South African Journal of Animal Science 2024, VOL 54

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

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(Submitted 29 August 2024; Accepted 26 September 2024; Published 9 December 2024)

Abstract

The effect of increasing non-protein nitrogen content on nutrient digestibility, performance, and carcass characteristics of South African Mutton Merino wether lambs on low-fibre finishing diets containing similar degradable protein was investigated. The production and digestibility studies consisted of 60 and 32 lambs, respectively. 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 production study was conducted over 71 d, and the digestibility study over 7 d. Crude protein digestibility increased with non-protein nitrogen content. Organic matter (40 g/kg and 51.7 g/kg), metabolizable energy content (51.7 g/kg), as well as the digestibility of neutral detergent fibre (40 g/kg) and acid detergent fibre (28.3 g/kg and 51.7 g/kg) also increased with a higher non-protein nitrogen content. A lower non-protein nitrogen content within the 16.6 g/kg and 28.3 g/kg treatments resulted in a higher average daily gain compared to the 51.7 g/kg treatment. Additionally, more effective energy was used for growth compared to treatments with 40 g/kg and 51.7 g/kg non-protein nitrogen. Dietary treatment left most of the carcass characteristics unaffected. In conclusion, a low non-protein nitrogen content of low-fibre lamb finishing diets with similar degradable protein content affected animal performance favourably, despite having an opposite effect on nutrient digestibility.

Keywords: carcass, growth, prime gluten meal, soybean meal, urea
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Introduction

Body tissues and cells require protein for growth, development, and maintenance. Nitrogen has a crucial role in the synthesis of hormones, enzymes, and other essential molecules (Zhao *et al.*, 2023). There are more than 700 amino acids in nature, but only 20 of them are building blocks for proteins in animal cells, some of which cannot be synthesized by some animal species (Wu *et al.*, 2014). Essential amino acids are defined as either those whose carbon skeletons cannot be synthesized or insufficiently synthesized *de novo* in animal cells relative to its needs for maintenance, growth, development, and health. These amino acids must then be provided in the diet to meet requirements (Hou *et al.*, 2015). Optimizing the efficiency of dietary crude protein requires selection of complementary feed protein supplements to provide the types and amounts of protein, which will meet, but not exceed, the nitrogen needs of rumen microorganisms and that of the animal (NRC, 2001). Over a century of ruminant nutrition research has been devoted to understanding nitrogen metabolism, processes, and practices to increase utilization efficiency (Hristov *et al.*, 2019).

Proteins provided to ruminants are composed of several sources, each with different properties that resist microbial degradation or are fermented at different rates (Polan, 1992). Protein quality and the

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v54i5.05

requirement of the animal *per se*, are either metabolized in the rumen and expressed as degradable ingested protein, or not available for rumen microbial fermentation and referred to as undegradable ingested protein (NRC, 2007). The degradable ingested protein includes non-protein nitrogen (NPN) compounds (Kellems & Church, 2010). Ultimately, metabolizable protein is the most appropriate method to define animal protein requirements (NRC, 2007).

Urea is normally added to ruminant diets as an NPN source, which benefits animal production and saves on nitrogen costs (Hailemariam *et al.*, 2021). Urea is hydrolysed to ammonia in the rumen by urease and used for microbial protein synthesis when enough carbohydrate is available (Mahmoudi-Abyane *et al.*, 2020). Hence, microbes supply their own amino acid requirements (Ali *et al.*, 2009), which become available to the host animal after they pass from the rumen to the small intestine (Cheeke, 2005). Sniffen & Robinson (1987), as well as Nichols *et al.* (2022), acknowledged that the protein requirements of ruminants can be met with the provision of urea as sole NPN source to the diet. Irrespective of protein quality, total nitrogen is generally considered to be the first limiting dietary component for the utilization of low-quality forages by microbes (Nolte *et al.*, 2000). For example, milk production was maintained in cows when microbial protein was synthesised in the rumen using a purified carbohydrate diet with urea and ammonium salts as the only nitrogen sources, compared to cows on a protein-rich silage diet (Nichols *et al.*, 2022). A greater by-pass protein source increases animal growth and muscle mass accretion compared to less by-pass protein. The inclusion of protein sources with the appropriate amino acid profiles catering to the needs of a growing animal results in greater growth performance and nitrogen utilization (El-Nomeary *et al.*, 2021). Hoover (1986) noted that urea as sole nitrogen source is not sufficient since microorganisms lack specific amino acids. However, if a diet contains too much protein, the animal will waste energy by producing more ammonia than needed, which is then converted to urea and excreted (Milis & Liamadis, 2008). Hence, the quantity of protein provided is just as important as the source thereof (Sniffen & Robinson, 1987).

Even though there are inconsistencies in the literature, protein source and the quality thereof affects ruminant performance. Recently the optimum degradable versus undegradable protein ratio in ruminant diets was evaluated (Putri *et al.*, 2021; Valizadeh *et al.*, 2021). Even though protein research on ruminant nutrition was evaluated extensively, no literature could be found on the quantity of NPN within a finishing diet fed to lambs with similar degradable protein and possible effects on performance. Where the NPN content in a finishing diet is manipulated, the total degradable protein content thereof is also altered. Therefore, the aim of this study was to determine the effect of increasing dietary NPN content within a standard, low-fibre finishing diet with similar degradable protein content on nutrient digestibility and performance of 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).

The production and digestibility studies consisted of 60 and 32 South African Mutton Merino (SAMM) wether lambs, respectively; the SAMM is a dual-purpose (meat and wool) sheep breed (Neser *et al.*, 2000). This breed was developed through selection for improved wool quality as well as carcass conformation, with high growth rate as the main characteristic (Skele *et al.*, 2024).

In the production study of 71 d, lambs with a mean (SD) weight of 25.5 ± 2.6 kg were fasted overnight, weighed, stratified according to descending mass, and randomly allocated to four dietary treatments. This culminated in a randomised trial design with n = 15 lambs per treatment (n = 1 lamb per experimental unit). For the digestibility study of 7 d, 32 wether lambs with a mean (SD) weight of 43.5 ± 4.1 kg were randomly allocated to the same dietary treatments (n = 8 lambs per treatment, n = 1 lamb per experimental unit) representing the same trial design.

All animals were subjected to a standard health and vaccination program prior to the study as commonly practiced in the commercial feedlot sector of South Africa. The animals 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. All animals were housed in pens (n = 1 lamb/pen; 1.404 m^2) on elevated, wooden slatted floors within a naturally-ventilated building. The metabolic building was properly washed and disinfected before the onset of the study. Each pen was cleaned twice a week.

Four finishing diets were formulated to facilitate an incremental increase in the NPN content, but also to contain a similar nutrient composition (Table 1).

Table 1 The physical and chemical composition of four experimental diets with incremental increases 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)

Feed grade urea was the protein source used to formulate the incremental NPN content of the diets. Soybean meal and prime gluten meal were included to ensure similar degradable, ingested protein content between treatments. The bypass protein potential of soybean meal is low (Cheeke, 2005) and is an example of a protein source with a high degradable, ingested 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). Urea, prime gluten meal, and soybean meal contain on average 288%, 2.01% and 5.94% NPN, and 288%, 27.47% and 35.10% degradable ingested protein, respectively (NRC, 1996).

Treatments were described according to NPN content in ascending order, henceforth control (CON: 16.6 g/kg NPN), NPN1 (28.3 g/kg NPN), NPN2 (40 g/kg NPN), and NPN3 (51.7 g/kg NPN). All other chemical parameters were formulated to be similar. Treatment diets were fed to the animals in the processed mash form. No feed additives nor rumen modifiers, which may have affected the rumen environment, were included in the diets.

At the onset of the production study (day 0), lambs were subjected to a standard adaptation period. Lucerne hay was provided on an *ad libitum* basis where each respective treatment diet was fed

to the lambs and increased incrementally 100 g/day/animal for 10 d. The animals were fed twice daily, at 08h00 and 16h00. Feed intake was recorded on a weekly basis, whereas total intake was used for statistical evaluation only.

The digestibility study of 7 d was conducted after adaption (10 d) to the treatment diets and faecal bags were fitted to the animals. The lambs were offered the same experimental diets as in the production study. To avoid variation in assessing the voluntary feed intake, a sequential method of feed allocation was followed by providing each animal a 15% refusal level of intake. Calculations were done daily by using a preceding three-day moving average of feed intake. The lambs were treated the same as during the production study and fed twice daily (08h00 and 16h00). Feed refusals (orts) were collected every morning just before the 08h00 feeding period. Faeces were collected twice daily for 7 d before feeding at 08h00 and 16h00. Each day's faecal output was amalgamated. Fresh, clean water was freely available to all the animals.

All samples were analysed for dry matter according to the Association of Official Analytical Chemists (AOAC) method 934.01 for chemical procedures (AOAC, 1990). The crude protein (method 990.03), ash (method 942.05), and organic matter were also analysed according to AOAC (1990), whereas non-structural carbohydrate content was determined as described by Van Soest *et al*. (1991). Neutral detergent fibre (aNDFom: Procedure A) analytical fraction was determined according to the method of Van Soest *et al*. (1991) using an ANCOM$^{200/220}$ Fibre Analyser (ANCOM Technology Corp., Fairport, NY, USA). Total acid detergent fibre (ADFom) was analysed according to AOAC method 973.18 (AOAC, 1990). Total lipid (ether extract) was extracted according to AOAC method 920.39 (AOAC, 1990). Gross energy (GE) was determined using a Leco® AC500 Isoperibol Calorimeter (Leco Corp., St. Joseph, MI) following ASTM (ASTM, 2009) standard D5865 (Cantrell *et al.*, 2010). Metabolizable energy was calculated by multiplying the digestible energy content of the diet by a factor of 0.81 to account for energy losses via urine and fermentation gasses (McDonald *et al.*, 2011). Non-protein nitrogen and degradable, ingested protein content of feeds were determined using published methods (NRC, 1996).

At the end of the production study, all lambs with a mean (SD) live weight of 49.1 ± 4.4 were slaughtered at a commercial abattoir after fasting overnight. Cold carcass weight was recorded 24 h after refrigeration at 2 °C to 4 °C according to the methods described by Fisher & De Boer (1994). The cold carcass weight was used to determine the dressing percentage (cold carcass weight divided by the empty stomach final live weight). The external length, shoulder circumference, and buttock circumference of each carcass was also recorded.

Meat evaluation was performed on the left side of each carcass. All carcasses were split between the 12th and 13th rib (thoracic vertebra) and fat depth was measured with a calliper (electronic digital calliper; Omni-Tech) 45 mm and 110 mm from the mid dorsal line (Carson *et al.*, 1999). The area of the longissimus muscle (*Musculus longissimus dorsi*) between the 12th and 13th rib was measured by tracing it directly onto transparent film (Edwards *et al.*, 1989). The traced outline was scanned with a scale bar and the eye muscle area measured using a video image analysis system (Soft Imaging System: Analysis® 3.0). The video image analyses system was calibrated with the scale bar.

Data were analysed as a completely randomized design using the General Linear Model (GLM) procedures in 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 at the 5% probability level. The description of the model used for ANOVA analysis was (Equation 1):

$$Yij = \mu + ti + \varepsilon ij \quad (1)$$

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) was defined and presented on a dry matter basis (Equation 2):

$$i_1 = 16.6 \text{ g/kg (CON)}, i_2 = 28.3 \text{ g/kg (NPN1)}, i_3 = 40 \text{ g/kg (NPN2)}, i_4 = 51.7 \text{ g/kg (NPN3)} \quad (2)$$

Results and Discussion

The effect of increasing NPN content in low-fibre finishing diets of wether lambs on apparent nutrient digestibility and metabolizable energy content are presented in Table 2. Treatment had no effect on the dry matter intake of the wethers in the digestibility study. The average dry matter intake of lambs fed the control diet (CON) was higher than in the other treatments. Even though the intake of treatments NPN1 to NPN3 was lower, it was not statistically lower than the control treatment. Voluntary intake therefore was not seen to influence apparent nutrient digestibility within the current study. A high intake decreases apparent total tract nutrient digestibility within ruminants due to a faster passage rate (shorter retention time) of digesta, and *vice versa* (McDonald *et al.*, 2011).

Table 2 The effect of increasing non-protein nitrogen (NPN) content in finishing diets of South African Mutton Merino wether lambs on nutrient apparent digestibility and diet metabolizable energy content

Parameter[#]	Treatment diets[1]								*P*-value	CV (%)
	CON		NPN1		NPN2		NPN3			
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD		
Dry matter intake (g/sheep/day)	1430	170	1261	110	1295	278	1278	198	0.3211	15.07
Digestibility coefficient:										
Dry matter	0.79	0.02	0.81	0.03	0.82	0.02	0.81	0.02	0.0958	2.89
Organic matter	0.80^{b}	0.02	0.82^{ab}	0.03	0.83^{a}	0.02	0.83^{a}	0.02	0.0475	2.79
Non-structural carbohydrate	0.94±	0.01	0.94	0.02	0.94	0.01	0.94	0.01	0.8471	1.40
Crude protein	0.69^{b}	0.03	0.74^{a}	0.03	0.73^{a}	0.02	0.76^{a}	0.02	0.0001	3.45
Neutral detergent fibre	0.43^{b}	0.08	0.52^{ab}	0.11	0.59^{a}	0.06	0.53^{ab}	0.06	0.0065	15.77
Acid detergent fibre	0.49^{b}	0.08	0.63^{a}	0.13	0.61^{ab}	0.08	0.62^{a}	0.05	0.0174	15.16
Ash	0.58	0.04	0.59	0.05	0.54	0.08	0.54	0.05	0.2207	10.22
Ether extract	0.79^{b}	0.03	0.83^{a}	0.03	0.84^{a}	0.02	0.79^{b}	0.01	<.0001	3.15
Metabolizable energy (MJ/kg DM)	10.76^{b}	0.36	11.01^{ab}	0.51	11.39^{a}	0.30	11.08^{ab}	0.20	0.0147	3.27

^{a,b}Mean values with different superscripts in the same row differ significantly (P <0.05)

[1]Treatment diets with NPN content of dry matter (DM): CON (control) = 16.6 g/kg, NPN1 = 28.3 g/kg, NPN2 = 40 g/kg, NPN3 = 51.7 g/ kg

There was a marked and increased effect of dietary treatment on organic matter (NPN2 and NPN3, compared to CON). This could, however, have been a result of NPN containing increased proportions of urea effecting faster ruminal nitrogen release. Generally, degradable true protein nitrogen is more slowly degraded in the rumen compared to an immediately-degraded protein source like urea. The nitrogen resulting from urea is incorporated into microbial protein less efficiently due to its speedy hydrolysis (McDonald *et al.*, 2011). The NPN content of low-fibre finishing diets had no effect (P = 0.0958) on total dry matter digestibility (Table 2).

Crude protein digestibility increased (P = 0.0001) with NPN content in the similar degradable, ingested protein diets. Urea within the rumen is quickly released (Niazifar *et al.*, 2024) and enhances fermentation (de Carvalho *et al.*, 2020). Therefore, nitrogen digestibility increases when NPN is included in the diet (Kropp *et al.*, 1977). Non-protein nitrogen compounds are highly soluble, rapidly converted to ammonia in the rumen and absorbed, thus yielding high digestibility values (Kellems & Church, 2010). This could be the case in the current study after increasing dietary urea content (NPN1, 2, and 3). The extent to which a feed protein is degraded depends on its inherent solubility in rumen liquid (Chalupa, 1975) and the time that it remains in the rumen (Dryden, 2008). Soto-Navarro *et al*. (2003) concluded that even with unlimited rumen ammonia from a concentrated diet, there are less benefits in microbial growth or digestion when using a true protein source (soybean meal) compared to an NPN source (urea).

Neutral detergent fibre digestibility was affected by NPN content (Table 2). An NPN content of 40 g/kg (NPN2) increased neutral detergent fibre digestibility compared to the CON (16.6 g/kg NPN). Similarly, acid detergent fibre digestibility increased substantially in treatments NPN1 (28.3 g/kg NPN) and NPN3 (51.7 g/kg NPN) compared to the CON. It is well documented that high levels of degradable protein enhance fibre fermentation considerably (Khandaker *et al.*, 2012) and thus, increase fibre digestibility (Allen, 2000). The substantial increases in neutral and acid detergent fibre digestibility in the current study could therefore have been related to protein degradability. Hence, NPN as the

degradable, ingested protein was comparable between diets. Belasco (1954) explained that at equivalent nitrogen levels, urea supported better cellulose breakdown than did any other protein meal (maize gluten, cottonseed, linseed, or soybean meal) of varying degradability, and was utilised more completely in an artificial rumen. The author showed that increasing urea from 1.3% to a maximum of 35% equivalent protein levels resulted in linear increases in cellulose digestion. Thus, within limits, the relationship between nitrogen supply, nitrogen utilisation, and cellulose breakdown was linear (Belasco, 1954). Polan (1992), however, stated that some rumen microorganisms were stimulated by more complex forms of nitrogen (amino acids or short peptides) than ammonia. *Fibrobacter succinogenes* (Matsui *et al.*, 1998) and *Butyrivibrio fibrisolvens* (French *et al.*, 2000) are cellulolytic organisms that prefer different nitrogen sources. Additionally, microorganisms acting on the non-structural (amylolytic) fraction derive ~65% of their nitrogen from amino acids or peptides, and the remainder from ammonia (NRC, 2007).

The digestibility of the ether extract fraction was affected by dietary treatment and increased with NPN1 (28.3 g/kg) and NPN2 (40 g/kg) compared to the CON (16.6 g/kg). There was no effect on ether extract digestibility with an NPN of 51.7 g/kg (NPN3). This effect of diet NPN content on ether extract digestibility is difficult to explain and requires more research.

Dietary treatment affected the metabolizable energy of the finishing diets in the current study. Comparable to organic matter digestibility, the metabolizable energy of treatment NPN2 was higher than the CON. This represented a 0.94% dietary urea inclusion (40 g/kg NPN). Nitrogen-free extract digestibility was not affected by the nature of the protein source (Milis & Liamadis, 2008) and this was reflected in the current study with no differences (P >0.05) in non-structural carbohydrate digestibility.

The effect of NPN in low-fibre finishing diets with similar degradable protein content on voluntary intake and performance of wether lambs are presented in Table 3.

Table 3 The effect of non-protein nitrogen (NPN) in finishing diets on intake and performance of South African Mutton Merino wether lambs

Parameter	Treatment diets[1]								*P*-value	CV (%)
	CON		NPN1		NPN2		NPN3			
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD		
Intake:										
Dry matter intake (g/sheep/day)	1306	139	1285	114	1227	130	1234	144	0.2948	10.48
Metabolizable energy intake (MJ/sheep/day)	13.46	1.43	13.54	1.19	13.36	1.41	13.05	1.55	0.7920	10.49
Production performance:										
Initial weight (kg)	25.59	2.83	25.53	2.71	25.49	2.56	25.44	2.36	0.9989	10.28
End weight (kg)	50.35	4.84	50.29	3.65	48.20	3.92	47.43	4.80	0.1669	8.84
Average daily gain (g/sheep/day)	349[a]	51	349[a]	31	320[ab]	36	310[b]	44	0.0196	12.40
Feed conversion ratio (kg DM feed intake/kg weight gain)	3.77	0.30	3.70	0.34	3.86	0.35	4.02	0.47	0.1132	9.65
MJ/kg live weight gain	38.87[b]	3.11	38.99[b]	3.58	41.98[a]	3.84	42.51[a]	4.85	0.0176	9.60

[a,b]Mean values with different superscripts in the same row differ significantly (P <0.05)
[1]Treatment diets with NPN content of dry matter (DM): CON (control) = 16.6 g/kg, NPN1 = 28.3 g/kg, NPN2 = 40 g/kg, NPN3 = 51.7 g/kg

Treatment had no effect on the dry matter intake (P = 0.2948) or metabolizable energy intake (P =0.7920) of finishing wether lambs (Table 3). The lack of treatment effect on metabolizable energy intake was not expected due to the significant effect of NPN on the metabolizable energy (Table 2). The dry matter intake corresponded with the lack of treatment effect (P = 0.3211) in the digestibility study (Table 2). The intake in the production study was, however, measured over a longer period (71 d). The lack of treatment effect on voluntary dry matter intake in the production study was not expected due to the possible influence of urea acceptability on voluntary intake of small stock (Dixon *et al.*, 2003). Urea supplementation either has no effect or decreases dry matter intake in sheep (Wahyono *et al.*, 2022). A lower intake of barley supplemented with urea presented to Merino wethers on low quality roughage

was associated with an adverse flavour or a conditioned feed aversion to the urea supplement (Dixon *et al.*, 2003). For finishing lambs, it is proposed that urea should be restricted to not more than 1% of the total diet because more than this could be unpalatable and decrease feed intake (Kellems & Church, 2010). Supplementing urea and molasses, however, increased dry matter intake of ram lambs, which was ascribed to higher dry matter and organic matter digestibility with the consequent reduction in the retention time of solid digesta in the reticulo-rumen (Can *et al.*, 2004). This was not the case in the present study. The influence of urea inclusion on the dry matter intake of ruminants presented in literature is inconsistent and variable. In addition, dry matter intake in lambs were reduced following an increased inclusion of a true and degradable protein source (casein) in their diets (Swanson *et al.*, 2004).

As in the current study, feed intake was similar with both NPN and soybean protein sources in purified diets fed to lambs, contrary to the slight decrease often found when urea is used in natural diets (Oltjen, 1969). Similar results were recorded by Shain *et al*. (1998) by feeding yearling cattle finishing diets containing either supplementary urea or other natural protein sources, as well as by Walker *et al*. (2006) who supplemented heifers' diets with urea or solvent-extracted soybean meal.

Increased neutral detergent fibre fermentation may reduce physical fill, stimulate flow from the rumen, and again allow for greater voluntary feed intake (Oba & Allen, 1999). An increased intake increases the liquid outflow of the rumen and feed particles in the early stages of digestion. This in turn results in more attached microorganisms (and thus also bacterial crude protein) flowing to the lower digestive system leading to an increase in microbial yield (Sniffen & Robinson, 1987). Even though both neutral and acid detergent fibre digestibility was affected by dietary NPN content, its low and similar content between diets (Table 1) was probably too low to affect the voluntary intake of the wether lambs.

A high NPN content (51.7 g/kg; NPN3) decreased (P <0.05) the growth efficiency (average daily gain) of wether lambs fed low-fibre finishing diets. The efficiency of gain presented as metabolizable energy utilized for the accretion of live weight gain (MJ metabolizable energy intake/kg live weight gain) of NPN2 and NPN3 decreased (P = 0.0176) compared to CON and NPN1 (higher values indicated less efficient growth). The positive effect of increased dietary NPN on organic matter digestibility and metabolizable energy (Table 2) did not, however, correspond to lamb growth efficiency. The lack of a treatment effect on the feed conversion ratio was not expected due to the strong influence of a high NPN on average daily gain. According to the NRC (2007), NPN sources should not provide more than one-third of the total nitrogen (crude protein) supplied for finishing ruminants. The recommendation for urea inclusion is no more than 15 to 25% of total crude protein in cattle and sheep fattening diets (Briggs, 1967). When urea inclusion provided more than 17.46% (NPN2) of the total crude protein in the current study, the average daily gain of the lambs declined (NPN3 compared to treatments CON and NPN1).

The amount and fermentability of organic matter in the diet complements the utilization of rumen ammonia concentration, i.e., fermentable energy in the rumen is required for efficient microbial use of ammonia-N for growth (Nichols *et al.*, 2022). It is possible that additional free nitrogen in the rumen is excreted as urea in urine (diets higher in crude protein digestibility), which negatively affects energy metabolism and decreases growth. This could have related to decreasing average daily gain and an increase in metabolizable energy utilized for growth purposes, as occurs on a higher NPN content. Productive ruminants require a higher percentage of true and bypass protein in their diets to meet the amino acid requirements of the post-ruminal stage (Putri *et al.*, 2021). Dietary protein sources that differ in the extent and rate of breakdown to ammonium and amino acids (NPN or true protein sources), affect animal performance (Kand & Dickhoefer, 2021). The degradability of crude protein in the rumen is related to the ruminal ammonia nitrogen concentration thereof (Mi *et al.*, 2022). The rapid decomposition of urea in the rumen to ammonia can occur at a faster rate than the utilization of ammonia by microbes, leading to the accumulation and escape of ammonia from the rumen (Mahmoudi-Abyane *et al.*, 2020). Energy required to process and excrete nitrogen in the animal's body includes energy lost through metabolic transformations, synthesis of urea, and excretion by the kidneys (Reed *et al.*, 2017).

The amount of various amino acids arriving in the duodenum plays a key role in the performance of growing and lactating ruminants (Shan *et al.*, 2007). Microbial synthesis supplies a decreasing proportion of the required protein, and substantial amounts must escape ruminal degradation to meet protein requirements of high yielding cows (Das *et al.*, 2014). Any process that increases the quantity of amino acids absorbed, may raise the efficiency of metabolizable energy for

growth purposes (Minson, 1990). From a quantitative perspective, true degradable protein appears to have a higher stimulatory effect on microbial essential amino acid flow to the duodenum compared to NPN (casein versus urea). This may affect the animal response and explains why natural protein is generally accepted as superior to NPN in terms of animal production (Nolte & Ferreira, 2005). A lower NPN content (more natural degradable proteins) seems to favour lamb growth (average daily gain) and efficiency within the current study (Table 3).

In contrast, Minson (1990) states it would be wrong to assume that a high proportion of bypass protein is always beneficial. Lambs were able to maintain growth with diets where virtually all the nitrogen was supplied by urea (Belasco, 1954). The addition of undegradable ingested protein (a feather and blood meal blend) to a diet also did not necessarily improve performance of finishing steers (McCoy *et al.*, 1998) nor lactation performance of goats (Soto-Navarro *et al.*, 2003). The same lack of response in feed efficiency was obtained in yearling cattle fed finishing diets containing either supplemental urea or other natural protein sources (Shain *et al.*, 1998). Walker *et al*. (2006) proposed, in accordance with the previous statements, that diets containing urea as the predominant protein were sufficient to optimize gain and efficiency of feedlot cattle. McCoy *et al*. (1998) mentioned that escape protein supplementation of finishing calf diets gave inconsistent results. Comparable to dry matter intake, the effect of protein quality on the production performance of ruminants presented in literature is also inconsistent and variable. None of these studies, however, formulated a comparable degradable, ingested protein content.

The effects of increasing NPN content in low-fibre finishing diets on the carcass characteristics of wether lambs are presented in Table 4. It was evident that dietary NPN content had a limited effect on the carcass characteristics.

Table 4 The effect of increasing non-protein nitrogen (NPN) content in finishing diets on the carcass characteristics of South African Mutton Merino wether lambs

Parameter[#]	Treatment diets[1]								*P*-value	CV (%)
	CON		NPN1		NPN2		NPN3			
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD		
Cold carcass weight (kg)	24.34	2.83	24.80	1.91	24.09	2.65	23.62	2.64	0.6394	10.46
Dressing percentage (%)	48.36	3.26	49.33	1.83	49.92	2.59	49.79	2.30	0.3334	5.17
Shoulder circumference (cm)	77.47	2.75	77.93	2.15	77.00	3.12	76.83	2.71	0.6814	3.50
Buttock circumference (cm)	68.60	3.19	67.63	2.49	67.17	2.70	66.40	3.54	0.2515	4.46
Carcass length (cm)	59.77	2.50	60.60	2.10	61.17	4.89	58.43	3.20	0.1421	5.58
M. longissimus dorsi width (mm)	63.35	5.96	62.74	4.59	59.96	7.20	62.21	3.54	0.3641	8.86
M. longissimus dorsi depth (mm)	31.24	3.47	31.28	4.15	30.46	3.26	31.21	3.15	0.9045	11.36
M. longissimus dorsi area (square mm)	1699[a]	251	1604[ab]	192	1509[b]	97	1562[ab]	163	0.0457	11.56
Fat thickness[2] (45 mm)	4.59	1.63	5.19	2.25	5.36	2.12	4.58	1.67	0.5800	39.29
Fat thickness[3] (110 mm)	9.36	3.47	9.99	2.56	10.79	2.74	9.82	2.82	0.6019	29.23

[a,b]Mean values with different superscripts in the same row differ significantly (P <0.05)
[1]Treatment diets with NPN content of diet dry matter (DM): CON (control) = 16.6 g/kg, NPN1 = 28.3 g/kg, NPN2 = 40 g/kg, NPN3 = 51.7 g/kg
[2]Fat thickness measured 45 mm from the mid dorsal line between the 12th and 13th thoracic vertebrae
[3]Fat thickness measured 110 mm from the mid dorsal line between the 12th and 13th thoracic vertebrae

Even though there was a dietary treatment effect on lamb growth efficiency (average daily gain) and metabolizable energy (weight gain) (Table 3), the lack of treatment effect on lamb dry matter intake, metabolizable energy intake, and final live weight corresponded to the lack of treatment effect on general carcass characteristics. However, increasing the NPN content of diets affected the area of the *musculus longissimus dorsi* (P = 0.0457). An NPN content of 40 g/kg diet dry matter (NPN2) negatively affected *longissimus dorsi* muscle area. In comparison to the current study, protein source did not alter carcass characteristics (dressing percentage, longissimus muscle area, 12th-rib fat thickness, and marbling score) of feedlot heifers on a soybean diet compared to those that were fed urea (Walker *et*

al., 2006). There seems to be inadequate literature regarding protein quality and its effect on lamb carcass composition. More research is required in this regard.

Conclusion

Increasing the NPN content of low-fibre finishing diets of South African Mutton Merino lambs positively affected nutrient digestibility and metabolizable energy. In contrast, the growth performance and efficiency of metabolizable energy utilization for growth decreased. It was thus apparent that there was an optimum NPN content in low-fibre finishing diets with comparable degradable, ingested protein content for small stock. A low dietary NPN content was beneficial for South African Mutton Merino production. It is accepted that contradictory literature exists on small stock growth response related to protein solubility. There appears to be inadequate recent literature regarding ruminant NPN research, let alone quality aspects thereof and its effect on small stock performance.

Acknowledgements

The support staff at the Department of Animal Science (University of the Free State) and Ms Liesl van der Westhuizen (Science Writer, University of the Free State) are gratefully acknowledged.

Funding

This study was funded by the Directorate Research and Development of the University of the Free State, Bloemfontein, South Africa.

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.

References

Ali, C. S., Islam-UD-DIN, Sharif, M., Nisa, M., Javaid, A., Hashmi, N. & Sarwar, M., 2009. Supplementation of ruminally-protected proteins and amino acids: Feed consumption, digestion, and performance of cattle and sheep. Int. J. Agric. Biol. 11, 477–482.

Allen, M. S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83, 1598–1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2.

Association of Official Agricultural Chemists (AOAC), 1990. AOAC Official Methods of Analysis. Washington DC..

American Society for Testing and Materials (ASTM), 2009. Standard test method for determining the axial disassembly force of taper connections of modular prostheses. ASTM International, West Conshohocken, PA.

Belasco, I. J., 1954. Comparison of urea and protein meals as nitrogen sources for rumen microorganisms. Urea utilization and cellulose digestion. J. Anim. Sci. 13, 739–747.

Briggs, M. H., 1967. Urea as a Protein Supplement. 1st ed. Pergamon, New York.

Can, A., Denek, N. & K, Yazgan, 2004. Effect of urea and molasses supplementation on nutrient intake and digestibility of sheep fed with straw. J. Anim. Vet. Adv. 3.

Cantrell, K. B., Bauer, P. J. & Ro, K. S., 2010. Utilization of summer legumes as bioenergy feedstocks. Biomass Bioenergy 34, 1961–1967. https://doi.org/10.1016/j.biombioe.2010.08.005.

Carson, A. F., Moss, B. W., Steen, R. W. J. & Kilpatrick, D. J., 1999. Effects of the percentage of Texel or Rouge de l'Ouest genes in lambs on carcass characteristics and meat quality. Anim. Sci. 69, 81–92. https://doi.org/10.1017/S1357729800051110.

Chalupa, W., 1975. Rumen bypass and protection of proteins and amino acids. J. Dairy Sci. 58, 1198–1218. https://doi.org/10.3168/jds.S0022-0302(75)84697-2.

Cheeke, P. R., 2005. Applied Animal Nutrition: Feeds and Feeding. Pearson Prentice Hall.

Das, L. K., Kundu, S. S., Kumar, D. & Datt, C., 2014. Metabolizable protein systems in ruminant nutrition: A review. Vet. World. 7, 622–629. https://doi.org/10.14202/vetworld.2014.622-629.

de Carvalho, I. P. C., Doelman, J. & Martín-Tereso, J., 2020. Post-ruminal non-protein nitrogen supplementation as a strategy to improve fibre digestion and N efficiency in the ruminant. J. Anim. Physiol. Anim. Nutr. 104, 64–75. https://doi.org/10.1111/jpn.13233.

Dixon, R. M., Hosking, B. J. & Egan, A. R., 2003. Effects of oilseed meal and grain-urea supplements fed infrequently on digestion in sheep 1. Low-quality grass hay diets. Anim. Feed Sci. Technol. 110, 75–94. https://doi.org/10.1016/S0377-8401(03)00204-9.

Dryden, G. M., 2008. Animal Nutrition Science. CABI. Cambridge University Press, UK.

Edwards, J. W., Cannell, R. C., Garrett, R. P., Savell, J. W., Cross, H. R. & Longnecker, M. T., 1989. Using ultrasound, linear measurements, and live fat thickness estimates to determine the carcass composition of market lambs. J. Anim. Sci. 67, 3322–3330. https://doi.org/10.2527/jas1989.67123322x.

El-Nomeary, Y. A. A., Abd El-Rahman, H. H. H., Shoukry, M. M., Abedo, A. A., Salman, F. M. & Mohamed, M. I., 2021. Effect of different dietary protein sources on digestibility and growth performance parameters in lambs. Bull. Natl. Res. Cent. 45, 40. https://doi.org/10.1186/s42269-021-00486-1.

Fisher, A. V. & De Boer, H., 1994. The EAAP standard method of sheep carcass assessment. Carcass measurements and dissection procedures. Livest. Prod. Sci. 38, 149–159.

French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J. & Moloney, A. P., 2000. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. J. Anim. Sci. 78, 2849–2855. https://doi.org//2000.78112849x.

Hailemariam, S., Zhao, S., He, Y. & Wang, J., 2021. Urea transport and hydrolysis in the rumen: A review. Anim. Nutr. 7, 989–996. https://doi.org/10.1016/j.aninu.2021.07.002.

Hoover, W. H., 1986. Chemical factors involved in ruminal fiber digestion1. J. Dairy Sci. 69, 2755–2766. https://doi.org/10.3168/jds.S0022-0302(86)80724-X.

Hou, Y., Yin, Y. & Wu, G., 2015. Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. Exp. Biol. Med. 240, 997–1007. https://doi.org/10.1177/1535370215587913.

Hristov, A. N., Bannink, A., Crompton, L. A., Huhtanen, P., Kreuzer, M., McGee, M., Nozière, P., Reynolds, C. K., Bayat, A. R., Yáñez-Ruiz, D. R., Dijkstra, J., Kebreab, E., Schwarm, A., Shingfield, K. J. & Yu, Z., 2019. *Invited review:* Nitrogen in ruminant nutrition: A review of measurement techniques. J. Dairy Sci. 102, 5811–5852. https://doi.org/10.3168/jds.2018-15829.

Kand, D. & Dickhoefer, U., 2021. The effects of rumen nitrogen balance on nutrient intake, nitrogen partitioning, and microbial protein synthesis in lactating dairy cows offered different dietary protein sources. J. Dairy Sci. 104, 4223–4235. https://doi.org/10.3168/jds.2019-18005.

Kellems, R. O. & Church, D. C., 2010. Livestock Feeds and Feeding. 6th ed. Pearson Prentice Hall.

Khandaker, Z. H., Uddin, M. M., Sultana, N. & Peters, K. J., 2012. Effect of supplementation of mustard oil cake on intake, digestibility and microbial protein synthesis of cattle in a straw-based diet in Bangladesh. Trop. Anim. Health Prod. 44, 791–800. https://doi.org/10.1007/s11250-011-9969-z.

Kropp, J. R., Johnson, R. R., Males, J. R. & Owens, F. N., 1977. Microbial protein synthesis with low quality roughage rations: Isonitrogenous substitution of urea for soybean meal. J. Anim. Sci. 45, 837–843. https://doi.org/10.2527/jas1977.454837x.

Mahmoudi-Abyane, M., Alipour, D. & Moghimi, H. R., 2020. Effects of different sources of nitrogen on performance, relative population of rumen microorganisms, ruminal fermentation, and blood parameters in male feedlotting lambs. Animal 14, 1438–1446. https://doi.org/10.1017/S175173111900291X.

Matsui, H., Ushida, K., Miyazaki, K. & Kojima, Y., 1998. Use of ratio of digested xylan to digested cellulose (X/C) as an index of fiber digestion in plant cell-wall material by ruminal microorganisms. Anim. Feed Sci. Technol. 71, 207–215. https://doi.org/10.1016/S0377-8401(97)00164-8.

McCoy, R. A., Stock, R. A., Klopfenstein, T. J., Shain, D. H. & Klemesrud, M. J., 1998. Effect of energy source and escape protein on receiving and finishing performance and health of calves. J. Anim. Sci. 76, 1488–1498.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A. & Wilkinson, R. G., 2011. Animal Nutrition. 7th ed. Pearson, Prentice Hall.

Mi, H., Ren, A., Zhu, J., Ran, T., Shen, W., Zhou, C., Zhang, B. & Tan, Z., 2022. Effects of different protein sources on nutrient disappearance, rumen fermentation parameters, and microbiota in dual-flow continuous culture system. AMB Express 12, 15. https://doi.org/10.1186/s13568-022-01358-1.

Milis, C. & Liamadis, D., 2008. Nutrient digestibility and energy value of sheep rations differing in protein level, main protein source, and non-forage fibre source. J. Anim. Physiol. Anim. Nutr. 92, 44–52. https://doi.org/10.1111/j.1439-0396.2007.00708.x.

Minson, D. J., 1990. Forage in Ruminant Nutrition. Academic Press, San Diego.

Neser, F. W. C., Erasmus, G. J. & Van Wyk, J. B., 2000. Genetic studies on the South African Mutton Merino: Growth traits. South Afr. J. Anim. Sci. 30, 172–177. https://doi.org/10.4314/sajas.v30i3.3849.

Niazifar, M., Besharati, M., Jabbar, M., Ghazanfar, S., Asad, M., Palangi, V., Eseceli, H. & Lackner, M., 2024. Slow-release non-protein nitrogen sources in animal nutrition: A review. Heliyon 10, e33752. https://doi.org/10.1016/j.heliyon.2024.e33752.

Nichols, K., de Carvalho, I. P. C., Rauch, R. & Martín-Tereso, J., 2022. Review: Unlocking the limitations of urea supply in ruminant diets by considering the natural mechanism of endogenous urea secretion. Animal 16, 100537. https://doi.org/10.1016/j.animal.2022.100537.

Nolte, J. V. E. & Ferreira, A. V., 2005. The effect of rumen degradable protein level and source on the duodenal essential amino acid profile of sheep. South Afr. J. Anim. Sci. 35, 162–171. https://doi.org/10.4314/sajas.v35i3.4055.

Nolte, J. V. E., Ferreira, A. V. & Köster, H. H., 2000. Effect of different levels of supplemental N from urea on intake and utilization of wheat straw by Dohne Merino wethers. South Afr. J. Anim. Sci. 30, 143–144.

National Research Council (NRC), 1996. Nutrient Requirements of Beef Cattle. 7th ed. National Academy Press, Washington D.C., USA.

National Research Council (NRC), 2001. Nutrient Requirements of Dairy Cattle. 7th Revised Edition. National Academy Press, Washington D.C., USA.

National Research Council (NRC), 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. The National Academies Press. Washington, DC, USA.

Oba, M. & Allen, M. S., 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82, 589–596. https://doi.org/10.3168/jds.S0022-0302(99)75271-9.

Oltjen, R. R., 1969. Effects of feeding ruminants non-protein nitrogen as the only nitrogen source. Am. Soc. Anim. Sci. 60, 673–682.

Polan, C. E., 1992. Protein and Amino Acids for Lactating Cows. In: Large Dairy Herd Management. Van Horn, H.H., Wilcox, C.J., eds. American Dairy Science Association. pp. 236–247.

Putri, E. M., Zain, M., Warly, L. & Hermon, H., 2021. Effects of rumen-degradable-to-undegradable protein ratio in ruminant diet on in vitro digestibility, rumen fermentation, and microbial protein synthesis. Vet. World. 14, 640–648. https://doi.org/10.14202/vetworld.2021.640-648.

Reed, K. F., Bonfá, H. C., Dijkstra, J., Casper, D. P. & Kebreab, E., 2017. Estimating the energetic cost of feeding excess dietary nitrogen to dairy cows. J. Dairy Sci. 100, 7116–7126. https://doi.org/10.3168/jds.2017-12584.

Statistical Analysis System (SAS), 1999. Statistical Analysis System User's Guide, 6th ed. SAS Institute Inc., Raleigh, North Carolina. USA.

Shain, D. H., Stock, R. A., Klopfenstein, T. J. & Herold, D. W., 1998. Effect of degradable intake protein level on finishing cattle performance and ruminal metabolism. J. Anim. Sci. 76, 242–248.

Shan, J. G., Tan, Z. L., Sun, Z. H., Hu, J. P., Tang, S. X., Jiang, H. L., Zhou, C. S., Wang, M. & Tayo, G. O., 2007. Limiting amino acids for growing goats fed a corn grain, soybean meal and maize stover based diet. Anim. Feed Sci. Technol. 139, 159–169. https://doi.org/10.1016/j.anifeedsci.2007.01.019.

Skele, N., Augustyn, W. C., O'Neill, H. A., Hugo, A. & Einkamerer, O. B., 2024. The effects of age of castration on carcass characteristics, meat quality, and fatty acid profile of finishing South African Mutton Merino lambs. Small Rumin. Res. 231, 107205. https://doi.org/10.1016/j.smallrumres.2024.107205.

Sniffen, C. J. & Robinson, P. H., 1987. Microbial growth and flow as influenced by dietary manipulations. J. Dairy Sci. 70, 425–441. https://doi.org/10.3168/jds.S0022-0302(87)80027-9.

Soto-Navarro, S. A., Goetsch, A. L., Sahlu, T., Puchala, R. & Dawson, L. J., 2003. Effects of ruminally-degraded nitrogen source and level in a high concentrate diet on site of digestion in yearling Boer × Spanish wether goats. Small Rumin. Res. 50, 117–128. https://doi.org/10.1016/S0921-4488(03)00120-2.

Swanson, K. C., Freetly, H. C. & Ferrell, C. L., 2004. Nitrogen balance in lambs fed a high-concentrate diet and infused with differing proportions of casein in the rumen and abomasum. J. Anim. Sci. 82, 495–501. https://doi.org/10.2527/2004.822495x.

Valizadeh, A., Kazemi-Bonchenari, M., Khodaei-Motlagh, M., Moradi, M. H. & Salem, A. Z. M., 2021. Effects of different rumen undegradable to rumen degradable protein ratios on performance, ruminal fermentation, urinary purine derivatives, and carcass characteristics of growing lambs fed a high wheat straw-based diet. Small Rumin. Res. 197, 106330. https://doi.org/10.1016/j.smallrumres.2021.106330.

Van Soest, P. J., Robertson, J. B. & Lewis, B., 1991. Polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Wahyono, T., Sholikin, M. M., Konca, Y., Obitsu, T., Sadarman, S. & Jayanegara, A., 2022. Effects of urea supplementation on ruminal fermentation characteristics, nutrient intake, digestibility, and performance in sheep: A meta-analysis. Vet. World. 15, 331–340. https://doi.org/10.14202/vetworld.2022.331-340.

Walker, D. K., Titgemeyer, E. C., Drouillard, J. S., Loe, E. R., Depenbusch, B. E. & Webb, A. S., 2006. Effects of ractopamine and protein source on growth performance and carcass characteristics of feedlot heifers. J. Anim. Sci. 84, 2795–2800. https://doi.org/10.2527/jas.2005-614.

Wu, G., Bazer, F. W., Dai, Z., Li, D., Wang, J. & Wu, Z., 2014. Amino acid nutrition in animals: Protein synthesis and beyond. Annu. Rev. Anim. Biosci. 2, 387–417. https://doi.org/10.1146/annurev-animal-022513-114113.

Zhao, X., Ali, S., Hassan, M. F., Bashir, M. A., Ni, X., Lv, C., Yang, H., Danzeng, B. & Quan, G., 2023. Effects of graded levels of dietary protein supplementation on milk yield, body weight gain, blood biochemical parameters, and gut microbiota in lactating ewes. Front. Vet. Sci. 10. https://doi.org/10.3389/fvets.2023.1223450.