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Effects of liquid protein feed on growth performance and ruminal metabolism of growing lambs fed low-quality forage and compared to conventional protein sources

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Abstract

The present study was conducted to assess the inclusion of liquid protein feed (maize steep liquor; MSL) in growing lambs fed low-quality forage (400 g/kg wheat straw, dry mater basis) compared with two protein sources (soybean meal; SBM, and cottonseed meal; CSM). Eighteen male Farahani lambs, average body weight 36 ± 3.3 kg, were allocated to individual pens for 9 weeks. Three protein sources were: (1) MSL; (2) SBM and (3) CSM. Feed intakes did not differ among treatments. Growth rate and feed conversion ratio were improved in SBM-fed lambs. Nitrogen efficiency was improved in MSL *v.* CSM-fed lambs. Digestibility of fibre was enhanced in lambs fed SBM diet. Ruminal short chain fatty acid was highest in lambs fed SMB and lowest in lambs fed CSM. The urinary allantoin concentration was greater in SBM-fed lambs, with improved microbial crude protein synthesis. Blood urea nitrogen tended to be reduced in SBM-fed lambs. Dressing percentage improved in SBM-fed lambs. Lambs fed with SBM also grew faster and more efficiently than lambs fed either of the other two diets. However, regardless of the positive effects of SBM on the performance and ruminal fermentation profile observed in the current study, the MSL diet could be a profitable protein source in comparison to CSM in growing lambs fed low-quality forage. In conclusion, the present study indicates a positive outlook for feeding a liquid protein source to growing lambs fed low-quality forage such as wheat straw.

Introduction

Cereal straws are agricultural by-products of poor nutritive value for ruminants because of low digestibility and low protein concentration (Antongiovanni *et al.*, 1991). Although applying chemical treatments to low-quality forage such as wheat straw has been evaluated before (Horton *et al.*, 1982; Antongiovanni *et al.*, 1991), there are some concerns related to animal and labour health while working with chemical compounds. Some other strategies to improve low-quality forage nutrient utilization provide enough ruminal degradable nitrogen (RDN) for microbial growth (Males, 1987; Salisbury *et al.*, 2004), in addition to providing adequate rumen undegradable protein levels (Batista *et al.*, 2016) to improve urea recycling into the gastrointestinal tract. Indeed, ammonia may be a first limiting nitrogen source for fibre-degrading bacteria (Griswold *et al.*, 1996). Therefore, providing an adequate RDN source could positively affect the nutrient utilization of low-quality forage through providing ruminal ammonia nitrogen. It has also been determined that addition of peptide and amino acid nitrogen sources improve digestion of fibre (Griswold *et al.*, 1996) and may have additional nutritional benefits for host animals (Reynal *et al.*, 2007). Males (1987) reported that natural protein sources were more effective than non-protein nitrogen sources by providing more amino acids as well as higher peptide contents when high levels of wheat straw are fed to growing beef cattle. Soybean meal (SBM) and cottonseed meal (CSM) are solid natural protein sources and have not been compared with a liquid protein source, although some studies have evaluated the availability of liquid protein feeds in the rumen (Elwakeel *et al.*, 2007) and evaluated the inclusion of liquid protein feeds in animal diets (Schingoenthe, 1976; Bowman *et al.*, 1995; Walker *et al.*, 2013). However, there is a lack of data regarding the inclusion of liquid protein in the diets of animals fed with a low-quality forage diet. The comparison of a liquid protein feed and conventional protein sources used in ruminant nutrition is not well documented.

Maize steep liquor (MSL) is an inexpensive, palatable and abundant by-product of wet-maize milling, which has been evaluated in cattle (Ribeiro-Filho and Trenkle, 2002), dairy

cows (Wickersham *et al.*, 2004) and growing lambs (Azizi-Shotorkhoft *et al.*, 2016). This feedstuff is a viscous slurry, which has a high soluble protein content, but data regarding the comparison of its inclusion with other conventional protein sources is scarce. The current study hypothesized that inclusion of MSL as a liquid natural protein source could improve nutrient utilization of a low-quality forage basal diet.

The current study aims to evaluate the effects of including MSL as a liquid protein source in the diets of growing lambs, compared to two conventional solid protein sources (SBM and CSM), on growth performance, ruminal fermentation, microbial protein synthesis, blood metabolites and liver enzymes, and carcass characteristics of growing lambs fed high dietary levels of wheat straw.

Materials and methods

Lamb management and diets

The present study was conducted in the Animal Production Station of Arak University, Arak, Iran, according to the guidelines of the Iranian Council of Animal Care (1995). The study lasted 9 weeks, with the first week for adaptation of the animals to the experimental diets and conditions. Average environmental conditions in the farm throughout the study were: temperature ranging between 25 and 31 °C, relative humidity in the range 61–67% and day length of 16–17 h/day. Eighteen male Farahani lambs (36 ± 3.3 kg body weight, age 5.8 ± 0.23 months) were used in a complete randomized design with three different protein sources (six animals per experimental group). The total mixed ration (TMR) fed to the lambs was formulated according to National Research Council (NRC, 2007 – small ruminant requirement recommendations for lambs) and its ingredient and chemical composition is shown Table 1. The animals were housed in individual pens and had free access to water. The basal diet contained the same amount of wheat straw [400 g/kg; dry matter (DM) basis] in all treatments. To obtain iso-nitrogenous experimental diets, the MSL, SBM and CSM were included at 130, 120 and 150 g/kg (DM basis) of TMR, respectively. The MSL was provided by a Glucosan factory (Qazvin, Iran). The TMR was fed to animals in two equal meals at 08.00 and 16.00 h. Orts were collected and weighed once daily at 07.30 h and the amount of feed offered to each animal adjusted to attain refusals of 10% intake. The DM intake was calculated depending on the DM composition of feed and Orts.

Sample collection, chemical and biochemical analysis

Body weights were recorded on the first day of the experiment and at 10 day-intervals thereafter until the end of the experiment (i.e. 9 weeks later). Animals were weighed before the morning meal to minimize the effect of digestive contents on body weight. Feed conversion ratio (FCR) was calculated as kg intake per kg of gain. The weight gain per 100 g of nitrogen (N) intake was considered as nitrogen conversion ratio into weight gain. Samples of each TMR were collected daily and DM was determined by drying at 60 °C for 48 h (AOAC, 1995). Composite samples of each TMR were ground in a Wiley mill through a 1 mm screen, and analysed to determine total nitrogen, ether extract and ash contents (AOAC, 1995). The methods of Van Soest *et al.* (1991) were used for the determination of neutral detergent fibre (NDF), which was assayed with a heat-stable amylase and

Table 1. Feed ingredients and chemical composition of experimental diets (g/kg dry matter; unless otherwise stated)

Ingredients	Protein sources ^a		
	MSL	SBM	CSM
Wheat straw, chopped	400	400	400
Wheat bran	140	150	120
Barley grain, rolled	315	315	315
Maize steep liquor ^b	130	0	0
Soybean meal ^c	0	120	0
Cottonseed meal ^d	0	0	150
Calcium carbonate	2.5	2.5	2.5
Salt	2.5	2.5	2.5
Mineral and vitamin premix ^e	10	10	10
Chemical composition			
Metabolizable energy ^f , MJ/kg	10.27	10.39	10.15
Non fibre carbohydrates ^f	369	360	357
Neutral detergent fibre	416	420	425
Ether extract	24.1	27.2	25.3
Ash	53.8	55.7	56.7
Calcium	6	6	6
Phosphorous	4	4	4
Crude protein	137	137	136
Rumen degradable protein	89.3	87.2	78.7
Rumen un-degradable protein ^f	48.2	50.4	58.1
Lysine, g/kg of CP ^f	42.2	46.1	38.2
Methionine, g/kg of CP ^f	14.8	14.9	15.4
Lucien, g/kg of CP ^f	60.0	64.7	57.6

^aProtein sources were: maize steep liquor (MSL), soybean meal (SBM) and cottonseed meal (CSM) feeding in growing lambs fed high level of wheat straw diet [400 g/kg dry matter (DM) basis].

^bMaize steep liquor contained 420 g/kg crude protein (DM basis). Dry matter content of MSL was 520 g/kg and its soluble protein content was 360 g per kg DM.

^cSoybean meal solvent contained 450 g/kg crude protein (DM basis).

^dCottonseed meal contained 380 g/kg crude protein (DM basis).

^eContained per kilogram of supplement: 2 500 000 IU vitamin A, 40 000 IU vitamin D₃, 1000 vitamin E, 750 g manganese, 110 g calcium, 2000 mg zinc, 45 g phosphorus, 20 g magnesium, 15 g sodium, 1000 mg iron, 8 mg cobalt, 500 mg copper, 20 mg iodine and 10 mg selenium.

^fCalculated based on NRC (2001).

expressed inclusive of residual ash. The soluble fraction of protein as the proportion of total protein (TP) in MSL was measured by a protein fractionation system (Licitra *et al.*, 1996) with some modifications described by Eghbali *et al.* (2011) in using phosphate buffer solution.

On the last 5 days of the experiment, two faecal grab samples were collected daily from each animal at 6 and 18 h after the morning meal (ten samples for each lamb). Faecal samples were dried in a forced draft oven (60 °C; 72 h), and then ground in a Wiley mill through a 1 mm screen. Aliquots of all faecal samples collected for each lamb were mixed to obtain one composite sample for each animal. These faecal samples were analysed to determine total nitrogen, ether extract and NDF based on the procedures mentioned above for TMR samples. Apparent total

tract digestibility coefficients of dietary components were measured using acid-insoluble ash as an internal marker (Van Keulen and Young, 1977). In this method, the acid-insoluble ash concentration was determined in feed and faeces by treating the ash with a 2 N hydrochloric acid (HCl) solution.

Samples of rumen fluid were collected using a stomach tube at 4 h after the morning feeding on days 28 and 56 of the experiment. The first 5 ml was eliminated because of possible saliva contamination and ruminal pH was measured immediately (HI 8314 membrane pH meter, Hanna Instruments, Villafranca, Italy). The rumen samples were squeezed through four layers of cheesecloth. Two samples of rumen fluid (8 ml each) were mixed with 0.2 ml sulphuric acid 50% and stored at -20°C . Just before analysis, samples were thawed, centrifuged at 10 000 g (4°C , 20 min). The clarified supernatant was then decanted and analysed for ruminal ammonia-N using a modified phenol-hypochlorite method adapted from Broderick and Kang (1980). In this method, phenol-hypochlorite and ninhydrin colorimetric procedures were used to measure ammonia concentration in the ruminal fluid of animals. The other sample was used for the analysis of short chain fatty acids (SCFA) using a gas chromatograph (model CP-9002; Chrom-pack, Delft, The Netherlands) with a 50 m (0.32 mm ID) silica-fused column (CP-Wax Chrompack Capillary Column Varian, Palo Alto, CA, USA) and using helium as the carrier gas, as described previously (Kazemi-Bonchenari *et al.*, 2016).

The spot urine sampling technique was used for the estimation of daily urine output from creatinine concentration (Chen *et al.*, 1995). On days 29 and 58 of the experiment, spot urine samples were collected from each animal during the morning (between 09.00 and 11.00 h). Samples were collected when lambs urinated spontaneously. An aliquot of 10 ml of each sample was diluted immediately with 90 ml of 0.036 N sulphuric acid and stored at -20°C for analysis. Later, urine samples were thawed at room temperature and analysed to determine the creatinine, uric acid and allantoin as described previously (Kazemi-Bonchenari *et al.*, 2017). In this method, total urine volume was estimated based on daily creatinine excretion, due to the close relationship between body mass and creatinine excretion. An average of 9.79 mg/kg BW for creatinine excretion was used for total urine volume estimated as previously found for sheep (David *et al.*, 2015). Thereafter, the total excretion of allantoin and uric acid was calculated from estimated daily urine output and determined metabolite concentrations. The synthesis of microbial N in the rumen was calculated from daily urinary purine derivative (PD) output using the following equation described by Chen and Gomes (1992);

$$\text{Microbial nitrogen } \left(\frac{\text{gN}}{\text{day}}\right) = \frac{X \left(\frac{\text{mmol}}{\text{day}}\right) \times 70}{0.116 \times 0.83 \times 1000}$$

where X is the microbial purine absorbed (mmol/day), 70 is the nitrogen content of purines coefficient (mg N/mmol), 0.116 is the ratio of purine nitrogen to total nitrogen in mixed rumen microbes which is 11.6:100, and 0.83 is the average digestibility of microbial purines.

Blood was sampled from the jugular vein of each lamb twice throughout the study (on days 30 and 60 of the experiment), 4 h after the morning feed. Blood samples were heparinized (Na-heparin) and stored at 2°C ; then centrifuged at 3000 g

(4°C , 15 min) and the plasma stored at -20°C . After thawing, the samples were analysed to determine the concentrations of glucose (Kit No. 9308), albumin (Kit No. 9307), TP (Kit No. 9304), blood urea nitrogen (BUN) (Kit No. 93013), aspartate aminotransferase (AST) (Kit No. 11840) and alanine aminotransferase (ALT) (Kit No. 11940) using commercial kits in accordance with the manufacturer's instructions (Pars Azmoon Co., Tehran, Iran).

At the end of the experiment (day 70), lambs were slaughtered and carcass characteristics measured. Dressing percentage (the ratio of hot carcass weight to live body weight) as well as different organ weights such as liver, heart and gastrointestinal tract were measured. Fat distribution in different deposition sites throughout the body was also measured. The deposited fat in fat-tail, mesenteric and abdominal sites was recorded by weighing the fat mass. However, the back-fat thickness was measured by fat depth (mm) coverage between ribs 12 and 13, measured by callipers (Esquivelzeta *et al.*, 2012). Moreover, the total fat deposited in the liver was measured by the method of Folch *et al.* (1957): briefly, fat deposited in the liver was extracted by a detergent containing chloroform, methanol and water in the ratio 85:14:1 (by volume).

Statistical analysis

The data were analysed applying SAS (version 9.1; SAS Inst. Inc., Cary, NC, USA) and using PROC MIXED using POLYANOVA routine for performance variables where there were repeated measurements over time (feed intake, daily gain, FCR, nitrogen intake and nitrogen conversion ratio) using the following model:

$$Y_{ijkl} = \mu + A_i + Pr_j + T_k + Pr T_{jk} + \beta(X_i - \bar{X}) + \varepsilon_{ijkl}$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, A_i is the effect of animal, Pr_j is the effect of protein source j (MSL, SBM and CSM), T_k is the effect of sampling time k (different sampling times), $Pr T_{jk}$ is the interaction between time k and protein source j , $\beta(X_i - \bar{X})$ is the covariate variable (used for BW with initial BW as covariate) and ε_{ijkl} is the residual error. The POLYANOVA model was considered for performance data because there were six time repetitions for each of these variables. Linear and quadratic contrasts were reported from this statistical analysis route output. The variables with two repetitions throughout the study (ruminal fermentation metabolites, microbial protein synthesis and blood metabolites) were analysed based on the above-mentioned model in PROC MIXED and linear contrast was reported as a general P value. The data for variables that did not have repeated measurements over time (apparent digestibility coefficient of components and carcass characteristics) were analysed using PROC GLM using the following model:

$$Y_i = \mu + Pr_i + \varepsilon_i$$

where Y_i is the dependent variable, μ is the overall mean, Pr_i is the effect of protein source i (MSL, SBM and CSM) and ε_i is the residual error. The model contained lamb effect within the treatment as a random effect. Differences between least squares means were considered significant at $P \leq 0.05$, and differences were considered to indicate a trend towards significance at $0.05 < P \leq 0.10$. In addition to overall treatment comparisons, orthogonal contrasts were constructed to compare (1) MSL *v.* SBM and CSM and (2) SBM *v.* CSM.

Table 2. Effects of maize steep liquor inclusion in growing lambs' diet fed high level of wheat straw on growth and component digestibility coefficient and compared to soybean meal and cottonseed meal ($n = 18$)

Item	Protein sources ^a				P value				Contrasts	
	MSL	SBM	CSM	S.E.M.	Treat	Treat × Time	Time		MSL v. SBM and CSM	SBM v. CSM
							Linear	Quadratic		
Intake, gain and efficiency										
Final body weight (kg)	49	51	48	1.7	0.519	–	–	–	0.459	0.509
Dry matter intake (g/day)	1598	1556	1611	24.5	0.259	0.465	0.011	0.152	0.651	0.301
Average daily gain (g/day)	188	207	176	7.1	0.026	0.319	0.191	0.252	0.693	0.060
FCR, DMI/ADG ^b	9.0	8.0	9.3	0.37	0.032	0.439	0.010	0.712	0.502	0.083
Nitrogen intake (g/day)	35.0	34.1	35.3	0.53	0.257	0.465	0.013	0.153	0.658	0.300
N to gain conversion ratio ^c	542	608	505	19.3	0.001	0.587	<0.001	0.558	0.550	0.022
Coefficient of digestibility										
Dry matter	0.64	0.64	0.63	0.026	0.854	–	–	–	0.962	0.879
Crude protein	0.67	0.74	0.67	0.024	0.083	–	–	–	0.336	0.077
Neutral detergent fibre	0.49	0.51	0.40	0.017	0.051	–	–	–	0.248	0.045
Ether extract	0.73	0.73	0.72	0.024	0.926	–	–	–	0.909	0.898

^aProtein sources were: maize steep liquor (MSL), soybean meal (SBM) and cottonseed meal (CSM) feeding in growing lambs fed high level of wheat straw diet (400 g/kg DM basis).

^bFeed conversion ratio is calculated as kg dry matter intake per kg of weight gain.

^cNitrogen to gain conversion ratio calculated as the amount of weight gain per 100 g of N intake.

Results

Chemical analysis, animal growth and components digestibility coefficient

The crude protein (CP) contents for MSL, SBM and CSM were 420, 450 and 380 g/kg (DM basis), respectively. The DM content of MSL was 520 g/kg and the soluble protein content for MSL was 360 g/kg DM.

Feed intake did not differ ($P = 0.259$) among different protein sources, while the average daily gain (ADG) ($P = 0.026$) and FCR were improved ($P = 0.032$) in SBM-fed lambs compared with CSM and MSL animals (Table 2). The lowest daily gain was observed in CSM-fed lambs (176 g/day), and in contrast, the greatest gain was seen in SBM-fed lambs (207 g/day). The FCR tended towards significance in SBM-fed lambs compared to CSM ($P = 0.083$). Although the nitrogen intake was constant among different protein sources in the current study ($P = 0.257$), the nitrogen conversion ratio (g weight gain per 100 g nitrogen intake) was greater in SBM ($P = 0.022$) v. CSM lambs. Dry matter intake, FCR, nitrogen intake and nitrogen conversion ratio were influenced with time ($P = 0.024$); however, the daily gain was not affected by time in the current study.

The total tract digestibility coefficient data showed an improvement in fibre digestibility for SBM compared with MSL and CSM lambs ($P = 0.051$) (Table 2). Digestibility of CP tended to be greater in SBM v. CSM lambs ($P = 0.077$). The other nutrient digestibility measurements (DM; $P = 0.854$; and EE; $P = 0.926$) were not affected by dietary protein sources (Table 2).

Rumen fermentation pattern

Ruminal fluid pH was not different ($P = 0.334$) among different protein sources fed to growing lambs (Table 3). Ruminal ammonia nitrogen was enhanced ($P = 0.010$) in both CSM and MSL v.

SBM lambs. Total SCFA production was different among different protein sources, with SBM shown to have the greatest concentration ($P = 0.026$). With the exception of isovalerate, which has relatively constant concentration among different protein sources in the current study ($P = 0.561$), the remaining individual SCFAs were shown to have a greater concentration in SBM-fed lambs v. the other experimental diets. The statistical contrasts showed that MSL-fed lambs produced less butyrate ($P = 0.001$) and had lower acetate to propionate ratios ($P = 0.034$) compared with either SBM or CSM diets (Table 3). Effect of time did not differ for rumen fermentation variables in the current study ($P = 0.846$).

Urinary purine derivatives excretion and microbial protein synthesis

The allantoin concentration was increased ($P = 0.011$) in SBM v. CSM- and MSL-fed lambs. Although uric acid concentration did not differ among different protein sources, total PD concentration was greater in SBM-fed lambs ($P = 0.001$). Microbial protein synthesis estimated through PD excretion was greater ($P = 0.001$) in SBM-fed lambs rather than those fed CSM or MSL (Table 3). Although MSL-fed lambs had lower PD excretion and lower microbial crude protein (MCP) production compared to SBM-fed lambs, the above-mentioned parameter values for this protein source were similar or greater rather than those observed for CSM-fed lambs (Table 3). Microbial protein synthesis variables were not influenced by sampling time in the current study ($P = 0.714$).

Blood metabolites and liver enzymes activities

The blood concentrations of glucose ($P = 0.915$), TP ($P = 0.254$), albumin ($P = 0.410$), triglyceride ($P = 0.211$) and cholesterol ($P = 0.581$) were not different among dietary protein sources.

Table 3. Effects of maize steep liquor inclusion in growing lambs' diet fed high level of wheat straw on ruminal fermentation pattern and microbial protein synthesis and compared to soybean meal and cottonseed meal ($n = 18$)

Item	Protein sources ^a				P value			Contrasts	
	MSL	SBM	CSM	S.E.M.	Treat	Linear	Treat × Time	MSL v. SBM and CSM	SBM v. CSM
Rumen fermentation pattern									
Ruminal pH	5.92	6.00	6.01	0.041	0.334	0.452	0.106	0.163	0.255
Ruminal ammoniacal nitrogen (NH ₃ -N; mg/dl)	16.8	13.5	15.1	0.46	0.010	0.513	0.965	0.001	0.001
Short chain fatty acids (mM)	78	88	71	2.6	0.001	0.669	0.846	0.809	0.026
Individual short chain fatty acids (mM)									
Acetate (A)	38	42	35	1.4	0.001	0.713	0.775	0.853	0.057
Propionate (P)	21	21	16	1.6	0.042	0.881	0.977	0.242	0.093
A:P ^b	1.9	2.1	2.4	0.13	0.034	0.976	0.990	0.036	0.316
Butyrate	16.4	20.5	17.1	0.70	0.001	0.692	0.521	0.001	0.001
Valerate	1.4	2.1	1.4	0.15	0.001	0.924	0.765	0.066	0.035
Isovalerate	1.2	1.3	1.1	0.12	0.561	0.202	0.884	0.977	0.605
BCVFA ^c	2.6	3.4	2.5	0.21	0.013	0.540	0.732	0.187	0.013
Purine derivatives and microbial protein synthesis									
Allantoin (mmol/day)	7.1	9.2	7.0	0.40	0.011	0.741	0.820	0.051	0.001
Uric acid (mmol/day)	0.38	0.39	0.40	0.043	0.957	0.147	0.556	0.822	0.743
TPD ^d (mmol/day)	7.5	9.6	7.4	0.42	0.001	0.714	0.875	0.063	0.001
MCP ^e (g/d)	40	51	39	2.2	0.001	0.714	0.875	0.064	0.001

^aProtein sources were: maize steep liquor (MSL), soybean meal (SBM) and cottonseed meal (CSM) feeding in growing lambs fed high level of wheat straw diet (400 g/kg dry matter basis).

^bA:P is the proportional ratio of acetate to propionate in the ruminal fluid.

^cBranched chain volatile fatty acids (BCVFA) are the molar proportion of valerate + molar proportion of isovalerate.

^dTotal purine derivatives (TPD) is allantoin + uric acid.

^eMicrobial crude protein synthesis calculated through urinary TDP excretion based on Chen and Gomes (1992).

Liver enzymes concentrations (AST and ALT) as well as other blood metabolites were not different among protein sources in the present study; however, the blood urea concentration was numerically increased in CSM and MSL compared to SBM-fed animals ($P = 0.073$) (Table 4). Time had no effect on blood biochemical variables in the present study ($P = 0.197$).

Carcass characteristics and fat deposition sites

Slaughter weight was no different among dietary protein sources ($P = 0.523$); while hot carcass weight ($P = 0.011$) and dressing percentage were greater in SBM-fed lambs ($P = 0.010$). Results indicated an improvement in carcass weight and efficiency when comparing MSL with SBM and CSM ($P = 0.042$). Internal organ weights [liver ($P = 0.614$); kidney ($P = 0.559$); heart ($P = 0.278$)] as well as gastrointestinal weight ($P = 0.742$) did not differ among different protein sources. There was no difference among different fat deposition sites for different protein sources in the current study ($P = 0.310$) (Table 5).

Discussion

Growth, total tract digestibility coefficients and ruminal fermentation pattern

Energy is the main factor controlling feed intake in animals (Allen *et al.*, 2009); therefore, the similar energy contents of the

experimental diets in the current study probably resulted in similar intakes in different treatments. Despite the similar intake among different protein sources, the greater gain was observed with SBM compared to CSM or MSL-fed lambs. This could be related to rumen fermentation patterns as well as the total tract digestibility coefficient. Ruminal SCFA concentration was greatest for lambs fed SMB and least for CSM, with MSL-fed lambs having intermediate SCFA concentrations. This shows greater energy extracted from the diet towards SCFA production, and as stated previously the main energy source for ruminant performance is SCFA (Bergman, 1990) which could subsequently increase gain and improve FCR in the SBM diet. The greater individual volatile fatty acids, especially propionate and butyrate, could also influence gain and efficiency from the stoichiometry point of view (Ørskov and Ryle, 1990) which was observed in the SBM diet in the current study. The improved fibre digestion in SBM-fed lambs could also be an effective factor in improving gain. Soybean meal is a protein source which could produce relatively high peptide levels (Wang *et al.*, 2017). Addition of a peptide source in continuous culture has been reported to increase branched chain volatile fatty acid (BCVFA) production (Griswold *et al.*, 1996), which has potential for increasing fibre digestion (Gorosito *et al.*, 1985). In the present study, BCFVA was increased with SBM feeding in comparison with the other two protein sources. The results of the present study revealed that feeding SBM resulted in greater BCVFA as well as greater

Table 4. Effects of maize steep liquor inclusion in growing lambs' diet fed high level of wheat straw on blood metabolites and liver enzymes and compared to soybean meal and cottonseed meal ($n = 18$)

Item	Protein sources ^a				P value			Contrasts	
	MSL	SBM	CSM	S.E.M.	Treat	Linear	Treat × Time	MSL v. SBM and CSM	SBM v. CSM
Glucose (mg/dl)	56	57	57	2.8	0.915	0.197	0.519	0.734	0.747
Total protein (g/dl)	5.6	6.1	5.8	0.20	0.254	0.596	0.357	0.372	0.186
Albumin (g/dl)	4.05	4.17	4.04	0.087	0.410	0.467	0.599	0.554	0.278
Triglyceride (mg/dl)	23	28	24	2.2	0.211	0.696	0.846	0.231	0.116
Cholesterol (mg/dl)	35	35	37	1.8	0.581	0.515	0.207	0.241	0.526
Blood urea nitrogen (mg/dl)	17.6	15.0	16.1	0.74	0.073	0.525	0.192	0.073	0.048
Aspartate aminotransferase (U/l)	54	51	53	2.2	0.664	0.395	0.854	0.644	0.590
Alanine aminotransferase, U/l	24	31	25	4.3	0.635	0.110	0.810	0.541	0.349

^aProtein sources were: maize steep liquor (MSL), soybean meal (SBM) and cottonseed meal (CSM) feeding in growing lambs fed high level of wheat straw diet (400 g/kg dry matter basis).

Table 5. Effects of maize steep liquor inclusion in growing lambs' diet fed high level of wheat straw on carcass characteristics and fat deposition (kg) and compared to soybean meal and cottonseed meal ($n = 18$)

Item	Protein sources ^a				S.E.M.	Treat	Contrasts	
	MSL	SBM	CSM	MSL v. SBM and CSM			SBM v. CSM	
Carcass characters								
Slaughter weight	49	51	48	1.7	0.523	0.354	0.264	
Hot carcass weight	20.2	22.8	19.6	0.69	0.011	0.043	0.056	
Dressing percentage ^b (no fat-tail)	41.1	44.9	40.9	0.76	0.010	0.045	0.027	
Dressing percentage (with fat-tail)	49	53	48	1.2	0.042	0.096	0.028	
Empty gastrointestinal weight	2.3	2.3	2.4	0.12	0.742	0.485	0.458	
Liver weight	0.59	0.64	0.62	0.032	0.614	0.931	0.677	
Kidney weight	0.21	0.18	0.19	0.021	0.559	0.896	0.860	
Heart weight	0.17	0.16	0.20	0.023	0.278	0.168	0.310	
Fat distribution in the body								
Fat-tail weight	4.0	4.1	3.6	0.36	0.645	0.369	0.383	
Mesenteric fat weight	0.57	0.63	0.53	0.058	0.435	0.310	0.544	
Abdominal fat weight	0.51	0.39	0.47	0.064	0.444	0.760	0.645	
Back-fat thickness ^c	6.4	7.0	7.7	0.54	0.276	0.151	0.187	
Fat deposited in liver ^d	7.0	6.1	6.4	0.35	0.218	0.754	0.499	

^aProtein sources were: maize steep liquor (MSL), soybean meal (SBM) and cottonseed meal (CSM) feeding in growing lambs fed high level of wheat straw diet (400 g/kg dry matter basis).

^bDressing percentage was calculated as hot carcass weight divided by slaughter weight.

^cBack-fat depth (mm) was considered as fat thickness covered between ribs 12 and 13 measured by callipers (Esquivelzeta *et al.*, 2012).

^dAs the per cent of liver weight which was measured based on Folch *et al.* (1957).

fibre digestibility compared to CSM and MSL. It seems that greater fibre digestibility supplies more organic matter as a substrate for the production of SCFA in the SBM diet. Regardless of the positive effects of SBM on growth and ruminal fermentation pattern in the current study, comparison of the MSL and CSM protein sources indicated that both ruminal SCFA as well as propionate concentrations were greater in MSL-fed lambs, which may partly explain the greater gain and improved FCR in this diet (8.96 v. 9.31 for MSL and CSM, respectively). Improved fibre digestion in MSL compared to CSM (0.49 v.

0.40 for MSL and CSM fibre digestibility coefficients, respectively) may be partly related to greater RDN for microbes, supplied through greater RDP in the diet, which had a positive influence on cellulolytic bacterial activity. The related mechanism for greater RDN effect on cellulolytic bacteria activity has been stated in previous works (Griswold *et al.*, 1996, 2003). Considering the rumen fermentation pattern and nutrient digestibility, these indicate that greater fibre digestibility resulted in greater SCFA concentration, and in turn, greater BCVFA caused greater fibre digestibility. Results show that although MSL as a liquid protein

feed was not as profitable as SBM in lambs' growth, it was more efficient than CSM in parameters such as rumen fermentation profile and fibre digestibility. Considering the effect of time on performance in the current study, it is clear that DM intake, FCR, nitrogen intake and nitrogen conversion ratio were influenced by different sampling times. The above-mentioned traits responded to time throughout the study. This clarifies that regardless of protein source in the current study, intake level, FCR and nitrogen efficiency may be influenced by animal age, which is mostly related to whole-body metabolism of the animal at different ages (Atti and Mahouachi, 2011). Zhao *et al.* (2016) stated that younger lambs have better nitrogen efficiency in comparison with older ones, which could consequently affect whole-body efficiency towards better FCR.

Ruminal pH fluid did not differ among different protein sources in the current study and all recorded pH values were in range for healthy animals (>5.8) (Penner *et al.*, 2007). Greater ruminal ammonia nitrogen concentration in both MSL and CSM diets showed lower nitrogen efficiency in these protein sources compared with SBM-fed animals. In agreement with rumen fermentation profile results in the current study, looking at the nitrogen conversion ratio into a gain (weight gain per 100 g of nitrogen intake) shows that this variable was greater for the SBM diet compared to MSL or CSM. Regardless of the greater nitrogen efficiency observed for SBM-fed lambs, comparing the results obtained for MSL and CSM indicates a greater nitrogen conversion ratio for MSL in comparison with CSM-fed lambs (542 *v.* 505 for MSL and CSM, respectively). The greater nitrogen conversion ratio for MSL compared to CSM-fed lambs may relate to improved fibre digestibility and increased ruminal SCFA production in this protein source.

Urinary purine derivatives excretion and microbial protein synthesis

Urinary allantoin was increased in lambs fed SBM and, because the main portion of excreted PD is allantoin, the urinary excreted PD was also greater for this protein source. The results of the current study indicated that SBM was the source of protein preferred by ruminal microbes in comparison with MSL and CSM. Based on the previous literature, optimal ammonia nitrogen as well as optimum peptide nitrogen in the ruminal fluid are necessary to have adequate MCP production (Griswold *et al.*, 1996). Based on the findings of the current study, it could be suggested that SBM provided enough ammonia nitrogen and peptide nitrogen to obtain greater MCP. Regarding the microbial protein synthesis, comparing the MSL and CSM diet results suggests that neither was highly preferred by ruminal microbes. Lower MCP observed in both MSL and CSM diets in comparison with SBM may be partly related to greater ruminal ammonia nitrogen concentration in these two treatments compared to SBM animals. The current results suggest that incorporating SBM with low-quality forage fed to growing lambs caused better nitrogen capturing than either MSL or CSM; therefore, we observed greater N efficiency in lambs fed SBM. The results of the current study indicate that ruminal ammonia concentration ~14 mg/dl was enough for optimum microbial growth when a high dietary level of wheat straw was fed to lambs and the greater MCP obtained in SBM was probably due to greater peptide concentration in ruminal fluid released from SBM. Future studies may need more consideration from the nitrogen efficiency point of view when liquid protein feed is supplemented to ruminants.

Blood metabolites and liver enzymes

Among the metabolites, BUN concentration was lower in SBM-fed lambs in comparison with other protein sources. As indicated previously, BUN can be used as an indicator of rumen nitrogen capture and associated with rumen ammonia concentration (DePeters and Ferguson, 1992). Lower BUN concentration observed in SBM-fed lambs revealed greater nitrogen capturing and subsequently its efficiency in this diet. The greater BUN in MSL and CSM diets compared to SBM-fed animals was in line with greater ruminal ammonia concentrations observed for these protein sources. Propionate concentration changed among diets and similar changes in blood glucose level were expected because propionate is the main glucose precursor in the liver (Bergman, 1990), but blood glucose concentration was similar among different protein sources. Even though blood glucose is a function of ruminal propionate concentration, it is an indicator of the energy status of the body and is a function of intake level that provides more energy to metabolism (Reynolds *et al.*, 2003), which was constant in the current study. Total protein and albumin were also constant among experimental diets, partly due to similar protein levels of the experimental diets. Similarly, due to the relatively constant amounts of ether extract in the diets, cholesterol and triglycerides concentrations were not different among dietary protein sources in the current study. Similar blood metabolite ranges were found for MSL- and CSM-fed lambs, indicating that MSL as a liquid protein source has similar effects to a solid protein source on body energy indicators; this was probably due to similar intake in lambs fed different protein sources. No blood variables were influenced by sampling time in the current study. Previous works have shown that sampling time can affect some blood metabolite concentrations in pre-weaned ruminants compared to mature ruminants (Kazemi-Bonchenari *et al.*, 2018). However, the experimental lambs used in the current study were mature ruminants and blood metabolites were mostly affected by experimental treatments rather than their physiological variations.

There were no differences between AST and ALT enzyme concentrations among lambs fed different experimental diets. Liver function is influenced by different situations such as reduced appetite and high fat deposition (Cebra *et al.*, 1997), or when feeding high-grain diets through reducing rumen pH (Nagaraja and Lechtenberg, 2007). The results of the present study show that liver function was not negatively influenced with either liquid or solid protein source in the current study.

Carcass characteristics and fat deposition sites

No differences were found regarding most carcass characteristics in different protein sources fed to animals. However, the hot carcass weight and dressing percentage were increased in SBM-fed lambs. Comparing the MSL and CSM results in carcass characteristics indicated a slight improvement in carcass weight with MSL-fed lambs. Previously, it has been stated that energy (Craddock *et al.*, 1974) and protein level (Prado *et al.*, 2014) are the most effective factors influencing carcass characteristics in meat production animals. However, the energy and protein levels were similar among different experimental diets in the current study and the only variable was the protein source included in the diet. Examining SCFA produced in the rumen of the animals indicated that the greatest value was observed for SBM-fed lambs. Indeed, SCFA is the main source of energy in ruminants

(Bergman, 1990) that can improve animal body gain. In addition, the greatest nitrogen conversion ratio to gain was observed in the SBM diet, indicating better nutrient partitioning into carcass deposition. Higher digestibility of amino acids in SBM than found in previous studies (Khorasani *et al.*, 1990) could also be an issue in the better dressing percentage found in SBM diet. Dabiri and Thonney (2004) found the best growth rate and efficiency in growing lambs fed SBM, compared with fish meal. Finally, better fibre digestibility was observed in SBM-fed lambs *v.* MSL and CSM diets, suggesting an improvement in dressing percentage. For all these reasons, it was found that SBM has the potential to improve dressing percentage compared to MSL and CSM in growing lambs fed low-quality forage diet.

It has been revealed in ruminants that greater energy intake (Beauchemin *et al.*, 1995) or greater glucose concentration could influence fat deposition throughout the body (Hocquette *et al.*, 1998). In the current study, both intake level and blood glucose concentration were in similar ranges among experimental diets; hence, fat accumulation in different deposition sites was relatively homogenous among different protein sources. The results indicate that a liquid protein source could change neither fat content nor fat deposition site in growing lambs in comparison with solid protein sources.

Conclusion

Based on the current results, SBM could be a better dietary protein source for lambs fed low-quality forage (wheat straw) in comparison with MSL and CSM. However, results show that liquid protein feed (MSL) is recommended *v.* CSM when low-quality forage is included in growing lambs' diets. Greater ruminal volatile fatty acid production and enhanced nitrogen efficiency were seen by feeding MSL compared to CSM, showing the profitability of a liquid protein source while feeding low-quality forage diet in growing lambs.

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Conflict of interest. None.

Ethical standards. The experiments in this work were carried out according to the guidelines of the Iranian Council of Animal Care (1995).

References

- Allen MS, Bradford BJ and Oba M (2009) Board-invited review: the hepatic oxidation theory of the control of feed intake and its application to ruminants. *Journal of Animal Science* **87**, 3317–3334.
- Antongiovanni M, Acciaoli A, Grifoni F, Martini A and Ponzetta P (1991) Effects of wheat straw treated with ammonia from urea hydrolysis in lamb diets. *Small Ruminant Research* **6**, 39–47.
- Association of Official Analytical Chemists (AOAC) (1995) *Official Methods of Analysis*, 16th Edn. Arlington, VA, USA: Association of Official Analytical Chemists.
- Atti N and Mahouachi M (2011) The effects of diet, slaughter weight and docking on growth, carcass composition and meat quality of fat-tailed Barbarine lambs. A review. *Tropical Animal Health and Production* **43**, 1371–1378.
- Azizi-Shotorkhoft A, Sharifi A, Mirmohammadi D, Baluch-Gharai H and Rezaei J (2016) Effects of feeding different levels of corn steep liquor on the performance of fattening lambs. *Animal Physiology and Animal Nutrition* **100**, 109–117.
- Batista ED, Detmann E, Titgemeyer EC, Valadares Filho SC, Valadares RFD, Prates LL, Rennó LN and Paulino MF (2016) Effects of varying ruminally undegradable protein supplementation on forage digestion, nitrogen metabolism, and urea kinetics in Nelore cattle fed low-quality tropical forage. *Journal of Animal Science* **94**, 201–216.
- Beauchemin KA, McClelland LA, Jones SDM and Kozub GC (1995) Effects of crude protein content, protein degradability and energy concentration of the diet on growth and carcass characteristics of market lambs fed high concentrate diets. *Canadian Journal of Animal Science* **75**, 387–395.
- Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* **70**, 567–590.
- Bowman JGP, Sowell BF and Paterson JA (1995) Liquid supplementation for ruminants fed low quality forage diets: a review. *Animal Feed Science and Technology* **55**, 105–138.
- Broderick GA and Kang JH (1980) Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *Journal of Dairy Science* **63**, 64–75.
- Cebra CK, Garry FB, Getzy DM and Fettman MJ (1997) Hepatic lipidosis in anorectic lactating Holstein cattle. a retrospective study of serum biochemical abnormalities. *Journal of Veterinary Internal Medicine* **11**, 231–237.
- Chen XB and Gomes MJ (1992) *Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of Technical Details*. Occasional Publication. Aberdeen, UK: Rowett Research Institute.
- Chen XB, Mejia AT, Kyle DJ and Ørskov ER (1995) Evaluation of the use of the purine derivative: creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: studies in sheep. *Journal of Agricultural Science, Cambridge* **125**, 137–143.
- Craddock BF, Field RA and Riley ML (1974) Effect of protein and energy levels on lamb carcass composition. *Journal of Animal Science* **39**, 325–330.
- Dabiri N and Thonney ML (2004) Source and level of supplemental protein for growing lambs. *Journal of Animal Science* **82**, 3237–3244.
- David DB, Poli CHEC, Savian JV, Amaral GA, Azevedo EB and Jochims F (2015) Urinary creatinine as a nutritional and urinary volume marker in sheep fed with tropical or temperate forages. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **67**, 1009–10015.
- DePeters EJ and Ferguson JD (1992) Nonprotein nitrogen and protein distribution in the milk of cows. *Journal of Dairy Science* **75**, 3192–3209.
- Eghbali M, Kafilzadeh F, Hozhabri F, Afshar S and Kazemi-Bonchenari M (2011) Treating canola meal changes *in situ* degradation, nutrient apparent digestibility, and protein fractions in sheep. *Small Ruminant Research* **96**, 136–139.
- Elwakeel EA, Titgemeyer EC, Drouillard JS and Armendariz CK (2007) Evaluation of ruminal nitrogen availability in liquid feeds. *Animal Feed Science and Technology* **137**, 163–181.
- Esquivelzeta C, Casellas J, Fina M and Piedrafito J (2012) Backfat thickness and longissimus dorsi real-time ultrasound measurements in light lambs. *Journal of Animal Science* **90**, 5047–5055.
- Folch J, Lees M and Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–503.
- Gorosito AR, Russell JB and Van Soest PJ (1985) Effect of carbon-4 and carbon-5 volatile fatty acids on digestion of plant cell wall *in vitro*. *Journal of Dairy Science* **68**, 840–847.
- Griswold KE, Hoover WH, Miller TK and Thayne WV (1996) Effect of form of nitrogen on growth of ruminal microbes in continuous culture. *Journal of Animal Science* **74**, 483–491.
- Griswold KE, Apgar GA, Bouton J and Firkins JL (2003) Effects of urea infusion and ruminal degradable protein concentration on microbial growth, digestibility, and fermentation in continuous culture. *Journal of Animal Science* **81**, 329–336.
- Hocquette JF, Ortigues-Marty I, Pethick D, Herpin P and Fernandez X (1998) Nutritional and hormonal regulation of energy metabolism in

- skeletal muscles of meat-producing animals. *Livestock Production Science* **56**, 115–143.
- Horton GMJ, Nicholson HH and Christensen DA** (1982) Ammonia and sodium hydroxide treatment of wheat straw in diets for fattening steers. *Animal Feed Science and Technology* **7**, 1–10.
- Iranian Council of Animal Care** (1995) *Guide to the Care and Use of Experimental Animals*, vol. 1. Isfahan, Iran: Isfahan University of Technology.
- Kazemi-Bonchenari M, Mirzaei M, Jahani-Moghadam M, Soltani A, Mahjoubi E and Patton RA** (2016) Interactions between levels of heat-treated soybean meal and prilled fat on growth, rumen fermentation, and blood metabolites of Holstein calves. *Journal of Animal Science* **94**, 4267–4275.
- Kazemi-Bonchenari M, Salem AZM and Lopez S** (2017) Influence of barley grain particle size and treatment with citric acid on digestibility, ruminal fermentation and microbial protein synthesis in Holstein calves. *Animal: An International Journal of Animal Bioscience* **11**, 1295–1302.
- Kazemi-Bonchenari M, Falahati R, Poorhamdollah M, Heidari SR and Pezeshki A** (2018) Essential oils improved weight gain, growth and feed efficiency of young dairy calves fed 18 or 20% crude protein starter diets. *Journal of Animal Physiology and Animal Nutrition* **102**, 652–661.
- Khorasani GR, Sauer WC, Ozimek L and Kennelly JJ** (1990) Digestion of soybean meal and canola meal protein and amino acids in the digestive tract of young ruminants. *Journal of Animal Science* **68**, 3421–3428.
- Licitra G, Hernandez TM and Van Soest PJ** (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* **57**, 347–358.
- Males JR** (1987) Optimizing the utilization of cereal crop residues for beef cattle. *Journal of Animal Science* **65**, 1124–1130.
- Nagaraja TG and Lechtenberg KF** (2007) Liver abscesses in feedlot cattle. *Veterinary Clinics of North America: Food Animal Practice* **23**, 351–369.
- National Research Council** (2001) *Nutrient Requirements of Dairy Cattle*, 7th revised Edn. Washington, DC, USA: National Academies Press.
- National Research Council** (2007) *Nutrient Requirements of Small Ruminants*. Washington, DC, USA: National Academies Press.
- Ørskov ER and Ryle M** (1990) *Energy Nutrition in Ruminants*, 1st Edn. Amsterdam, The Netherlands: Elsevier Science Publishers, Ltd.
- Penner GB, Beauchemin KA and Mutsvangwa T** (2007) Severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. *Journal of Dairy Science* **90**, 365–375.
- Prado IN, Campo MM, Muela E, Valero MV, Catalan O, Olleta JL and Sañudo C** (2014) Effects of castration age, dietary protein level and lysine/methionine ratio on animal performance, carcass and meat quality of Friesian steers intensively reared. *Animal: An International Journal of Animal Bioscience* **8**, 1561–1568.
- Reynal SM, Ipharraguerre IR, Liñeiro M, Brito AF, Broderick GA and Clark JH** (2007) Omasal flow of soluble proteins, peptides, and free amino acids in dairy cows fed diets supplemented with proteins of varying ruminal degradabilities. *Journal of Dairy Science* **90**, 1887–1903.
- Reynolds CK, Aikman PC, Lupoli B, Humphries DJ and Beever DE** (2003) Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *Journal of Dairy Science* **86**, 1201–1217.
- Ribeiro-Filho CC and Trenkle A** (2002) Evaluation of feeding value of the corn steep liquor as an energy and protein source for finishing cattle diets. *Journal of Animal Science* **80**(suppl. 1), 232–238.
- Salisbury MW, Krehbiel CR, Ross TT, Schultz CL and Melton LL** (2004) Effects of supplemental protein type on intake, nitrogen balance, and site, and extent of digestion in whiteface wethers consuming low-quality grass hay. *Journal of Animal Science* **82**, 3567–3576.
- Schingoenthe DJ** (1976) Whey utilization in animal feeding: a summary and evaluation. *Journal of Dairy Science* **59**, 556–570.
- Van Kuelen J and Young BA** (1977) Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science* **44**, 282–287.
- Van Soest PJ, Robertson JB and Lewi BA** (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597. Available at <https://www.ncbi.nlm.nih.gov/pubmed/15483174>.
- Walker RS, LaMay D, Davis JR and Bandyk CA** (2013) Method of feeding a liquid-protein supplement with low- to medium-quality hay affects hay waste and cow performance. *The Professional Animal Scientists* **29**, 552–558.
- Wang Z, Cui Y, Liu P, Zhao Y, Wang L, Liu Y and Xie J** (2017) Small peptides isolated from enzymatic hydrolyzate of fermented soybean meal promote endothelium-independent vasorelaxation and ACE inhibition. *Journal of Agricultural Food and Chemistry* **65**, 10844–10850.
- Wickersham EE, Shirley JE, Titgemeyer EC, Brouck MJ, DeFrain JM, Park AF, Johnson DE and Ethington RT** (2004) Response of lactating dairy cows to diets containing wet corn gluten feed or a raw soybean hull-corn steep liquor pellet. *Journal of Dairy Science* **87**, 3899–3911. Available at <https://www.ncbi.nlm.nih.gov/pubmed/15483174>.
- Zhao YG, Gordon AW, O'Connell EO and Yan T** (2016) Nitrogen utilization efficiency and prediction of nitrogen excretion in sheep offered fresh perennial ryegrass (*Lolium perenne*). *Journal of Animal Science* **94**, 5321–5331.