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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

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ABSTRACT

The present study aimed to evaluate the effects of various rumen undegradable to rumen degradable protein ratios (RUP:RDP) on the performance, rumen fermentation pattern, urinary purine derivatives, and carcass characteristics in growing lambs when received a high dietary wheat straw level (420 g/kg, DM basis). Lambs (average initial live weight = 27.2 ± 2.4 kg) were individual housed (n = 18, 6 animals/treatment) to evaluate the effects of treatments. The RUP:RDP ratios tested were obtained through replacing extruded soybean meal (ESBM; RUP = 64 %, based on total CP) instead of conventional soybean meal (CSBM; RUP = 26 %, based on total CP) in proportion of 0, 50, and 100 % in order to obtain ratios of (1) low RUP:RDP ratio as LR diet (25:75); (2) moderate RUP:RDP ratio as MR diet (30:70) (3) high RUP:RDP ratio as HR diet (35:65). The study lasted 10 weeks. Dry matter intake did not differ among experimental treatments ($P = 0.80$), while average daily gain (ADG) and final BW were linearly improved ($P < 0.05$) with increased RUP:RDP ratio. Accordingly, the lowest feed efficiency observed in LR lambs and it was reduced linearly when RUP:RDP ratio was increased ($P = 0.01$). Although the amount of nitrogen intake was constant across experimental treatments ($P = 0.69$); however, the nitrogen to gain conversion ratio was increased as RUP:RDP being increased ($P = 0.02$). Digestibility of organic matter ($P = 0.02$) and crude protein ($P = 0.03$) as well as ruminal concentration of acetate ($P = 0.05$), urinary concentration of allantoin ($P = 0.01$), and blood glucose and insulin concentrations ($P = 0.01$) were linearly increased when lambs received diets contained high RUP:RDP ratio. In contrast, ruminal propionate concentration ($P = 0.02$) and urinary nitrogen concentration ($P = 0.02$) were reduced as RUP:RDP being increased. The dressing percentage was increased ($P = 0.02$) but mesenteric fat content ($P = 0.03$) and back-fat thickness ($P = 0.01$) were reduced when diets with higher RUP:RDP ratio being fed to lambs. In summary, high dietary RUP:RDP level is recommendable when growing lambs received wheat straw based-diet due to improvement in nutrient digestibility, nitrogen efficiency, and preventing high fat accretion within animal body.

1. Introduction

The quantity and quality of dietary protein has been shown to have pivotal role on the performance (Santos et al., 2015), nutrients digestibility (Chegini et al., 2019), and carcass characteristics (Wood et al., 2008) in growing lambs. Regardless the dietary protein content, growth performance of growing lambs influenced with ruminal protein degradability (Haddad et al., 2005; Karamnejad et al., 2019). As a general categorizing, protein in ruminant nutrition, is divided into rumen undegradable protein (RUP) and rumen degradable protein (RDP) that various ratios of RUP:RDP can influence animal performance (NRC, 2001). Although various RUP:RDP ratios has been shown to impact ruminant animals' performance (Haddad et al., 2005; Atkinson et al., 2010; Kazemi-Bonchenari et al., 2020); however, its optimum ratio is still controversial in growing lambs. Providing the optimum level of dietary RDP has potential to improve N utilization efficiency (McGuire et al., 2013), ruminal fermentation (Cappellozza et al., 2013) and performance in ruminants (Santos et al., 2015). This

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supplementation is more critical when ruminants received low-quality forage (LQF; Marini and Van Amburgh, 2003; Soliva et al., 2015). This is related to providing adequate N requirement of fiber-digesting bacteria in diets contained low-quality forage-based diet (Griswold et al., 2003). High ruminal concentration of NH_3-N which is resulted in high dietary RDP content, from the other side, increases urinary N excretion which can be an indicator of the lower N utilization efficiency in farm animals (McGuire et al., 2013). The higher content of RUP in the diet also has been indicated to improve growing animal performance due to providing higher amino acid (AA) content entered into small intestine (Rastgoo et al., 2020). The AA provided through RUP may even be more digestible in small intestine rather than protein with microbial origin in sheep (Bartocci and Terramoccia, 2006). This condition opens an interesting field for ruminant nutritionists to formulate growing lambs' diet with optimum RUP:RDP ratio. Extrusion of feedstuffs has the potential of reducing protein degradability in the rumen (Cros et al., 1992), which could improve the nutritive value of feedstuff as a source of ruminally undegradable protein. Because main source of protein in animal nutrition is soybean meal, making different dietary RUP:RDP ratios can be obtained by replacing extruded soybean meal (ESBM) instead of conventional soybean meal (CSBM) (Cros et al., 1992; Berenti et al., 2020).

Reviewing the feedstuffs that have been considered as low-quality forage sources in ruminant' diets, wheat straw (WS) is a harvest residue and as a LQF that is easily available with low cost that has been evaluated as the sole-fed feedstuff in ruminant (El-Meccawi et al., 2009). However, its low N content as well as low nutrient digestibility (Chegini et al., 2019) remains as a concern for animal nutritionists when formulating the rations. Although the chemical treating of LQF improves its quality of feeding (Khan et al., 2008); however, treating process is time-consuming and make the diet more expensive due to the high-cost of treatment (Eghbali et al., 2011). Furthermore, some concerns related to animal and labor health may limit chemical treating of feedstuffs in animal nutrition (Kazemi-Bonchenari et al., 2017; Bhatt and Sahoo, 2019). Providing optimum dietary RUP:RDP ratio can be a replacement strategy rather than chemical treating, while feeding LQF to ruminants (McGuire et al., 2013; Santos et al., 2015; Soliva et al., 2015). For instance, feeding high amount of LQF for steers (Wickersham et al., 2008), beef steers (Bandyk et al., 2001), feedlot cattle (Batista et al., 2016) or heifers (Koch et al., 2017) showed greater nutrient digestibility and improved performance when animals received higher dietary RUP:RDP level. In addition, the N availability rate can affect carcass quality in lambs (Wood et al., 2008); however, the effects of different RUP:RDP ratios on lambs' carcass quality fed WS based-diet is not well documented.

The aim of the current study was to evaluate the effects of different dietary RUP:RDP ratios obtained through replacing ESBM instead of CSBM on performance, ruminal fermentation, urinary purine derivatives (PD), blood metabolites, liver enzymes, and carcass characteristics of growing lambs fed a high WS based-diet (420 g/kg, DM basis).

2. Materials and methods

2.1. Animals, management, and experimental diets

The present study was carried out at Animal Production Station under supervision of Arak University, Arak, Iran. All the animal procedures were approved by the Animal Care and Use Committee of Arak University (IACUC Protocol #IR2018011) outlined by the Iranian Council of Animal Care (ICAC, 1995). Eighteen male Farahani lambs (avg. BW 27.2 ± 2.43 kg, age 3.9 ± 0.19 months) were used in a complete randomized design with three different experimental treatments (6 animals/ experimental group) in a 10 weeks trial. The lambs received total mixed ration (TMR) which was formulated according to NRC (2007) small ruminant requirements recommendations for lambs. The ingredient and chemical composition of experimental diets are given Table 1. The animals were housed in individual stalls bedded with concrete surface which was cleaned every 24 h. Constant amount of wheat straw (420 g/kg; DM basis) was considered across all treatments. Wheat straw was chopped (geometric mean particle size of 3.1 ± 0.14 mm) and then well-mixed with experimental diets before feeding to lambs.

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

Ingredients	Treatments[1]		
	LR	MR	HR
Wheat straw, chopped	420	420	420
Barley grain, rolled	340	340	340
Wheat bran	70	70	70
Conventional soybean meal	150	75	0
Extruded soybean meal (Profidam)	0	75	150
Calcium carbonate	5	5	5
Salt	5	5	5
Mineral and vitamin premix[2]	10	10	10
Chemical composition			
Metabolisable energy, Mcal/kg	2.47	2.47	2.47
Non fiber carbohydrates[3]	356	358	359
Neutral detergent fiber	399	397	396
Ether extract	24.5	24.6	24.8
Ash	65.4	65.3	65.1
Calcium	7	7	7
Phosphorous	4	4	4
Crude protein	155	155	155
Rumen degradable protein	116.3	108.5	100.9
Rumen undegradable protein	38.7	46.5	54.2
RUP:RDP ratio, (% of dietary CP)[3,4]	25:75	30:70	35:65
Lysine, g/kg of CP[3]	44.3	44.7	44.9
Methionine, g/kg of CP[3]	13.4	13.6	13.9

[1] Treatments were; (1) low RUP:RDP ratio (LR) = 25:75, (2) moderate RUP:RDP ratio (MR) = 30:70, and (3) high RUP:RDP ratio (HR) = 35:65.
[2] Contained per kilogram of supplement: 500,000 IU vitamin A, 80,000 IU vitamin D_3, 2000 vitamin E, 750 g Mn, 110 g Ca, 2000 mg Zn, 45 g P, 20 g Mg, 15 g Na, 1000 mg Fe, 8 mg Co, 500 mg Cu, 20 mg I, and 10 mg Se.
[3] Calculated based on NRC, 2001.
[4] The RUP:RDP content of CSBM and ESBM was evaluated through *in situ* experiment and total mixed ration RUP:RDP ratio was calculated based on NRC, 2001.

The content of the crude protein was constant across the treatments (15.5, % of DM), and the ratios for RUP:RDP were based on the total crude protein content (Table 1). The alteration of RUP:RDP ratios were performed by substituting conventional soybean meal by extruded processed soybean meal (Yasna-Mehr Company, Tehran, Iran). A batch of the SBM separated into 2 equal portions, with the first portion being used as a CSBM. The second portion of SBM was ESBM at a high temperature (150 ± 2 °C) along with moisture (25–30 %) and at a high pressure for 15 s by a single screw (speed of 450 rpm, diameter of 10 cm) double conditioner extruder system (Amandus Kahl, Expander, OEE 32, GmbH and Co., KG, Germany) at Yasna Mehr Co. (Tehran, Iran) according to Berenti et al. (2020). After identification of the RUP contents of CSBM and ESBM in an *in situ* study that is explained hereafter, the RUP:RDP ratios tested were obtained through replacing extruded soybean meal (ESBM) contained 64 % RUP (based on total CP) instead of conventional soybean meal (CSBM) contained 26 % RUP (based on total CP) in proportion of 0, 50, and 100 % order to obtain ratios of (1) low RUP:RDP ratio as LR diet (25:75); (2) moderate RUP:RDP ratio as MR diet (30:70) (3) high RUP:RDP ratio as HR diet (35:65). The total mixed ration (TMR) was fed to animals in two equal meals at 0800 and 1600 h. Experimental treatments were fed *ad libitum* to let the animals have at least 10 % orts (the amount of feed not consumed over a 24-h period).

2.2. In situ experiment

The protein degradability (% CP) of CSBM and ESBM was determined by *in situ* experiment for having more precise RDP and RUP content of experimental diets. The degradability trial was conducted on two ruminal cannulated Farahani sheep (avg. BW 46 ± 2.9 kg). The bags (polyester, 5 cm × 7 cm, with a 45-μm pore size) containing 3 g of either soybean meal or processed soybean meal were placed in triplicates in the rumen for 0, 2, 4, 8, 16, 24, 36, and 48 h. The nonlinear model was used to calculate degradation kinetics (Ørskov and McDonald, 1979). The RDP and RUP values (% CP) were calculated (NRC, 2001) using the following equations: RDP = A + B × [kd/(kd + kp)] RUP = C + B × [kp/(kd + kp)], that A is the soluble which is equal to rapidly degraded fraction, B is the slowly degradable which is potentially degradable fraction, C is the undegradable or unavailable fraction, kp is the ruminal passage rate which is assumed to be 0.08/h, and kd is the degradation rate of the B fraction.

2.3. Performance and digestibility trial

The BW of the animals was recorded at the first of the trial and at 10 d intervals thereafter until the last day (*n = 7*). The lambs were weighted before the morning meal (0700) to eliminate the effects of empty/full status of the gastrointestinal tract on BW. The average daily gain (kg) divided by kg of intake was considered as gain to feed ratio (feed efficiency). The weight gain per 100 g of N intake was considered as N conversion ratio into weight gain (Chegini et al., 2019). The TMR Samples were collected weekly and DM was determined by drying at 60 °C for 48 h (AOAC, 2002). Subsamples of dried feeds and orts were mixed thoroughly and ground in a mill (Ogaw Seiki CO., Ltd., Tokyo, Japan) to passing a 1-mm screen were analyzed for CP (method 988.05; AOAC, 2002), ether extract (method 920.39; AOAC, 2002), neutral detergent fiber (NDF; Van Soest et al., 1991) and non-fibrous carbohydrate (NFC) was computed as 100 – (CP + NDF + EE + ash) as described by NRC (2001).

At the end of the experiment (last 5 days) three faecal grab samples were collected daily from each animal at 6, 12, and 18 h after offering the morning meal. The samples were then analysed to determine total N, ether extract and NDF based on procedures earlier mentioned for TMR samples. Apparent nutrient total tract digestibility coefficients were measured by using acid insoluble ash (AIA) as an internal marker (Van Kuelen and Young, 1977).

2.4. Ruminal fermentation profile

The ruminal samples were taken from all experimental lambs using a stomach tube at 4 h after the morning feeding on day 32 and 64 of the experiment. For avoiding the possible saliva contamination, the first 5 ml was eliminated, and then ruminal pH was measured immediately (HI 8314 membrane pH meter, Hanna Instruments, Villafranca, Italy). The rumen samples, thereafter, were squeezed using 4-layers of cheesecloth. The aliquots of ruminal liquid samples (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 NH_3-N using a modified phenol-hypochlorite method adapted from Broderick and Kang (1980). The other aliquot was used for the analysis of short-chain fatty acids (SCFA) using a gas chromatograph (model CP-9002; Chrom-pack, Delft, The Netherlands) as described in our previous work (Kazemi-Bonchenari et al., 2016).

2.5. Urinary purine derivatives

Daily urine output was estimated through spot sampling technique from creatinine concentration that extensively explained in previous work on growing lambs (Chegini et al., 2019). On days 22, 44, and 66 of the experiment, urine samples were collected from each animal during the morning (between 0900 and 1100 h). Samples were collected when lambs urinated spontaneously. An aliquot (10 ml) was diluted immediately with 90 ml of 0.036 N sulphuric acid and stored at −20 °C for later analysis. After thawing, the creatinine, allantoin, UN, and uric acid concentrations were determined based on procedures described extensively in our previous work (Kazemi-Bonchenari et al., 2017). An average creatinine excretion (9.79 mg/kg BW) was used for total urine volume estimated as previously suggested for sheep (David et al., 2015). The total excretion of allantoin and uric acid was calculated through estimated daily urine output and determined metabolite concentrations. The ruminal microbial N synthesis was calculated from daily urinary purine derivative (PD) output using the following equations presented by Chen and Gomes (1992).

2.6. Blood metabolites and liver enzymes

Blood sampled from the jugular vein of each lamb at 3−4 h after morning feeding (at 1100 h) on days 34 and 68 into 10 mL tubes. Serum subsamples stored at −20 °C and then were analyzed to determine concentrations of glucose (Kit No. 93008), albumin (Kit No. 9307), TP (Kit No. 9304), and urea nitrogen (BUN; Kit No. 93013) using commercial kits in accordance with manufacturer's instruction protocols (Pars Azmoon Co., Tehran, Iran). Liver function indicator enzymes, aspartate aminotransferase (AST; Kit No. 92005) and alanine aminotransferase (ALT; Kit No. 92004), were measured using ELISA (Auto Analyzer Hitachi 717, Japan) usng commercial laboratory kits provided by Pars Azmoon Co., Tehran, Iran. Serum insulin concentration was determined using ELISA (Auto Analyzer Hitachi 717, Japan) by commercial kits (Monobind, CA, USA).

2.7. Carcass characteristics

Lambs were slaughtered at the end of the experiment (d 70) and carcass characteristics were measured. The ratio of hot carcass weight to live body weight, which was considered as dressing percentage. The deposited fat in fat-tail, mesenteric, and abdominal, was recorded by weighing the total fat mass collected from the organ (Jiriaei et al., 2020). However, the thickness of back-fat was measured by fat depth (mm) covered between ribs 12 and 13 measured by caliper as previously descried by Esquivelzeta et al. (2012). Furthermore, the total deposited fat content in liver was measured by method explained by Folch et al. (1957).

2.8. Statistical analysis

The data were analysed using PROC MIXED regarding the performance items which there were repeated measurements over time (amount of intake, average daily gain, gain to feed ratio, and N conversion ratio) using the following model:

$$Y_{ijkl} = \mu + A_i + RUP_j + T_k + RUP \times T_{jk} + \beta(X_i - \overline{X}) + \varepsilon_{ijkl}$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, A_i is the effect of animal, RUP_j is the effect of ruminal undegradable protein to degradable protein ratio *j* (LR, MR, and HR), T_k is the effect of recording time *k*, $RUP \times T_{jk}$ is the interaction between time *k* and RUP:RDP ratio *j*, $\beta(Xi - \overline{X})$ is the covariate variable (used for BW with initial BW as covariate) and ε_{ijkl} is the residual error. The model was contained lamb effect within treatment as random effect. The data were analysed using PROC GLM for variables that did not have repeated measurements over time (apparent digestibility coefficient of components and carcass characteristics) using the following model:

$$Y_{ij} = \mu + RUP_i + \varepsilon_{ij}$$

Differences between least squares means were considered significant at $P \leq 0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P \leq 0.10$. In addition to overall multiple treatment comparison, orthogonal polynomial contrasts were conducted to obtain linear and quadratic effects of different RUP:RDP ratios regarding different statistically analysed items.

3. Results

3.1. Chemical composition of experimental feedstuffs

The ruminal undegradable protein content for CSBM and ESBM was 26 % and 64 % (based on total CP), respectively. The contents of NDF and CP for wheat straw were 74.6 % and 5.2 % (DM basis).

3.2. Performance and nutrient digestibility

There was no effect of RUP:RDP ratio on DM intake ($P = 0.80$). However, increasing RUP:RDP ratio increased ADG (Fig. 1; $P = 0.05$); therefore, gain to feed ratio was improved ($P = 0.01$) as RUP:RDP level was increased. Even when N intake was not differing between treatments ($P = 0.69$), N to gain conversion ratio was linearly increased ($P = 0.02$) as RUP:RDP level was increased.

Total tract digestibility of organic matter ($P = 0.02$) and crude protein ($P = 0.03$) were linearly increased as RUP:RDP ratio being increased in diets. The other nutrient digestibility (NDF, and EE) was not affected ($P > 0.10$) among experimental treatments (Table 2).

3.3. Ruminal fermentation profile, urinary purine derivatives, and urinary N

Ruminal pH was constant across different experimental treatments offered into growing lambs ($P = 0.54$). The greatest ruminal NH_3-N was found for LR diet which was linearly reduced as RUP:RDP level being increased ($P < 0.01$). Total SCFA production was not differ among experimental treatments ($P = 0.46$). However, results showed that ruminal acetate ($P = 0.05$) and BCVFA ($P = 0.03$) concentrations were linearly reduced and ruminal propionate concentration was linearly increased ($P = 0.02$) when RUP:RDP ratio was increased. The acetate: propionate ratio was tended to be lower as RUP:RDP level being increased in diets ($P = 0.09$).

The allantoin concentration in the urine was linearly increased ($P = 0.01$) when lambs received higher RUP:RDP ratio in diets. Urinary uric acid concentration did not differ among treatments; however, total PD concentration linearly increased as RUP:RDP level was increased ($P = 0.01$). The estimated microbial protein synthesis was linearly increased when lambs fed diets with higher RUP:RDP ratio ($P = 0.01$). The greatest

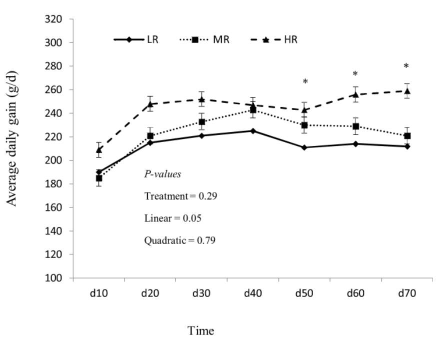

Fig. 1. Average daily gain in lambs receiving different diets: (◆) low RUP:RDP ratio (LR) = 25:75; (■) moderate RUP:RDP ratio (MR) = 30:70; (▲) high RUP: RDP ratio (HR) = 35:65. Asterisks indicate a significant difference (*$P < 0.05$) between groups at a given time point. Error bars represent SEM.

UN excretion was observed in LR diet that was linearly reduced when lambs received diets with higher RUP:RDP ratios ($P = 0.02$), hence the UN proportion of total N intake was linearly reduced when RUP:RDP level being increased ($P = 0.02$; Table 3).

3.4. Blood metabolites, liver enzymes, and insulin

The blood concentration of glucose was linearly increased when lambs received higher RUP:RDP diets ($P = 0.01$). The blood concentrations of TP, albumin, and TG were not different among different experimental treatments ($P > 0.10$); however, BUN concentration was linearly reduced as RUP:RDP ratio was increased ($P = 0.03$). Liver enzymes concentrations (AST and ALT) were constant across experimental treatments ($P > 0.10$). Blood insulin concentration was linearly increased when lambs fed diets with higher RUP:RDP ratios ($P = 0.01$; Table 4).

3.5. Carcass characteristics and fat deposition

The hot carcass weight ($P = 0.04$) and dressing percentages (carcass efficiency with no fat-tail; $P = 0.02$) were linearly increased when RUP: RDP ratio being increased (Table 5). The weights of internal organs (liver, kidney, heart) and gastrointestinal weight did not change among experimental treatments ($P > 0.10$). Results showed that the fat-tail weight as well as the amount of fat deposited in the heart and the liver were constant across experimental treatments ($P > 0.10$). However, the mesenteric fat weight ($P = 0.03$) and the back-fat thickness ($P = 0.01$) were linearly decreased as RUP:RDP being increased in experimental diets. (Table 5).

4. Discussion

Extruding the SBM increased ruminal undegradable protein content (64 % vs. 26 % based on total CP content for ESBM and CSBM, respectively). Giallongo et al. (2015) resulted that RUP content of conventional solvent SBM increased from 25.9%–58.9% (based on total CP). This is related to changes in molecular structures, weight and size of protein which these changes may be attributed to changes in protein sub-fractions (Berenti et al., 2020). Variations found between RUP content of extruded soybean meals can be related to temperature applied for performing extrusion process (Giallongo et al., 2015).

In the current study, a similar amount of intake was found among the experimental probably due to a similar dietary energy content. Allen et al. (2009) stated that the energy is the most limiting factor of intake in ruminants. The NDF content in the basal diet (avg. = 39.8 %) is also probably contributed to constant DMI across experimental treatments. It has been stated that when the content of dietary digestible energy is low in sheep nutrition, feed intake has potential to be limited by gut fill (Dinius and Baumgardt, 1969). Therefore, under this condition providing higher dietary RUP content cannot affect the animal intake (Haddad et al., 2005). Despite the similar amount of intake using different treatments, the ADG and gain to feed ratio were linearly increased, when higher RUP:RDP ratio was offered into lambs. In this regard, lower weight gains in LR diet can be related to lower digestibility of OM and CP, which was found for this diet in the present study. Notably, the main sources of N in the rumen are ruminal ammonia, amino acid, and peptide. Accordingly, optimum ratios of these sources required obtaining the optimum nutrient digestibility (Griswold et al., 2003). Our results show that LR diet provides greater ruminal NH_3-N concentration that did not affect nutrient digestibility, providing higher dietary RUP content that improves OM and CP digestibility. In agreement with previous works (Haddad et al., 2005), the higher dietary undegradable protein content had more digestibility, which consequently improves ADG in growing lambs. The higher efficiency when higher RUP content is formulated in ruminants' diets is probably related to by-passing more AA into small intestine as well as higher digestibility

Table 2
Effects of different ruminal undegradable protein to degradable protein ratios (RUP:RDP) on performance and nutrient digestibility of growing lambs fed wheat straw-based diet.

Item	Treatments[1]			SEM	*P-value*	*Polynomial Contrast*	
	LR	MR	HR			Linear	Quadratic
Performance, *n* = 18							
Initial body weight, kg	27.3	27.4	27.6	0.79	0.92	0.87	0.89
Final body weight, kg	42.2	43.8	44.9	0.96	0.08	0.04	0.83
Dry matter intake, g/d	1443	1456	1424	34.48	0.80	0.70	0.63
Average daily gain, g/d	212	233	246	16.07	0.29	0.05	0.79
Gain to feed ratio[2]	0.14	0.16	0.17	0.03	0.02	0.01	0.71
Nitrogen intake, g/d	32.3	32.4	31.5	0.76	0.69	0.52	0.63
N to gain conversion ratio[3]	672	702	769	41.89	0.05	0.02	0.73
Coefficient of components digestibility, %							
Organic matter	72.51	75.68	78.23	1.61	0.04	0.02	0.87
Crude protein	63.17	66.21	71.12	2.39	0.07	0.03	0.75
Neutral detergent fiber	52.53	52.91	51.31	2.46	0.89	0.73	0.76
Ether extract	82.95	82.30	83.46	1.42	0.84	0.80	0.59

[1] Treatments were; (1) low RUP:RDP ratio (LR) = 25:75, (2) moderate RUP:RDP ratio (MR) = 30:70, and (3) high RUP:RDP ratio (HR) = 35:65.
[2] Gain to feed ratio (feed efficiency) is calculated as kg of weight gain per kg dry matter intake.
[3] Nitrogen to gain conversion ratio calculated as the amount of weight gain per 100 g of N intake.

Table 3
Effects of different ruminal undegradable protein to degradable protein ratios (RUP:RDP) on ruminal fermentation, urinary purine derivatives and nitrogen excretion, and microbial protein synthesis in growing lambs fed wheat straw-based diet.

Item	Treatments[1]			SEM	*P-value*	*Polynomial Contrast*	
	LR	MR	HR			Linear	Quadratic
Rumen fermentation profile, *n* = 18							
Ruminal pH[2]	6.08	6.20	6.11	0.07	0.54	0.88	0.29
Ruminal NH_3-N, mg/dL	16.1	14.2	11.8	0.93	0.01	<0.01	0.92
Short chain fatty acids, mM	75.8	77.0	84.8	5.46	0.46	0.39	0.63
Individual short chain fatty acids, mM							
Acetate (A)	63.2	57.3	56.9	2.11	0.05	0.05	0.22
Propionate (P)	23.1	27.9	29.6	1.75	0.08	0.02	0.14
A:P[3]	2.75	2.13	2.03	0.18	0.03	0.09	0.22
Butyrate	9.5	11.5	10.9	0.65	0.14	0.36	0.08
BCVFA[4]	4.10	3.23	2.51	0.45	0.04	0.03	0.71
Purine derivatives and microbial protein synthesis							
Allantoin, mmol/d	10.6	11.8	14.6	0.97	0.03	0.01	0.49
Uric acid, mmol/d	1.1	1.2	1.4	0.09	0.17	0.10	0.38
Total PD[5], mmol/d	11.8	13.0	16.0	1.03	0.04	0.01	0.47
MPS[6], g/d	63.4	69.7	85.9	5.54	0.04	0.01	0.47
Urinary N, g/d	10.6	9.4	8.1	0.66	0.03	0.02	0.94
Urinary N, proportion of total N intake	33.4	29.5	26.9	2.07	0.07	0.02	0.92

[1] Treatments were; (1) low RUP:RDP ratio (LR) = 25:75, (2) moderate RUP:RDP ratio (MR) = 30:70, and (3) high RUP:RDP ratio (HR) = 35:65.
[2] The values are average of samples were taken days 32 and 64 (4-h post-feeding).
[3] A:P is the proportional ratio of acetate to propionate in the ruminal fluid.
[4] Branched chain volatile fatty acids (BCVFA) are the molar proportion of valerate + molar proportion of isovalerate.
[5] Total purine derivatives (TDP) is allantoin + uric acid.
[6] Microbial protein synthesis calculated through urinary total PD excretion based on Chen and Gomes (1992).

of AA in the small intestine (McGuire et al., 2013). Therefore, higher intestinal AA digestibility in extruded soybean meal can more contribute to the improved total tract digestibility of CP compared to un-processed soybean meal which subsequently improves gain and efficiency in dairy calves (Berenti et al., 2020) or productivity in dairy cows (Giallongo et al., 2015).

Higher ruminal acetate concentration and subsequently higher acetate: propionate ratio on the other hand, found in LR diet can partly contribute to lower ADG and efficiency in this treatment. It is notable that propionate and butyrate provide more energy from the stoichiometry point of view in ruminants compared to acetate which affects gain and efficiency (Ørskov and Ryle, 1990). Ruminal acetate concentration has increased in low RUP:RDP ratio, probably because of higher un-processed soybean meal content incorporated in this diet. Previous works stated that greater degradable true protein content in the diet increases the iso-acids concentration in the rumen, which consequently affects acetate concentration (Yang, 2002; Griswold et al., 2003). Previous studies indicated the fiber-digesting bacteria require BCVFA for maximum activity (Bryant, 1973) and supplemented the diets with these iso-acids can stimulate fiber digestibility (Gorosito et al., 1985; Yang, 2002) that consequently influence the acetate concentration in the rumen (Soltani et al., 2017). In contrast, feeding HR diet into growing lambs increased propionate concentration in the rumen. Soliva et al. (2015) in their study indicated that the requirement of RDP for optimum ruminal fermentation of OM and fiber may differ when feeding various fibrous feeds.

The ruminal pH values were in the range for healthy animals (>5.8; Penner et al., 2007) probably due to adequate level of fiber consumed in experimental treatments. Indeed, ruminal SCFA concentration contributes in pH alteration (Soltani et al., 2017) and unchanged SCFA across experimental treatments caused unchanged ruminal pH values. The greatest ruminal NH_3-N concentration found in LR diet that expectedly had the highest ruminal protein degradation in the rumen. Assessing the BUN concentration (14.6 mg/dL) as well as the urinary N excretion rate (10.6 g/d) which is found in the LR diet is linked to the high concentration of ruminal NH_3-N in this diet. It has been indicated previously that BUN is an indicator of ruminal N capturing and has close relationship with ruminal NH_3-N concentration (DePeters and Ferguson, 1992). Higher BUN concentration which was found in LR-fed lambs suggested the lower N capturing and subsequently its utilization efficiency compared to other diets. This is in agreement with Atkinson et al. (2010) who stated that provision of higher dietary RUP content may provide a delay in ureagenesis in lambs fed low-quality forage that was improved N use efficiency. Greater urinary N excretion also could be an indicator of lower N efficiency in ruminants (Kauffman and St-Pierre, 2001). Therefore, the results show that the lowest N utilization efficiency was found for growing lambs when LR diet was offered in the current study. From the other side, urinary allantoin and total PD were linearly increased in lambs fed HR diets. It has been stated that optimal ruminal NH_3-N and peptide-N concentrations are necessary to have

Table 4
Effects of different ruminal undegradable protein to degradable protein ratios (RUP:RDP) on blood metabolites, liver enzymes, and insulin concentration in growing lambs fed wheat straw-based diet.

Item	Treatments[1]			SEM	*P-value*	*Polynomial Contrast*	
	LR	MR	HR			Linear	Quadratic
Blood metabolites, *n* = *18*							
Glucose, mg/dL	53.8	57.3	59.1	1.46	0.04	0.01	0.64
Total protein, g/dL	6.6	6.8	7.4	0.27	0.15	0.11	0.59
Albumin, g/dL	3.9	4.1	4.7	0.26	0.13	0.13	0.33
Triglyceride, mg/dL	25.6	23.1	22.8	3.01	0.77	0.51	0.77
Blood urea nitrogen, mg/dL	14.6	13.3	10.9	0.90	0.04	0.03	0.59
Liver enzymes and insulin, *n* = *18*							
Aspartate aminotransferase, IU/L	55.2	51.4	54.7	2.99	0.63	0.90	0.34
Alanine aminotransferase, IU/L	23.0	25.1	22.6	1.25	0.39	0.83	0.18
Insulin, IU/L	5.2	5.9	6.4	0.34	0.02	0.01	0.87

[1] Treatments were; (1) low RUP:RDP ratio (LR) = 25:75, (2) moderate RUP:RDP ratio (MR) = 30:70, and (3) high RUP:RDP ratio (HR) = 35:65.

Table 5
Effects of different ruminal undegradable protein to degradable protein ratios (RUP:RDP) on carcass characteristics in growing lambs fed wheat straw-based diet.

Item	Treatments[1]			SEM	*P-value*	*Polynomial Contrast*	
	LR	MR	HR			Linear	Quadratic
Carcass characteristics, *n* = *18*							
Slaughter weight, kg	42.2	43.8	44.9	0.96	0.08	0.04	0.83
Hot carcass weight, kg	17.27	18.61	19.27	0.60	0.09	0.04	0.65
Dressing percentage (with fat-tail), %	51.12	50.58	52.0	1.16	0.69	0.63	0.52
Dressing percentage[2] (no fat-tail), %	40.91	42.40	42.98	0.56	0.05	0.02	0.49
Empty gastrointestinal weight, kg	3.66	2.98	3.06	0.56	0.65	0.46	0.59
Liver weight, kg	0.65	0.61	0.64	0.03	0.71	0.84	0.43
Kidney weight, kg	0.28	0.23	0.27	0.01	0.15	0.67	0.10
Heart weight, kg	0.21	0.21	0.22	0.02	0.94	0.88	0.78
Fat distribution in body							
Fat-tail weight, kg	4.30	3.60	3.96	0.36	0.44	0.53	0.27
Fat content in heart, kg	0.037	0.045	0.042	0.01	0.87	0.80	0.66
Mesenteric fat weight, kg	0.97	0.71	0.62	0.09	0.05	0.03	0.50
Back-fat thickness[3], cm	5.70	5.05	4.67	0.25	0.03	0.01	0.67
Fat deposited in liver[4], kg	6.81	6.52	5.85	0.38	0.25	0.11	0.72

[1] Treatments were; (1) low RUP:RDP ratio (LR) = 25:75, (2) moderate RUP:RDP ratio (MR) = 30:70, and (3) high RUP:RDP ratio (HR) = 35:65.
[2] Dressing percentage was calculated as hot carcass weight divided by slaughter weight.
[3] Back-fat depth (mm) was considered as fat thickness covered between ribs 12 and 13 measured by caliper (Esquivelzeta et al., 2012).
[4] As the percent of liver weight which was explained by Chegini et al. (2019).

maximum microbial protein production (Griswold et al., 2003). The minimum ruminal NH_3-N concentration range from greater than 1 (Satter and Slyter, 1974) up to 16 mmol/L which is proposed by Mehrez et al. (1977) has been suggested to obtain optimum ruminal microbial activities. Moreover, evaluating the fibrous feedstuff digestibility as *in vitro* system, Soliva et al. (2015) suggested that OM and fiber digestibility approached a plateau when ruminal NH_3-N concentration was in the range of 1.73–2.54 mmol/L. It can be suggested that, in the current study, higher RUP:RDP ratios provided adequate ruminal NH_3-N concentration and its greater concentration provided by LR diet cannot be utilized efficiently by ruminal microbes. Therefore, the approximate ruminal NH_3-N concentration of 11.8 mg/dL was adequate when high level of WS as LQF was fed into growing lambs to obtain maximum MPS and greater concentration caused greater urinary N excretion and thus reduced N utilization efficiency. Previous works conducted on heifers (Koch et al., 2017) or steers (Wickersham et al., 2008) reported greater nutrient digestibility and MPS when animals feed higher RUP level. Koch et al. (2017) suggested that feeding LQF source in diet depressed microbial protein flow and N utilization in heifers; however, greater RUP supplement was helpful in improving N use efficiency. The suggested mechanism is related to increased ruminal available N which is provided by higher urea recycling into the gastrointestinal tract that was found when higher dietary RUP:RDP content was fed in to animals (Wickersham et al., 2008; Koch et al., 2017). Gathering all results together in the current study, it can be concluded that ruminal fermentation pattern, digestibility, and improvement in N utilization efficiency, all contributed in improvement in ADG, feed efficiency, and N utilization efficiency when growing lambs received various RUP:RDP ratios.

Results show that glucose concentration was linearly increased in lambs fed higher RUP:RDP diets probably because of higher propionate concentration in the ruminal fluid which is the main precursor of glucose in ruminants (Soltani et al., 2017). The greater blood glucose concentration in lambs fed diets contained higher RUP:RDP ratios had potential to sparing on AA utilization and expend them into better performance instead of entering into gluconeogenesis pathway (Young, 1970). This mechanism also may be involved in better gain and efficiency in high RUP:RDP ratios. The least (5.2 IU/L) and the greatest (6.4 IU/L) insulin concentration was found for LR and HR, respectively, likely due to greater both glucose (Kazemi-Bonchenari et al., 2017) and AA (Lal and Chungh., 1995) available for metabolism when higher RUP:RDP ratio was offered in the lambs. Irrespective the mechanism related to increased insulin in blood concentration when lambs fed diets contained higher RUP:RDP ratios, previous works on growing lambs cleared that higher insulin involves in higher growth through improving AA uptake (Wester et al., 2000). There were no changes found for AST and ALT enzymes among animals fed on different experimental treatments. It has been cleared that high-fat deposited in liver (Cebra et al., 1997) or feeding high-grain diets (Nagaraja and Lechtenberg, 2007) put the liver in dysfunction condition. Our results show that liver function did not negatively influence with experimental diets.

The hot carcass weight and dressing percentage (without fat-tail) were reduced when lambs fed low RUP:RDP ratio. Dietary energy content (Craddock et al., 1974) and protein level (Prado et al., 2003) are the most effective factors impact the carcass characteristics in meat production animals. The dietary energy and protein contents as well as the amount of intake were similar among different experimental treatments in the current experiment, and the dietary RUP:RDP ratio was the only variable. More intestinal digestibility of AA in higher RUP content that was found in previous studies (Bartocci and Terramoccia, 2006) could improve dressing percentage found in HR diet.

Assessing the SCFA profile in the present study clears that the greatest concentrations for ruminal acetate and BCVFA were observed for LR-fed lambs that may involve in fat distribution through body (Brennand and Lindsay, 1992). Acetate is the primary precursor of fat in ruminants (Wood et al., 2008). Furthermore, previous results suggested that high BCVFA content in the rumen increases the subcutaneous fat accretion (Wood et al., 2008). Albeit, some literatures also believe that high proportion of propionate derived from the high-grain diet could increase fat deposition in fattening animals (Wood et al., 2008). The more fat deposited in mesenteric and back-fat was found in animals fed low RUP:RDP ratio may be more related into higher ruminal acetate and BCVFA concentrations. Our results suggest that when lamb received high level of WS, providing higher RUP:RDP ratio could prevent the carcass being fattier likely through changing the ruminal fermentation and SCFA profile. Regardless the ruminal fermentation end-products, deposited fat in fattening lambs also was influenced with dietary N condition (Wood et al., 2008). For instance, Doran et al. (2006) stated that inadequacy of AA can increase the stearoyl Co-A desaturase expression inside longissimus muscle which there is a linear relationship between this enzyme activity and fat deposition. Our results suggest that low RUP:RDP ratio expectedly reduced metabolizable protein reached into small intestine due to lower RUP content as well as lower microbial protein synthesized, and thus fattier carcass has been acquired by feeding lower RUP:RDP ration. Our results suggest that higher dietary RUP content can be an applicable strategy to prohibit high fat accretion in fat deposition sites of growing lambs when they received wheat-straw based diets.

5. Conclusions

Feeding higher dietary RUP:RDP ratios linearly improved feed efficiency, OM and CP digestibility, blood glucose and insulin concentrations, and dressing percentage when growing lambs received a high WS based-diet (420 g/kg, DM basis). Moreover, higher dietary RUP:RDP ratio linearly reduced ruminal NH_3-N concentration and consequently decreased urinary N excretion indicating higher N utilization efficiency and less deposited fat content in mesenteric and back-fat indicating higher energy efficiency. In summary, high RUP:RDP ratio can be strategy to obtain high nutrient digestibility, greater N utilization efficiency, and less deposited fat within the animal body in growing lambs when fed a high WS based-diet.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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