Iranian Journal of Applied Animal Science

Growth Performance, Rumen and Cecum Fermentation Parameters, and Microbial Protein Synthesis in Kermani Lambs with Divergent Residual Feed Intake Fed Forage and Concentrate Diets

Research Article

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Received on: 23 Aug 2019
Revised on: 16 Nov 2019
Accepted on: 30 Nov 2019
Online Published on: Dec 2020

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Online version is available on: www.ijas.ir

ABSTRACT

It was hypothesized that some of the variations in rumen and cecum fermentation parameters can form a share of individual differences resulting in feed efficiency, which may be altered based on the type of diet. This research aimed to determine the effects of dietary effect and feed efficiency of growing lambs (Kermani lambs) on their rumen and cecum indices and also microbial protein synthesis in gastrointestinal tract. Lambs (n=40; bodyweight (BW)=16±1.5 kg) were fed either a concentrate (CONC; 11.8% crude protein (CP), 18% neutral detergent fiber (NDF), 2.66 Mcal/kg metabolizable energy (ME); n=20) or a forage-based diet (FOR; 15.6% CP, 36.8% NDF, 2.15 Mcal/kg ME; n=20). Individual intake was recorded and residual feed intake (RFI) was determined over 42 days. The 8 highest (Low-RFI) and 8 lowest efficiencies (High RFI) records of lambs from each dietary group were selected (n=16; average BW=20±2.1 kg), and the samples of rumen and cecum fluid, and also urine were collected at the end of the trial. Data were analyzed as a 2 × 2 factorial design with RFI class (high *vs.* low efficiency), their type of diet (FOR *vs.* CONC), and their interaction in the defined model. Based on the results, high-efficiency lambs had a higher level (P<0.01) of total volatile fatty acid (VFA), proportional concentrations of acetate, propionate, and ammonia nitrogen in the rumen in comparison to low-efficiency lambs. Higher (P<0.01) amounts of allantoin, xanthine + hypoxanthine, total purine derivative (PD), microbial nitrogen and microbial protein were observed in the high efficiency than low-efficiency lambs. The low efficiency lambs had greater (P<0.01) proportional acetate, cecal pH and cecal ammonia N compared to high-efficiency lambs. The RFI class × diet type interaction was significant (P<0.01) for the majority of parameters of the rumen, cecum, and microbial protein synthesis. The results of this experiment exhibited that hindgut fermentation especially cecum played a key role in the efficiency of feed utilization in lambs which have consumed larger amounts of fermentable substrates.

KEY WORDS cecum, feed efficiency, fermentation, residual feed intake, sheep.

INTRODUCTION

Residual feed intake (RFI) is the most important criterion in the calculation of feed efficiency, which is defined as the difference between the animal's actual intake and its expected feed intake, based on the average daily gain (ADG) of an animal in a particular test period (Koch *et al.* 1963). The RFI has been reported to be independent of growth and mature size and is moderately heritable, so selection for lower RFIs will result in generating higher feed efficiency and production that lead to consuming less feed in animals. Diet composition is an effective factor in the rumen microbial population (Carberry *et al*. 2012). Ellison *et al*. (2017) have found that residual feed intake may be affected by diet through alteration in rumen microbiome. The rumen microbial population provides end products as volatile fatty acid (VFA), ammonia (NH_3), methane gas and microbial mass (Ampapon *et al*. 2019). Therefore, these fermentation products affect the feed efficiency of animals. Guan *et al*. (2008) indicated that there is a linkage among the rumen microbiome and its fermentation parameters with RFI in cattle. Carberry *et al*. (2012) reported that the ruminal bacterial population may be altered with diet, and it also differs among animals with dissimilar RFI. There is a relationship between the high-energy diet and *Methanobrevibacter smithii* and RFI in cattle fed with high concentrate diet (Zhou *et al*. 2010). Although in ruminant the main site of microbial action is rumen but fermentation occurs in hindgut especially in cecum (Vitorino *et al*. 2012). The cecum has microbial mass and activity of epithelial tissue as rumen (Elliott and Little, 1977). It has been specified that cecal VFA supplies about 8.6% of total metabolizable energy in steers (Siciliano-Jones and Murphy, 1989). Microbial fermentation in the cecum of ruminants elevates the efficiency of the digestive tract (Lewis and Dehority, 1985). Gressley *et al*. (2011) indicated hindgut fermentation supplied a considerable quantity of energy requirement in ruminants. Furthermore, this additional fermentation contributes to health disorders connected with ruminal acidosis, thus it can affect the metabolism and health of the ruminants. There is little information to determine the effect of diet type on the RFI in the sheep. These studies on the sheep are less than other domestic ones (Zhang *et al*. 2017). The Kermani sheep is a fat-tail breed for meat production. This breed is one of the most important of Iranian sheep adapted to the semi-dry environment in the southeastern region of Iran. The quantity and quality of feed and pasture are limited, so improving feed efficiency can improve Kermani sheep products. We hypothesized that some of the variations in rumen and cecum fermentation can form a share of individual differences observed in feed efficiency, which may be altered based on the diet type. The objectives of this research were: (1) to determine the RFI of Kermani lambs (2) to determine the relationship between RFI and diet type on the rumen and cecum parameters and microbial protein synthesis.

MATERIALS AND METHODS

Animals and diet

All animal procedures were approved by the University of Zabol Animal Care and Use Committee. A total of 40 growing male lambs of Kermani breed types were used (average initial body weight (BW)= 16±1.5 kg). These lambs were individually penned to record individual feed intake. Lambs were randomly grouped according to their BW to receive either a concentrate-based diet (CONC; Table 1; n=20) or forage-based diet (FOR; Table 1; n=20). A 14-d period was carried out for adaptation to diets. The lambs were fed *ad libitum* formulated ration at 07:00 and 15:00 daily, and had free access to water. Feed intake of lambs was measured using an automated feed intake system (GrowSafe Systems Ltd.; Airdrie, Alberta, Canada). Body-weight was recorded weekly, and 2-d average initial and final BW were collected to determine average daily gain (ADG). The lambs were selected and penned according to their RFI. Individual feed intake was recorded over a 42-d trial period. Cockrum *et al*. (2013) suggested that at least a 42-day period is an essential time to compute RFI in sheep. RFI was calculated as the expected feed intake subtracted from the actual feed intake. Expected feed intake for each was determined by regressing ADG and metabolic mid-weight (MBW) on actual feed intake. Metabolic mid-weight was calculated using the average BW calculated using 2 d initial and final BW and raised to the 0.75 power. The amounts of experimental diet-fed and refusals were individually recorded daily. The base model (Koch *et al*. 1963) used was:

$$Y_j = \beta0 + \beta1\ ADGj + \beta2\ MBW_j + e_j$$

Where:

Y_j: dry matter intake (DMI) of the jth animal.

β0: regression intercept.

β1: partial regression coefficient of the DMI on average daily gain (ADG).

β2: partial regression coefficient of the DMI on MBW.

e_j: uncontrolled error of the jth animal.

These 40 lambs were ranked within diet type by their RFI (CONC, n=20; FOR, n=20). Lambs were grouped on RFI (most negative to most positive RFI) within each diet, and the 10% highest group were selected high feed efficiency (Low-RFI; n=4) and the 10% lowest ranking were selected low feed efficiency (Low-RFI; n=4). Eight lambs were selected for each RFI class in two groups based on diet type (n=16 total) for collection experimental samples and data on the last day of the experimental period. Diet samples were dried at 60 ˚C for 48 h and calculated the percentage of DM. Dried samples were ground with a Wiley mill (2-mm screen). Diet samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to procedures of Van Soest (1994) and dry matter (DM), organic matter (OM), ash, ether extract (EE) and nitrogen (N) by the AOAC (2005) methods.

Table 1 Ingredient and nutrient composition of diets fed to experiment growing lambs

Ingredients (% dry matter, DM)	CONC[1]	FOR[2]
Alfalfa	-	68.3
Corn	51.1	-
Wheat middlings	30.1	27
Corn gluten	10.9	-
Cane molasses	2.5	2.50
Salt	1.8	1.30
Calcium carbonate	2.26	0.62
Calcium sulfate	0.75	-
Potassium chloride	0.22	-
Trace minerals and vitamins	0.38	0.36
Analyzed nutrient composition		
Dry matter, % as fed	92.1	93.40
Crude protein, % DM	11.8	15.6
Neutral detergent fiber, % DM	18	36.8
Acid detergent fiber, % DM	7.2	25.4
Metabolizable energy, Mcal/kg DMc	2.66	2.15

CONC: concentrate based diet and FOR: forage based diet.

Ruminal fluid collection and analysis

Ruminal fluid samples were collected from the individual animal on the last day of the experimental period to determine ruminal PH, ammonia and VFA. Samples were collected with the stomach tube (Flora Rumen Scoop, Profs-Products, Guelph, Canada), between 2 and 4 h after morning feeding. The rumen fluid pH was determined immediately using an Orion digital pH meter (Orion SA 720, Thermo Fisher Scientific, Waltham, MA) after collection. Samples (50 mL) of rumen fluid were strained through 4 layers of cheesecloth and mixed with 0.5 mL of 9 M sulfuric acid and stored at -20˚ for later analysis for ammonia determinations (AOAC, 2005). In preparation for VFA, the samples were thawed and mixed with 4 mL mercuric-2-chloride at a ratio of 1:4 to stabilize the VFA (Pierce *et al.* 2006). VFA concentration samples were then determined by gas chromatography using a flame ionization detector (Supelco Bulletin 749D, Supelco, Inc., Bellefonte, Pa.).

Urine collection and analysis

Urine was collected daily into a container with approximately 100 mL of 10% H_2SO_4. Daily subsamples were stored at -20 ˚C until being analyzed. Urine samples were analyzed for allantoin, uric acid, xanthine and hypoxanthine following the procedure reported by Chen and Gomes (1992). The total PD excretion (in sheep sum of all 4 compounds allantoin, uric acid xanthine and hypoxanthine) is used for the estimation of microbial protein synthesis.

Cecal fluid collection and analysis

The method used for insertion cecal cannula by surgery was the same as used by Myers *et al.* (1967). All of the lambs (n=40) cannulated before the experiment.

Samples of approximately 20 mL were collected from cecum using a cannula between 2 and 4 h after morning feeding to measure pH, ammonia and VFA concentration. The samples were preserved with 0.5 mL of 9 M sulphuric acid and stored at -20 ˚C for subsequent analysis of NH_3-N. The methods used for measure pH, cecal fluid VFA and ammonia concentration were similarly used in rumen fluid.

Statistical analysis

Data were analyzed using the PROC MIXED of SAS 9.2 (SAS, 2004) as a 2 × 2 factorial in a completely randomized design with RFI class (high efficiency vs. low efficiency), diet type (FOR *vs.* CONC), and their interaction as fixed effects in the model. Significance was determined using Tukey's test" at P < 0.05 level of significance and the values were presented in the tables. The model is:

$$Y_{ij}= \mu + A_i + B_j + AB_{ij} + e_{ij}$$

Where:
μ: population mean.
A_i: main effects of A factor.
B_j: main effects of B factor.
AB_{ij}: interaction effects of A and B.
e_{ij}: experimental error effects.

RESULTS AND DISCUSSION

Animal performance

Performance parameters are presented in Table 2. High-efficiency animals consumed 25.2% less feed (P<0.01) compared to high-RFI animals. However, average daily gain and final body weight were the same (P>0.1).

Table 2 Effect of residual feed intake class and diet type on intake and performance in Kermani lambs

Item	RFI class		Diet type		RFI class × diet type				SEM	P-value		
	Low RFI	High RFI	CONC	FOR	Low RFI × CONC	Low RFI × FOR	High RFI × CONC	High RFI × FOR		RFI class	Diet type	RFI × diet
No. of animals	8	8	8	8	-	-	-	-	-	-	-	-
Residual feed intake (RFI), kg/d	-0.51	0.52	0.00	0.01	-0.57	-0.42	0.53	0.52	0.007	< 0.001	0.133	0.080
Average daily gain (ADG), kg/d	0.12	0.12	0.14	0.11	0.14	0.10	0.13	0.11	0.040	0.694	< 0.001	0. 400
Average daily feed intake (ADFI), kg/d	0.80	1.07	0.83	1.04	0.73	0.87	0.93	1.21	0.010	< 0.001	< 0.001	0.096
Initial body weight, kg	17.08	17.06	17.07	17.06	17.01	17.13	17.10	17.03	0.008	0.840	0.421	0.176
Metabolic mid-weight (MMW), kg	9.33	9.34	9.44	9.23	9.46	9.20	9.41	9.26	0.057	0.933	0.801	0.361
Final body weight, kg	22.22	22.27	22.83	21.66	23.00[a]	21.44[b]	22.66[a]	21.88[b]	0.143	0.744	< 0.001	0.021
Feed conversion ratio, kg of DM/kg of BW gain (FCR)	6.84	8.76	6.12	9.48	5.19	8.50	7.05	10.47	1.136	< 0.001	< 0.001	0.900

Low-RFI: 20% lowest RFI lambs of each diet (most efficient); High-RFI: 20% highest RFI lambs of each diet (least efficient); CONC: concentrate based diet and FOR: forage based diet.
SEM: standard error of the means.

Feed conversion ratio (FCR) was better in the high efficiency compared to low-efficiency lambs (P<0.001). The high level of concentrate in the diet led significantly to more daily gain and final body weight, less feed intake and improvement in the FCR in the lambs (P<0.001). Interactions between the efficiency of feed intake and diet types were not significant except for final body weight (P<0.05).

Rumen fermentation parameters

The effects of RFI class and diet type on the ruminal parameters are reported in Table 3. The high-efficiency lambs had a significantly higher level (P<0.01) of total VFA, proportional concentrations of acetate, propionate, and ammonia nitrogen compared with the low-efficiency lambs. However, the low-efficiency lambs had greater (P<0.01) proportional concentration of butyrate than high-efficiency lambs. The RFI class did not affect (P>0.1) on ruminal pH. The diet based on forage increased significantly (P<0.001) proportional concentration of acetate, butyrate, ruminal pH, and ammonia nitrogen in the content of rumen lambs. However, CONC-fed lambs had significantly greater (P<0.001) ruminal concentration of total VFA and proportional concentration of propionate. The RFI class × diet type interaction affected (P≤0.001) total VFA, the proportional concentration of propionate, butyrate and ammonia nitrogen although there was no interaction on the acetate or ruminal pH.

Cecum fermentation parameters

Cecal fermentation data are summarized in Table 4. The Low-RFI lambs had significantly greater (P<0.001) concentrations of total VFA and proportional concentrations of propionate in cecal fluid compared to High-RFI lambs. However, the low-efficiency lambs had significantly greater (P<0.01) proportional acetate, cecal pH and cecal ammonia nitrogen than high-efficiency lambs.

Lambs fed by the FOR-diet had greater (P<0.001) proportion of acetate, butyrate, pH of cecum and ammonia concentration than CONC-diet according to Table 4. The total VFA and proportion of propionate were significantly greater (P<0.001) in CONC-fed compared to FOR-fed lambs. The interaction of RFI class × diet type was significantly for (P<0.001) total VFA, proportion concentration of acetate, propionate, butyrate, and ammonia nitrogen.

Microbial protein

Purine derivatives and microbial nitrogen data are shown in Table 5. The high-efficiency lambs had higher (P<0.01) amounts of allantoin, xanthine + hypoxanthine, total purine derivative (PD), microbial nitrogen and microbial protein compared to low-efficiency lambs. The RFI class had no (P=0.964) significant effect on the amount of uric acid. The amount of all purine derivatives in the urine (allantoin, xanthine+hypoxanthine and uric acid), microbial nitrogen and thus gram per day of microbial protein synthesis were greater (P<0.001) in CONC-fed than FOR-fed lambs. The interaction affected significantly (P<0.001) the levels of all PDs in urine so that the Low-RFI, CONC-fed lambs had the greatest amount of allantoin, xanthine+hypoxanthine and total PD, microbial nitrogen and protein. The lowest level of allantoin and uric acid was for High-RFI, FOR-fed lambs. However, the uric acid was the greatest in the High-RFI, CONC-fed lambs.

The same average daily gain for less feed intake in Low-RFI versus High-RFI is consistent with the concept of residual feed intake. Similarly, other researchers obtained the same results in sheep (Zhang *et al*. 2017), cattle (Steyn *et al*. 2014; Sharma *et al*. 2016) and steer (Smith *et al*. 2010). Zhang *et al*. (2017) showed smaller rumen size and longer duodenum in the Low-RFI than High-RFI lambs lead to the less feed intake and more sufficient absorption rate of Low-RFI lambs.

Table 3 Effects of residual feed intake (RFI) class, diet type on ruminal volatile fatty acids (VFAs), pH, ammonia and nitrogen

Item	RFI class		Diet type		RFI class × diet type				SEM	P-value		
	Low RFI	High RFI	CONC	FOR	Low RFI × CONC	Low RFI × FOR	High RFI × CONC	High RFI × FOR		RFI class	Diet type	RFI × diet
Total VFA (mM/L)	71.35	62.93	70.55	63.73	73.98^{a}	68.72^{b}	67.13^{b}	58.73^{c}	1.421	< 0.001	< 0.001	< 0.001
VFAs (mol/100 mol)												
Acetate, C2	64.25	63.34	60.52	67.08	61.20	67.31	59.83	66.86	0.634	0.012	< 0.001	0.175
Propionate, C3	25.47	22.47	28.55	19.39	28.79^{a}	22.15^{b}	28.3^{a}	16.64^{c}	0.473	< 0.001	< 0.001	< 0.001
Butyrate, C4	10.49	13.94	10.91	13.52	10.45^{c}	10.53^{c}	11.38^{b}	16.50^{a}	0.222	< 0.001	< 0.001	< 0.001
Ruminal pH	6.92	6.76	6.46	7.21	6.50	7.33	6.43	7.08	0.055	0.116	< 0.001	0.353
Ruminal NH_3-N (mg/dL)	7.89	5.25	6.06	7.10	7.22^{b}	8.56^{a}	4.90^{d}	5.60^{c}	0.241	< 0.001	< 0.001	0.01

Low-RFI: 20% lowest RFI lambs of each diet (most efficient); High-RFI: 20% highest RFI lambs of each diet (least efficient); CONC: concentrate based diet and FOR: forage based diet.
SEM: standard error of the means.

Table 4 Effects of residual feed intake (RFI) class, diet type on fermentation parameters in the cecum

Item	RFI class		Diet type		RFI class × Diet type				SEM	P-value		
	Low RFI	High RFI	CONC	FOR	Low RFI × CONC	Low RFI × FOR	High RFI × CONC	High RFI × FOR		RFI class	Diet type	RFI × diet
Total VFA (mM/L)	37.68	24.89	34.00	28.57	31.13^{c}	44.24^{a}	36.88^{b}	12.90^{d}	0.811	< 0.001	< 0.001	< 0.001
VFA mol/100 mol												
Acetate, C2	52.38	56.88	52.09	57.16	48.37^{c}	56.39^{b}	55.82^{b}	57.94^{a}	0.258	< 0.001	< 0.001	< 0.001
Propionate, C3	33.38	29.21	35.02	27.57	35.63^{a}	31.13^{c}	34.40^{b}	24.01^{d}	0.266	< 0.001	< 0.001	< 0.001
Butyrate, C4	13.13	13.01	9.81	16.32	6.42^{c}	19.60^{a}	13.22^{b}	13.05^{b}	0.231	0.527	< 0.001	< 0.001
Cecal pH	6.72	7.10	6.60	7.22	6.45	7.00	6.75	7.45	0.036	< 0.001	< 0.001	0.346
Cecal NH_3-N (mg/dL)	15.56	18.31	15.30	18.56	14.71^{c}	16.40^{b}	15.90^{b}	20.72^{a}	0.385	< 0.001	< 0.001	< 0.001

Low-RFI: 20% lowest RFI lambs of each diet (most efficient); High-RFI: 20% highest RFI lambs of each diet (least efficient); CONC: concentrate based diet and FOR: forage based diet.
SEM: standard error of the means.

Table 5 Effects of residual feed intake (RFI) class, diet on the purine derivative in urine and microbial protein synthesis

Item	RFI class[1]		Diet type[2]		RFI class × Diet type				SEM	P-value		
	Low RFI	High RFI	CONC	FOR	Low RFI × CONC	Low RFI × FOR	High RFI × CONC	High RFI × FOR		RFI class	Diet type	RFI × diet
Allantoin (mmol/d)	6.51	4.39	5.62	5.28	7.33^{a}	5.70^{b}	4.86^{c}	3.91^{d}	0.078	< 0.001	0.007	< 0.001
Xanthine + hypoxanthine (mmol/d)	0.96	0.86	0.97	0.85	1.14^{a}	0.78^{c}	0.80^{c}	0.91^{b}	0.021	< 0.001	< 0.001	< 0.001
Uric acid (mmol/d)	2.05	2.04	2.24	1.86	1.91^{c}	2.19^{b}	2.58^{a}	1.52^{d}	0.081	0.964	< 0.001	< 0.001
Total purine derivative (mmol/d)	9.53	7.30	8.84	7.99	10.38^{a}	8.67^{b}	7.31^{c}	7.29^{c}	0.093	< 0.001	< 0.001	< 0.001
Microbial N (g/d)	6.93	5.31	6.42	5.81	7.56^{a}	6.30^{b}	5.30^{c}	5.31^{c}	0.049	< 0.001	< 0.001	< 0.001
Microbial protein synthesis (g/d)	43.30	33.17	40.16	36.31	47.18^{a}	39.42^{b}	33.15^{c}	33.19^{c}	1.930	< 0.001	< 0.001	< 0.001

Low-RFI: 20% lowest RFI lambs of each diet (most efficient); High-RFI: 20% highest RFI lambs of each diet (least efficient); CONC: concentrate based diet and FOR: forage based diet.
SEM: standard error of the means.

The lower level of intake in high-efficiency animals improved FCR and decreased maintenance requirements. This can be due to the decrease in the amount of energy required for the digestion of feed, lower heat rise of feeding (Ferrell and Jenkins, 1998), as well as energy expenditure for maintenance and growth (Steyn *et al.* 2014). Different results were obtained using the diet based on concentrates for feed intake and average daily gain (ADG). Similar to the current result, Borton *et al.* (2005) reported higher ADG in CONC-fed lambs compared to FOR-fed lambs. In contrast to these results, other researchers found that average daily feed intake was lower in CONC-fed lambs compared with FOR-fed lambs while ADG was the same (Ellison *et al.* 2017).

This difference in the results can be explained by the greater crude protein and metabolizable energy (ME) content in the diet based on concentrate than on forage. Livestock is fed mainly to supply their energy (Van Soest *et al.* 1991). Reducing feed intake and increasing average daily again via concentrate-based ration led to improved FCR in CONC-fed than in FOR-fed lambs. These results were consistent with the findings of Hatfield *et al.* (1997) where high levels of cereals were used in the diet of lambs.

The total VFA was the greatest in Low RFI, CONC-fed lambs. Similar to the results of this study, Guan *et al.* (2008) observed a significant difference in the profile of rumen volatile fatty acids in the cattle with different RFIs.

Low-RFI steers were reported to have significantly higher concentrations of butyrate ($P<0.001$) and valerate ($P=0.006$) and tended to exhibit greater concentrations of total VFAs ($P=0.06$) and acetate ($P=0.07$). The total VFA concentration is an important indicator of microbial fermentation, which is largely determined by the type of diet and affects the feed efficiency of ruminant animals. Probably, higher levels of microbial fermentation in the rumen of high-efficiency animals and providing greater levels of fermentable carbohydrate by increasing the level of concentrate elevated the total VFA concentration.

A negative relationship was observed between ruminal pH and VFA concentration given the diet type (Table 3). The high grain diet leads to altered ruminal status benefits to subacute acidosis (Kiyoshi *et al.* 2000).

The Low-RFI, CONC-fed lambs had the highest level of propionate (Table 3). Similarly, Ellison *et al.* (2017) reported that the proportional concentration of propionate tended to be greater in highly efficient sheep fed with a concentrate-based diet. The propionate is a more energetically efficient end product of fermentation compared to acetate and butyrate for ruminants. The higher production of propionate in high-efficiency animals can be associated with a reduction in the loss of gas energy, especially methane. On the other hand, the propionate formation of the dicarboxylic pathway requires the consumption of hydrogen through NADH and FADH provided by different biochemical pathways. It can therefore reduce methane production and improve the energy status of livestock (Morris *et al.* 2019). In ruminant animals, the use of concentrate often increases the proportional of propionate in the rumen (Van Soest, 1994).

The greatest butyrate proportion was observed in High-RFI, FOR-fed lambs while its lowest value was recorded in Low-RFI lambs regardless of the diet type. The possible reason for the diminishing of butyrate is that high-efficiency lambs can convert more co butyrate into important energy metabolites (Guan *et al.* 2008; Liang *et al.* 2017).

High ruminal pH is expected in lambs fed with a high forage diet. Forage intake leads to higher salivation and ruminal pH compared to concentrate diet. Similarly, some of the researchers recorded that the rumen pH did not affect the RFI groups (Ellison *et al.* 2017). The effect of factors such as ruminal buffering capacity and microbial population on ruminal pH led to a diminishing effect of VFA concentration on rumen acidity in high-efficiency lambs (Dijkstra *et al.* 2012).

In this study, a higher concentration of ammonia N in Low-RFI than in High-RFI lambs was similar to the results of Rius *et al.* (2012) as well as Lawrence *et al.* (2011) in lactating cows and heifers, respectively. The high concentration of ammonia in high effective lambs can be due to several reasons. High fermentation activity in the high effective animals because of use microbes of peptide and amino acids following lack of energy conducted high level ammonia in Low-RFI lambs. Also, it was hypothesized that Low-RFI animals would have greater activity of urealytic and oxygen scavenging bacteria that would provide more ammonia for microbial protein synthesis and better fermentation conditions (Li *et al.* 2019). The protein degradation and microbial protein synthesis in the rumen are the main events of N metabolism in ruminants (Bach *et al.* 2005). Differences in rumen microbial activity or absorption from the rumen could account for the discrepancy of NH_3 concentrations (Rius *et al.* 2012). The supplying fermentable carbohydrates by concentrate could lead to more nitrogen utilization for microbial growth in the rumen (Hungate, 1966), thereby reducing the level of N ammonia in animal fed concentrate. According to the results of Cardozo *et al.* (2000) and Cardozo *et al.* (2002), the lower proteolytic activity of rumen bacteria with high-concentrate diets caused a reduction of the ruminal level N ammonia in CONC-fed lambs.

Sparse studies have been done on the effect of cecal fermentation on the divergence in feed efficiency. According to the results of Gressley *et al.* (2011) of the 1.77 kg of organic matter injected into the ileum of male calves, 0.27 kg (15%) of organic matter reached the ileum to feces. Cecum is also important as a small fermentation tank for digestion and absorption of the digestive tract. The greatest concentration of total VFA and butyrate was observed in Low-RFI, FOR-fed lambs. This result suggested more activity of the bacterial population in the cecum of efficiency animals (Siciliano-Jones and Murphy, 1989). Probably the bacterial population of the cecum in the Low-RFI lambs exploited nutrients very effectively especially carbohydrates reaching the cecum for improving the energy efficiency.

The cecal proportion of propionate was the greatest in Low-RFI, CONC-fed lambs. Similarly, previous studies have indicated a decline in acetate to propionate ratio (De Gregorio *et al.* 1984) and higher propionate (Orskov *et al.* 1970) in cecum of sheep as well as diminished cecal pH in steer (Siciliano-Jones and Murphy, 1989) which consumed high grain than high forage levels. In CONC-fed lambs, the higher share of digestible carbohydrates approaching the cecum (Mann and Orskov, 1973) caused a greater proportion of cecal propionate compared to FOR-fed lambs.

The High-RFI, FOR-fed lambs had the greatest cecal ammonia in contrast to the rumen. Reduction of the amount of cecal NH3 in the CONC-fed was consistent with results obtained by Siciliano-Jones and Murphy (1989) where a decline in cecal NH_3 concentration was observed in steers fed with high grain diets. Reduction of the concentration of cecal ammonia observed with increased concentrate consumption and Low-RFI is probably due to the synchronization of carbohydrate and nitrogen in the cecum and further utilization from nitrogen for the growth and production of microbial protein by microorganisms (Orskov *et al*. 1970). The elevated absorption of ammonia of the cecal epithelium (Dixon and Nolan, 1986) can be another possible explanation for its decline in the cecum of CONC-fed than that of FOR-fed lambs. An increase in the rate of absorption was observed for acetate and propionate as well as butyrate in proportion with the rise in their concentration (Myers *et al.* 1967). The production of fermentable compounds in the cecum is indicative of the utility of the processes in the interest of improving the energy metabolism in animals, and the absorption of products is also important. This has been revealed in the cecal absorption of VFA and NH3 (Elliott and Little, 1977).

The high molar proportion of acetate produced in the cecum of low-efficiency lambs was probably due to the greater escape of fibers of the rumen digestion; thus a high level of the substrate was available for cecal bacteria. Also, the low level of acetate produced in the cecum of Low-RFI lambs could be due to more cecal absorption of acetate compared to high-RFI lambs.

Purine derivatives are indicators for microbial protein synthesis (MPS). Measurement of the synthesis of microbial protein in the rumen can reveal the nitrogen metabolism status in ruminants. In this trial, the proportion of the purine derivatives in the total purine derivatives was consistent with the range proposed by Chen and Gomes (1992). In a study, urinary loss of the purine derivatives increased with the utilization of a high energy diet in sheep and cattle (Fujihara *et al*. 2007). Thus, we observed higher concentrations of individual and total purine derivatives in the urine of CONC-fed lambs than FOR-fed lamb.

An elevated level of microbial protein synthesis was observed in Low-RFI, CONC-fed lambs. The amounts and type of carbohydrate and concentrations of available nitrogen and synchrony among available nutrients in the rumen are some important factors for microbial protein synthesis (Bach *et al*. 2005). The higher levels of fermentable carbohydrates were supplied by diet-based concentrate rather than by the forage could synchrony at providing fermentable carbohydrates and available nitrogen (Horadagoda *et al*. 2008) led to the more effective usage of nitrogen sources and promoting microbial growth in the rumen of Low-RFI lambs.

In spite of the existence of a negative relationship between ammonia concentration and microbial protein synthesis (Bach *et al*. 2005) in the current research, a positive relationship was observed for the lambs of different classes of RFI. A large number of protozoa in Low-RFI lambs and the probability of backflow of some engulfed insoluble protein to the rumen fluid by protozoa can justify this positive relationship (Eugene *et al*. 2004).

CONCLUSION

As the results showed, high-efficiency lambs had a higher level of total VFA, proportional concentrations of acetate, propionate, and ammonia nitrogen in the rumen than low-efficiency lambs. Higher amounts of microbial nitrogen and microbial protein in both simple and interaction effects were observed in the high efficiency than low-efficiency lambs that it indicated rumen microbes in high effective animals had maximum operation of synchronization of energy and nitrogen sources. The greater proportional acetate, pH and ammonia nitrogen in cecum of High-RFI lambs expressed more escape feed of rumen fermentation than Low-RFI lambs. The RFI class × diet type interaction affected the most of the parameters of the rumen, cecum, and microbial protein synthesis. The results of the present study indicated that rumen and cecum fermentation parameters play a role in the efficiency of feed (RFI) utilization in particular lambs which had taken larger amounts of fermentable substrates.

ACKNOWLEDGEMENT

The authors wish to thank the Iran National Science Foundation (INSF) for the Financial support for this research.

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