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Effects of elevated moisture content of total mixed ration on nutrient digestibility, rumen fermentation, microbial enzyme activity and growth performance in fattening lambs

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Abstract The objective of the current study was to determine the effects of increased water content in the total mixed ration (TMR) on feed intake, apparent nutrient digestibility, rumen fermentation parameters, microbial enzyme activity, blood metabolites, performance and feeding behavior in fattening lambs. Twenty-eight fat-tailed Lori-Bakhtiari male lambs [120±6.5 d of age; 33.4±2.24 kg body weight (BW)] were randomly assigned to four experimental diets containing 10% [control], 20%, 30% and 40% moisture content. Increased moisture content of TMR up to 30%, increased the intake of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and non-fiber carbohydrates, total weight gain and average daily gain [linearly (L), $P<0.05$] while acid detergent fiber (ADF) intake, final BW and feed conversion ratio remained consistent across the experimental diets ($P>0.05$). Increasing the moisture content of TMR up to 30% linearly increased the digestibility of DM, OM and CP and increased ruminal NH_3-N, total volatile fatty acid content, molar proportion of propionate and activities of CMCase and α-amylase (L, $P<0.05$) while NDF and ADF digestibility, individual VFA contents and selected blood metabolites were unchanged ($P>0.05$). Feeding-related behaviors were unaffected, but rumination and chewing times increased linearly (L, $P<0.05$). Overall, increasing the water content of the TMR diet by adding water increased the feed intake and digestibility and improved the ruminal condition and growth performance in finishing lambs.

Keywords: dietary moisture, lamb, performance, rumen parameters

Introduction

Insufficient animal feed production in many parts of the world, including Iran, has increased the feed costs of animal production (Sabertanha et al., 2012; Karimizadeh et al., 2017). Therefore, improvement in feed utilization will increase the revenues from livestock production. Feeding high concentrate diets to fattening lambs is a common strategy to promote faster, more efficient growth and heavier carcasses (Papi et al., 2011; Chen et al., 2012; Arjmand et al., 2022). This mainly arises because high-concentrate diets are rapidly fermented by ruminal microbes to produce volatile fatty acids (VFA), predominantly propionate and butyrate, which are required to improve growth performance and feed efficiency (Kim et al., 2018; Plaizier et al., 2018). However, feeding high-concentrate diets modifies the ruminal environment, including a decrease in ruminal pH (Penner et al., 2009; Wang et al., 2020), change in VFA pattern (Gudla et al.,

2012) and rumen microbial population (Sun et al., 2010), decreased ruminal motility (Owens et al., 1998), keratinization of the ruminal villi and decreased VFA absorption (Nocek and Kesler, 1980; Zitnan et al., 2003).

Addition of water to the total mixed ration (TMR) has been suggested as one potential strategy to decrease feed sorting in ruminants (Shaver, 2002; Fish and DeVries, 2012), but the findings are contradictory. Through adding water to a dry TMR and decreasing the dietary dry matter (DM) from 81% to 64%, the degree of sorting against long particles and the preferential consumption of concentrate particles decreased in dairy cows (Leonardi et al., 2005). Recently, Beiranvand et al. (2018) reported that adding water to the starter diet of suckling calves up to 25% of DM improved their growth performance during the winter. During summer, adding water to the starter diet of suckling calves to 50% DM improved their feed intake and growth performance (Beiranvand et al., 2016). Also, in the experiment of Lahr et al. (1983) feeding diets differing in DM contents (*i.e.,* 78, 64, 52 and 40% DM) to dairy cows improved the diet palatability. These positive responses are probably due to the increased adhesion of feed particles, resulting in increased particle condensation and decreased dietary followed by increased palatability and feed consumption (Arzola-Álvarez et al., 2010). This may compensate a large part of the reduction in DM intake (Beiranvand et al., 2018). Despite these positive responses, Miller-Cushon and DeVries (2009) indicated that increasing the dietary moisture content from 42 to 52%, increased the feed sorting and reduced DM intake (DMI) in dairy cows. In another experiment, increasing TMR moisture content from 44 to 56% increased the sorting behavior and reduced DMI in dairy cows (Felton and DeVries, 2010). To our knowledge, there is a lack of information about the effects of increasing moisture content of TMR rations on growth performance and rumen microbial enzyme activity in fattening lambs. Therefore, the aim of the present study was to investigate the effects of adding different levels of water to TMR ration on growth performance, microbial enzyme activity, ruminal and blood parameters as well as feeding activity of the fattening lambs.

Materials and methods

Animals and diets

All experimental procedures were conducted according to The Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) guidelines. All procedures and guidelines involving the animal experimentation were approved by the Animal Experiment Committee at Lorestan University, Lorestan, Iran. Twenty-eight fat-tailed Lori-Bakhtiari male lambs at an average age of 120±6.5 days and initial body weight (BW) of 33.4±2.24 kg were randomly assigned to four experimental diets in a balanced completely randomized design. Lambs were housed in individual pens (1.3×1.2 m). The feeding trial lasted 70 days with two weeks acclimatization to the pens and diets, and 56 days for the fattening period. During the adaptation period, lambs were vaccinated against enterotoxaemia (3 mL per lamb; Razi Vaccine and Serum Research Institute, Iran). They were also treated with anthelmintic for external (1 mL of Azantole 10% per 7 L of water, as spraying method; Bayer, Germany) and internal (Triclabendazole + levamisole, 12 mL per lamb; Darou-Pakhsh Co., Iran) parasites.

Ingredients and chemical composition of the basal diet (forage:concentrate ratio 30:70) is presented in Table 1. The ration was formulated to meet the nutrient requirements of lambs according to National Research Council recommendations (NRC, 2007). The dietary treatments which differed in moisture contents consisted of the control diet (containing 10% moisture; without adding water), and those of 20, 30 and 40% moisture content by adding incremental water quantities to the control diet. The diets were provided *ad libitum* as a TMR in two equal meals daily at 08:00 and 16:00 h for 56 days with a 5% of daily refusal. The quantity of water required to yield the desired moisture content of each experimental diet was added 1 h before the feed was offered to the lambs. This process was repeated daily, ensuring that the ration for each lamb had sufficient moisture content. Feed offered and orts per lamb were collected and recorded daily during the experimental period. All animals had free access to drinking water. Lambs were weighed at the beginning and end of the experimental period at 8:00 h after 16 h of feed and water deprivation. Total weight gain (TWG) was calculated based on the difference between the final BW and the initial BW. The average daily gain (ADG) was calculated by divided TWG by the number of days (56 days), and the feed conversion ratio (FCR) calculated by dividing daily DMI by ADG.

Table 1. Ingredients and chemical composition of the basal diet (g/kg DM, unless otherwise stated)

Ingredients	Content
Alfalfa hay, ground	200
Wheat straw	50.0
Beet pulp, dried	50.0
Ground corn grains	230
Ground barley grains	230
Soybean meal	110
Wheat bran	100
Premix[1]	28.0
Urea	2.0
Chemical composition	
Dry matter (g/kg fresh weight)	900
Organic matter	931
Crude protein	162
Neutral detergent fiber	299
Acid detergent fiber	158
Ether extract	26.5
Non-fiber carbohydrates	443
Ca	8.0
P	4.0
Metabolizable energy[2] (Mcal/kg DM)	2.67

[1] Vitamin–mineral premix contained per kg: vitamin A 250,000 IU; vitamin D_3 500,000 IU; vitamin E 1000 IU; Mn 1250 mg; Zn 2500 mg; Cu 375 mg; Se 25 mg; Ca 140000 mg; P 2500 mg; Co 20 mg; I 25 mg; Mg 25000 mg; Na 25000 mg as NaCl; Na 25000 mg as $NaHCO_3$ and 1000 mg antioxidant.
[2] Calculated from feed ingredients ME content (NRC, 2007).

From day 43 until 48 of the experiment, fecal samples (50 g) were collected for 5 consecutive days from all lambs and pooled (5 samples) to determine total-tract apparent digestibility of the nutrients using acid-insoluble ash as an internal marker (Van-Keulen and Young, 1977).

Rumen fluid samples (RF, 50-60 mL) were collected from all lambs on day 45 of the trial, 3 h after the morning feed by using a stomach tube. To avoid saliva contamination, the first 10-20 mL of RF form each animal was discarded (Jasmin et al., 2011). The obtained RF was strained through 4 layers of cheese cloth and pH was immediately determined using a pH meter (Sentron, model A102-003). For ammonia (NH_3-N) determination, 5 mL of the strained RF was acidified with 1 mL of 0.2 N HCl to stop fermentation and immediately frozen (−20 ^oC). For VFA analysis, 1 mL of the strained RF was mixed with 0.25 mL of an acid solution containing 200 mL/L of orthophosphoric acid and 20 mM 2-ethyl-butyric acid and frozen at -20 ^oC. Another sub-sample of the strained RL was used for determination of hydrolytic fiber-degrading enzymes.

On day 50, blood samples were collected from all lambs using jugular venipuncture containing lithium heparinate 3 h after the morning feed. Plasma was harvested by centrifugation at 3000 × *g* for 15 min and kept at −20 ^oC pending further analyses.

During the sampling period (on day 40), the chewing activities of each lamb were monitored visually for 24 h continuously (08:00 to 08:00) at 5-min intervals. The total number of minutes eating and ruminating as well as the resting activity were then estimated by the sum of each observation and multiplied by a factor of five (Kononof et al., 2002). Chewing activities were adjusted for DM and neutral detergent fiber (NDF) intake (Beauchemin, 1991).

Analytical procedures

Samples of the diets and orts were oven-dried at 55 °C to reach a constant weight, and ground to pass through 1-mm sieve (Wiley mill, Swedesboro, NJ, USA). Samples were analyzed for DM (# 930.15; AOAC, 2004), ash (# 924.05; AOAC, 2004), crude protein (CP; #984.13; AOAC, 2004), NDF (Mertens, 2002), acid detergent fiber (ADF) (without sodium sulfite and with amylase treatment; Van Soest et al., 1991) and Lignin(sa) (Robertson and Van Soest, 1981).

Non-fiber carbohydrate (NFC) was calculated as (Hall, 2000):

NFC (g/kg DM) = 1000 – (NDF g/kg DM + CP g/kg DM + EE g/kg DM + ash g/kg DM)

The ruminal concentration of NH_3-N was determined in the thawed strained RL (Broderick and Kang, 1980) using phenol and hypochlorite reagents. Volatile fatty acid concentrations, in centrifuged samples, were determined by gas liquid chromatography with ethyl-butyric acid as the internal standard (Stewart and Duncan, 1985). The activities of microbial fiber-degrading enzymes including carboxymethyl cellulase (CMCase), microcrystalline cellulase (MCCase), filter paper-degrading (FPD) activity and α-amylase were estimated in the extracellular (EC) fraction of the ruminal fluid (Agarwal, 2000). Approximately 10 mL of RL was centrifuged (27000 × g, 20 min; 4 °C) and the clear supernatant (CS) used as an enzyme source for the EC fraction. For estimation of CMCase, the reaction mixture containing 0.1 M phosphate buffer (pH 6.8) 1 mL, CS 0.5 mL and 1% carboxymethyl cellulose 0.5 mL, was incubated at 39 °C for 60 min. To determine MCCase, the reaction mixture containing 0.1 M phosphate buffer (pH 6.8) 1 mL, CS 1 mL and 1% microcrystalline cellulose 1 mL, was incubated at 39 °C for 60 min. To measure α-amylase, the reaction mixture containing 0.1 M phosphate buffer (pH 6.8) 1 mL, CS 0.5 mL and 1% starch 0.5 mL was incubated at 39 °C for 30 min. The reaction mixture which contained 1 mL buffer (0.1 M, pH 6.8), 1 mL CS and 0.5 g Whatman No. 1 filter paper was incubated at 39 °C for 60 min to estimate FPD activity. In these assays, the reaction was stopped by adding 3 mL of 1% dinitrosalicylic acid solution. Glucose, liberated due to enzyme activities, was quantified according to the method described by Miller (1959), using glucose as the standard. Enzyme activities were calculated considering that one unit of enzyme produced 1 µmol of glucose/mL per min under the assay conditions.

Plasma samples were analyzed by colorimetric methods for total protein (TP), blood urea-N (BUN) and glucose using Pars Azmun Diagnostic kits (Tehran, Iran). Total protein was measured after reaction with copper sulfate in sodium hydroxide to form a violet biuret complex (biuret method; Johnson et al., 1999), BUN based on urease and glutamate dehydrogenase reactions (Thomas, 1998) and glucose using glucose oxidase method (Barham and Trinder, 1972).

Statistical analysis

Data on ruminal and blood plasma parameters and chewing activity were analyzed using the GLM procedures, and nutrient digestibility data were analysed using the MIXED procedures (SAS Institute, 2001). The model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the dependent variable, μ is the population mean for the variable, T_i is the treatment effect (*i.e.,* nutrient digestibility, blood and ruminal parameters and chewing activity) and e_{ij} the error associated with the observation $_{ij}$.

Data on growth performance were analyzed using the following model:

$$Y_{ij} = T_i + ß_i(X_{ij} - \bar{X}) + e_{ij}$$

whereY_{ij} is observation parameters, T_i is the fixed effect of treatment, $ß_i$ is the regression coefficient, X_{ij} is the initial BW with mean $\bar{X}$ (covariate) and e_{ij} is the standard error of the term. The initial BW was used as the covariate for analyzing the changes in BW gain.

Orthogonal polynomials were used to test the linear (L) or quadratic (Q) effects of the moisture levels on assessed parameters. Significance was declared at P<0.05.

Results

Nutrient intake and growth performance

Intake of DM, organic matter (OM), CP, NDF and NFC increased [linearly (L), P<0.05; Table 2] with elevated moisture content although intake of ADF was similar among the experimental diets (P>0.05). The TWG and ADG improved linearly (L, P<0.05) with increasing moisture up to 30%; however, final BW and FCR were not affected by the diets.

Table 2. Effect of dietary moisture content on nutrient intake and growth performance of fattening lambs

	Level of moisture in the diet (% DM)[1]				SEM	*P*-value	
	10	20	30	40		Linear	Quadratic
Nutrient intake (g/d)							
Dry matter	1610^{c}	1686^{abc}	1795^{a}	1777^{ab}	51.9	0.04	0.23
Organic matter	1496^{c}	1569^{abc}	1671^{a}	1654^{ab}	45.5	0.03	0.19
Crude protein	262^{c}	273^{abc}	291^{a}	288^{ab}	7.66	0.04	0.25
Neutral detergent fiber	483^{c}	505^{abc}	541^{a}	533^{ab}	15.5	0.04	0.21
Acid detergent fiber	255	266	285	281	11.9	0.13	0.19
Non-fiber carbohydrates	713^{c}	747^{abc}	795^{a}	788^{ab}	23.4	0.02	0.24
Growth performance							
Initial body weight (kg)	33.2	33.2	33.5	32.7	1.88	0.87	0.81
Final body weight (kg)	51.4	52.6	54.1	52.7	1.06	0.22	0.31
Total weight gain (kg)	18.2^{b}	19.5^{ab}	20.7^{a}	20.1^{ab}	0.71	0.04	0.18
Average daily gain (g)	325^{c}	347^{abc}	371^{a}	359^{ab}	11.2	0.03	0.22
Feed conversion ratio	4.97	4.99	4.84	4.95	0.37	0.36	0.48

[1]Diets contained 10, 20, 30, and 40% moisture.
SEM, standard error of the mean.
a,b; Within rows, means with common letter(s) are not different (P>0.05).

Apparent nutrient digestibility

By increasing the dietary moisture content up to 30%, the digestibility of nutrients increased linearly but NDF and ADF digestibility remained stable (Table 3).

Rumen fermentation parameters

Increasing the level of moisture in the TMR had no effect on ruminal pH (Table 4). Rumen concentration of NH_3-N, total VFA and molar proportion of propionate increased linearly with increasing diet moisture content up to 30%, while acetate, butyrate, iso-butyrate, valerate, isovalerate levels, and the acetate:propionate ratio were similar among the experimental diets. The effects of experimental diets on rumen hydrolytic enzyme activities are presented in Table 5. Activity of CMCase and α-amylase increased linearly with the dietary moisture content 30%. The MCCase and FPD activities were not influenced by the experimental treatments.

Table 3. Effect of dietary moisture content on apparent nutrient digestibility of a total mixed ration fed to fattening lambs (g/kg DM)

	Level of moisture in the diet (% DM)[1]				SEM	*P*-value	
	10	20	30	40		Linear	Quadratic
Dry matter	781^{b}	796^{ab}	824^{a}	803^{ab}	11.5	0.04	0.19
Organic matter	785^{b}	810^{ab}	829^{a}	815^{ab}	12.2	0.03	0.22
Crude protein	801^{b}	814^{ab}	833^{a}	821^{ab}	9.55	0.05	0.32
Neutral detergent fiber	571	585	597	585	9.12	0.11	0.18
Acid detergent fiber	525	527	542	535	8.45	0.22	0.36

[1]Diets contained 10, 20, 30, and 40% moisture.
SEM, standard error of the mean.
a,b; Within rows, means with common letter(s) are not different (P>0.05).

Table 4. Effect of dietary moisture content on rumen fermentation parameters in fattening lambs

	Level of moisture in the diet (% DM)[1]				SEM	*P*-value	
	10	20	30	40		Linear	Quadratic
pH	6.26	6.20	6.14	6.19	0.039	0.17	0.15
NH_3-N (mg/dL)	19.1^{c}	20.1^{bc}	21.5^{a}	20.7^{ab}	0.46	0.02	0.21
Total VFA (mmol/L)	90.1^{b}	93.1^{b}	96.9^{a}	93.3^{ab}	2.11	0.04	0.18
Acetate (mmol/L)	55.3	56.5	58.9	57.6	2.07	0.41	0.65
Propionate (mmol/L)	17.2^{b}	18.5^{ab}	19.9^{a}	18.9^{ab}	0.64	0.04	0.23
Butyrate (mmol/L)	12.2	12.4	12.8	11.6	0.49	0.57	0.19
Isobutyrate (mmol/L)	2.42	2.48	2.53	2.31	0.13	0.63	0.31
Valerate (mmol/L)	1.32	1.34	1.29	1.27	0.04	0.31	0.62
Isovalerate (mmol/L)	1.06	1.14	1.10	1.04	0.08	0.76	0.35
Acetet:Propionate	3.24	3.06	2.97	3.05	0.15	0.34	0.47

[1]Diets contained 10, 20, 30, and 40% moisture.
SEM, standard error of the mean.
a,b; Within rows, means with common letter(s) are not different (P>0.05).

Blood metabolites

Plasma metabolites including glucose, TP and BUN were not impacted by the TMR moisture level (Table 6).

Feeding activity

The eating, rumination and chewing activities, individually or adjusted for nutrient intake [DMI and neutral detergent fiber intake (NDFI)], are presented in Table 7. Duration times for rumination and chewing were increased linearly when the dietary level of moisture increased up to 30% although the duration of eating, rumination and chewing per gram of DMI and NDFI was similar among the experimental diets.

Table 5. Effect of dietary moisture content on rumen microbial enzyme activity in fattening lambs (U/mL/min)

Item	Level of moisture in the diet (% DM)[1]				SEM	*P*-value	
	10	20	30	40		Linear	Quadratic
Carboxymethyl cellulase	22.8[b]	24.7[ab]	28.6[a]	26.5[ab]	1.44	0.04	0.36
Microcrystalline cellulase	5.13	5.16	5.68	5.73	0.36	0.14	0.96
Filter paper degrading activity	19.1	20.2	24.6	21.2	1.71	0.13	0.21
α-Amylase	60.1[b]	61.2[b]	72.8[a]	68.2[ab]	3.14	0.03	0.34

[1]Diets contained 10, 20, 30, and 40% moisture.
SEM, standard error of the mean.
a,b; Within rows, means with common letter(s) are not different (P>0.05).

Table 6. Effect of dietary moisture content on blood plasma metabolites in fattening lambs

	Level of moisture in the diet (% DM)[1]				SEM	*P*-value	
	10	20	30	40		Linear	Quadratic
Glucose (mg/dL)	77.3	77.7	78.5	77.9	0.46	0.22	0.29
Total protein (g/dL)	8.21	8.25	8.41	8.25	0.16	0.40	0.76
Blood urea nitrogen (mg/dL)	3.68	3.79	3.83	3.77	0.16	0.67	0.59

[1]Diets contained 10, 20, 30, and 40% moisture.
SEM, standard error of the mean.
There was no effect of treatments on these measurements.

Table 7. Effect of dietary moisture content on chewing activity in fattening lambs (min/day)

Item	Level of moisture in the diet (% DM)[1]				SEM	*P*-value	
	10	20	30	40		Linear	Quadratic
Eating	294	298	315	302	6.92	0.37	0.15
Rumination	262[b]	266[b]	286[a]	277[ab]	6.79	0.04	0.53
Chewing	555[b]	564[b]	599[a]	579[ab]	8.49	0.03	0.12
Eating per kg DMI	181	176	179	174	4.38	0.28	0.94
Ruminating per kg DMI	163	158	162	161	4.92	0.97	0.67
Chewing per kg DMI	345	334	342	335	6.11	0.42	0.77
Eating per kg NDFI	608	591	600	580	14.6	0.28	0.94
Ruminating per kg NDFI	544	529	541	539	16.5	0.97	0.67
Chewing per kg NDFI	1153	1119	1141	1120	20.4	0.41	0.76

[1]Diets contained 10, 20, 30, and 40% moisture.
SEM, standard error of the mean; DMI, dry matter intake, NDFI, neutral detergent fiber intake.
a,b; Within rows, means with common letter(s) are not different (P>0.05).

Discussion

The increase in nutrient intakes (*i.e.,* DM, OM, CP, NDF and NFC) by increasing the TMR moisture content up to 30% was probably due to the positive effects on diet palatability through improving the dietary texture or diluting the undesirable flavors (Lahr et al., 1983), resulting in higher feed consumption. Improved nutrient digestibility may explain the increase in feed intake although literature data detailing the effects of dietary moisture level on feed intake in ruminants are controversial. Consistent to our results, Beiranvand et al. (2016) indicated that adding water to a dry starter diet up to 50% dietary moisture content decreased the dustiness and aggregation of the smaller particles which improved the feed palatability and increased the starter intake in dairy calves. Moreover, Lahr et al. (1983) showed that as the level of DM decreased from 78 to 40%, diet palatability improved in dairy cows. In contrast, in a study conducted by Khan et al. (2014), feed intake tended to decrease as the level of dietary moisture increased from 35 to 65% in dairy heifers. In other studies, feeding diets differing in moisture contents to different classes of ruminants did not affect the feed intake (Leonardi et al., 2005; Fish and DeVries, 2012, Ghasemi Nejad et al., 2017; Beiranvand et al., 2018). These conflicting findings may be due to differences in feed composition (*i.e.,* source and level of forage, forage to concentrate ratio and feed particle size), environmental conditions (*i.e.,* temperature and humidity), moisture levels and animal age (Beiranvand et al., 2016).

A parallel pattern was obtained between performance traits including TWG and ADG with feed intake (Table 2) because feed intake is one of the most important parameters influencing animal growth performance. In agreement with our results, Beiranvand et al. (2016) reported that ADG was improved during the pre-weaning stage and throughout the experimental period in calves fed starter diets containing increasing levels of moisture

up to 50%. They postulated this was probably due to the higher solid feed intake by the calves and improved uniformity, adhesiveness and lower dustiness of the moist starter. Additionally, in another experiment, increasing the moisture content of starter diets up to 25% improved pre- and post-weaning ADG in dairy calves (Beiranvand et al., 2018).

In the present study, the beneficial effects of added moisture content on DM, OM and CP digestibility in lambs fed with TMR containing 30% moisture in comparison with those fed the control diet might be related to increased palatability by improving the texture or reducing undesirable flavors (Lahr et al., 1983). Another explanation could be a decreased feed sorting with increasing dietary moisture level (Shaver, 2002; DeVries and Gill, 2012) as it optimizes rumen fermentation by providing a variety of nutrients for maximum microbial growth. This is supported by the observed increase in rumen MCCase and α-amylase activity (Table 5) with increasing level of moisture in the diet. Increased rumen enzyme activity indicates also that digestion of cellulose was increased, thereby justifying the increased OM and DM digestibility. Unfortunately, there is a scarcity of information regarding the effects of TMR moisture content on nutrient digestibility in ruminant animals and to the best of our knowledge, the only study done is that of Ghasemi Nejad et al. (2017). However, contrary to the results of the present study, Ghasemi Nejad et al. (2017) did not report any significant effect on nutrient digestibility in Corriedale ewes, except for ether extract digestibility which was increased as the level of moisture was elevated from 40 to 60% in the diet.

In all experimental diets, mean ruminal pH values were within the normal physiological range of 6.1–6.8 stated by Van Soest (1994). The average pH was 6.20, indicating that lambs were not acidotic. Despite the increase in total ruminal VFA (acid load; Table 4) in lambs that were fed with the diets containing higher moisture levels in comparison to the control diet, which possibly exceeded the capacity of absorption for VFA from the rumen (Dijkstra et al., 1993), ruminal pH was unchanged among the experimental treatments. This was probably due to greater NDF intake (Table 2) which by stimulating the salivary secretion neutralizes the ruminal acids. Similar work conducted by Leonardi et al. (2005) with dairy cattle, indicated no effect on rumen pH from feeding experimental diets of different moisture contents (including 19.2 and 35.6% moisture). Additionally, Lahr et al. (1983) stated that ruminal pH was unchanged in dairy cows with increments of diet moisture content from 22 to 60%. On the contrary, Beiranvand et al. (2018) reported that ruminal pH significantly decreased in Holstein male calves as the DM of starter diet decreased from 90 to 50% during winter. In other work, ruminal pH remained unaffected when the DM of starter diet decreased from 90 to 50% during summer in Holstein male calves (Beiranvand et al., 2016).

Ruminal NH_3-N concentration in all lambs remained above the 5 mg/dL, which is the minimum required by rumen microbes to support optimum growth (Satter and Slyter, 1974). The increased ruminal NH_3-N concentration in lambs that were fed with 30% moisture TMR was probably due to higher DM intake (especially CP; Table 2) compared to other experimental groups. On the contrary, in a study on dairy cows, reducing the dietary DM from 80.8 to 64.4% did not affect the NH_3-N concentration (Leonardi et al., 2005).

Increased DMI (Table 2) in the experimental lambs feeding on the high-moisture diets up to 30% may explain the greater total VFA production. The likely reason for the higher propionate concentration in the rumen of these lambs might be attributed to their increased intake of smaller particles with lower fiber content. Consistent with the results obtained in the present study, experiments with dairy calves during winter and summer demonstrated that the total ruminal VFAs concentration and molar proportion of propionate increased linearly as the DM content of the starter diets decreased from 90 to 50% (Beiranvand et al., 2016; 2018); this was justified by the increased starter feed consumption. Conversely, in some studies rumen fermentation parameters were not changed when different dietary moisture contents were fed to dairy cattle. In the experiment conducted by Leonardi et al. (2005), decreasing the diet DM content from 80.8% to 64.4% had no effect on VFA concentrations in dairy cows. Moreover, Robinson et al. (1990) indicated that feeding experimental diets containing 65, 55 and 35% moisture had no effect on total VFA and molar proportions of major VFA in dairy cattle.

Differences amongst the experimental diets in ruminal enzymatic activity could reflect the changes in microbial communities affected by the diet (Kamra et al., 2010). The improvement in ruminal CMCase activity as an indicator of fiber degradation and α-amylase as an indicator of starch degradation by feeding wet diets compared to control group was probably due to greater available substrate (*i.e.,* NFC for α-amylase and NDF for MCCase) through increased feed intake (Table 2). Another possible explanation is the reduction of the lag phase of the rumen microflora to colonize and then ferment feed particles by feeding wet the diets. Indeed, in ruminants, dietary feed particles must be hydrated via liquid uptake before being colonized and digested by rumen microbes (Teimouri Yansari et al., 2004; Teimouri Yansari, 2017).

Despite increased nutrient intake (*i.e.,* NFC and CP; Table 2) with increasing TMR moisture level, concentrations of blood metabolites including glucose, TP and BUN were not affected by the experimental diets. The reason for this inconsistency is not clear. Consistent with the results obtained in the present study, Ghasemi Nejad et al. (2017) indicated that adding water to TMR from 40% to 60% moisture content had no significant effect on serum TP, BUN, glucose or triglycerides in Corriedale ewes. Findings of Beiranvand et al. (2016)

also indicated that adding water to starter (up to 50% DM) had no significant effect on serum glucose or BUN concentration in young calves on days 30, 45 and 70. In another experiment, serum glucose and BUN concentrations were not influenced by feeding TMR containing different moisture levels (*i.e.,* 35, 55 and 65% on DM basis) in dairy cows (Robinson et al., 1990).

Increased mean average time devoted to rumination and chewing in lambs fed with the diets containing higher moisture content probably relates to greater feed intake, especially NDF, as it simulates the rumination activity. Despite these results, no differences were obtained by Beiranvand et al. (2016) among the experimental groups in time spent on eating, ruminating, standing, lying or non-nutritive oral behavior when dairy calves were fed with starter diets differing in moisture content.

Conclusions

Based on the present findings, adding water to total mixed rations up to 30% (DM basis) linearly increased the digestibility of DM, OM and CP and improved the daily gain in finishing lambs. Increasing the moisture content up to 30% modified the rumen parameters including an increase in total VFA and molar proportion of propionate as well as in CMCase and α-amylase activities. However, moisture content of the diet did not significantly impacted on blood metabolites and feeding behavior. Overall, adding water to dry diets appeared to be a cost effective management practice that could be implemented in small ruminant hisbandry to improve the diet palatability and animal performance.

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