.460	2.34 ± 0.366	2.38 ± 0.609	2.58 ± 0.455	0.3364	20.1

[1]Treatment diets containing NPN content per dry matter (DM): CON (control) = 1.66%, NPN1 = 2.83%, NPN2 = 4.00%, NPN3 = 5.17%

CV = coefficient of variation (%); L* = Lightness; a* = Redness; b* = Yellowness; Chroma = saturation index

Carvalho *et al*. (2014) found an increase in both L* (lightness) and b* (yellowness) indices in animal muscles as a result of higher lipid levels. Fat is lighter than muscle and its increased presence could contribute to an increase the luminosity value (Carvalho *et al*., 2014). Even though muscle lipid content in the current study was affected ($P = 0.0021$) by dietary NPN inclusion (Table 3), a different result than that mentioned by Carvalho *et al*. (2014) was recorded. In addition, the b* index has been correlated with the presence of carotenoids in diets (Carvalho *et al*., 2014). Carvalho *et al*. (2014) ascribed higher yellowness values in diets to the addition of maize gluten, an ingredient rich in beta-carotene. Even though prime gluten 60 was used in this study and included in treatments NPN1, NPN2, and NPN3 (Table 1), its addition was probably too low to effect any substantial differences.

The colour indices (L*, a*, and chroma) of *longissimus dorsi* muscle from animals consistently decrease during ground soybean meal consumption (Ladeira *et al*., 2014) and the consumption of concentrate diets. Concentrate diets produce muscle with a brighter red colour (a* value) (Cooke *et al*., 2004), whereas pasture-fed lambs have higher b* values (Hajji *et al*., 2016). The higher yellow colour of grass-fed animals is ascribed to the presence of carotenoids (Priolo *et al*., 2002). Hue angle, however, gives a more realistic perspective on meat browning than any other single parameter (Luciano *et al*., 2009). The closer the hue angle is to 90°, the more yellow the colour is. All measured values were also lower than this recommended value.

The effect of increasing NPN content in low-fibre finishing diets on the oxidative stability (malonaldehyde content) of wether lamb muscle tissue is presented in Table 6.

Table 6 The effect of incremental non-protein nitrogen (NPN) content of finishing diets on the malonaldehyde content of South African Mutton Merino wether lamb muscle tissue (mean ± SD)

	Treatment diets[1]					
Thiobarbituric acid reactive substances (mg malonaldehyde/kg muscle tissue)	**CON**	**NPN1**	**NPN2**	**NPN3**	***P*-Value**	**CV[#] (%)**
Day 0 (refrigerated storage at 4°C)	$0.0517^{b} \pm 0.0151$	$0.0659^{a} \pm 0.0140$	$0.0620^{ab} \pm 0.0110$	$0.0544^{ab} \pm 0.0147$	0.0240	23.54
Day 8 (refrigerated storage at 4°C)	0.542 ± 0.487	0.510 ± 0.371	0.600 ± 0.438	0.414 ± 0.262	0.6356	77.14
Day 90 (frozen storage at -18°C)	$0.0476^{ab} \pm 0.0175$	$0.0563^{ab} \pm 0.0187$	$0.0583^{a} \pm 0.0187$	$0.0411^{b} \pm 0.0120$	0.0264	33.35

[a,b] Mean values with different superscripts in the same row differ significantly (P <0.05); CV = coefficient of variation (%)
[1]Treatment diets containing NPN content per dry matter (DM): CON (control) = 1.66%, NPN1 = 2.83%, NPN2 = 4.00%, NPN3 = 5.17%

Dietary treatment had an effect on the malonaldehyde content of lamb muscle tissue (P <0.05). Malonaldehyde of NPN1 increased compared to CON on day 0 (fresh sample). In contrast, malonaldehyde content of NPN2 was higher than NPN3 on day 90 of refrigerated storage. Even though NPN content had an effect on the oxidative stability of lamb muscle tissue, the results were not consistent. The stability of meat is related to its FA composition (Scollan *et al*., 2006). Any alteration of the FA profile of meat to more UFA, increases oxidative breakdown during post-mortem storage (Vatansever *et al*., 2000; Ladeira *et al*., 2014). Therefore, there is a positive correlation with the degree of UFA and meat oxidative stability (Carvalho *et al*., 2014). The PUFA content is especially susceptible to lipid oxidation (Yagoubi *et al*., 2018). Due to the marked effect on muscle UFA composition (Table 3), a related response in lamb muscle oxidation was expected. This result was, however, not consistent with the UFA content of lamb muscle tissue.

A malonaldehyde content of 0.5 mg/kg and above is considered negative and indicates lipid oxidation, which causes the rancid odour and taste of meat (Wood *et al*., 2008). Higher malonaldehyde values than this were only evident for treatment CON, NPN1, and NPN2 (P >0.05) stored for 8 d. Fortunately, these values were much lower than the proposed threshold value of 1 mg/kg (Ripoll *et al*., 2011), and even the high 2 mg/kg value presented in other research (Vatansever *et al*., 2000; Wood *et al*., 2003).

Tissue lipids of ruminants are more saturated than non-ruminants (French *et al*., 2000; Jenkins *et al*., 2008). Sheep meat is high in SFAs (Chikunya *et al*., 2004) and lamb fat is particularly high in stearic acid content (Wood *et al*., 2003). Meat with a higher SFA content is less susceptible to lipid oxidation (Yagoubi *et al*., 2018).

Changes in the a* and the oxymyoglobin values of meat are driven by lipid oxidation and are strongly correlated with malonaldehyde content (Ladeira *et al*., 2014). Even though only the malonaldehyde content of fresh (day 0) lamb muscle tissue and that of frozen storage (90 d) were substantially affected by dietary treatment, the lack of effect (P >0.05) regarding the redness index of the same cuts on day 0 seemed not to support this hypothesis. A lack of literature regarding protein content and quality related to oxidative stability of meat as well as quality parameters is evident, and more research is required in this regard.

Conclusions

A higher diet NPN content of low-fibre finishing SAMM lamb diets increases the lipid and SFA content of muscle tissue but decreases UFA and CLA content of adipose tissue. Diet NPN content had a limited effect on the tenderness and stability of SAMM lamb muscle tissue. There appears to be inadequate literature regarding protein research and its effect on finishing lamb meat quality, which

requires more attention. This study should also be repeated using the same treatments to determine the effect on lamb rumen pH and microbial composition.

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Authors' contributions

OBE, AVF, and AH designed the experiment and OBE carried out the research trial. MDF completed the statistical analyses. OBE and AH structured the scientific content and OBE drafted the manuscript. AVF and AH assisted with the experimental design and research trial, while all authors provided editorial suggestions and approved the final manuscript. AH conducted the meat quality analysis.

Conflict of interest

The authors declare that there is no conflict of interest.

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