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Original Research Article

Alterations in nutrient digestion and utilization associated with different residual feed intake in Hu sheep

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ABSTRACT

Improving feed efficiency is crucial to the animal industry. Residual feed intake (RFI) is now regarded as an index of feed efficiency evaluation and is independent of growth characteristics. Our study aims to explore the alterations in growth performance and nutrient digestion in Hu sheep with different RFI phenotypes. Sixty-four male Hu sheep (body weight = 24.39 ± 1.12 kg; postnatal days = 90 ± 7.9) were selected for the study. After an evaluation period of 56 days and power analysis, samples were collected from 14 low RFI (L-RFI group, power = 0.95) and 14 high RFI sheep (H-RFI group, power = 0.95). The L-RFI sheep yielded a lower ($P < 0.05$) feed conversion ratio and dry matter intake; however, both groups exhibited similar average daily gain ($P > 0.05$). The acid detergent fiber, neutral detergent fiber, organic matter, and crude protein apparent digestibility were higher ($P < 0.05$) in L-RFI sheep. N intake and fecal N output (% of N intake) were lower ($P < 0.05$) and N retention (% of N intake) was higher ($P < 0.05$) in L-RFI sheep, whereas no difference ($P > 0.05$) was found in urine N output (% of N intake) between the 2 groups. Furthermore, L-RFI sheep gave lower ($P < 0.05$) serum glucose concentrations and higher ($P < 0.05$) non-esterified fatty acid concentrations. Meanwhile, a lower ruminal acetate molar proportion ($P < 0.05$) and higher propionate molar proportion ($P < 0.05$) were observed in L-RFI sheep. In summary, these results revealed that despite having lower dry matter intake, L-RFI sheep possess higher nutrient digestibility, N retention, ruminal propionate production and serum glucose utilization, in order to meet energy demands. Selection for low RFI sheep could reduce feed costs, which in turn provides economic benefits to the sheep industry.

1. Introduction

One of the main challenges that livestock production systems currently face is the conflict between food supply and its demand by a growing population (O'Hara et al., 2020). To mitigate this, one efficient approach is via selecting animals with greater feed efficiency (FE) (Jackson, 2014; Muir et al., 2018). While growing up at a similar rate as other animals, animals with high FE consume less feed and produce lower methane and manure (Basarab et al., 2003). Residual feed intake (RFI) describes the variance between actual and expected daily feed intake (Koch et al., 1963; Hernandez-Sanabria et al., 2012) based on the body weight (BW) and average daily gain (ADG) of the animal (Koch et al., 1963; Kennedy et al., 1993; Crews, 2005). Previous works regarded the FE as the feed conversion ratio (FCR) in growing sheep, however, there is growing evidence that RFI could be another efficient way to evaluate the FE in sheep (Paula et al., 2013; Zhang et al., 2017; Goldansaz et al., 2020).

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The RFI method has been used in FE evaluation of sheep, including lambs and ewes (Paula et al., 2013; Redden et al., 2014). Sharifabadi et al. (2012) found that dry matter intake (DMI) of low RFI lambs was 12% lower than that of high RFI lambs, whilst having comparable growth performance to high RFI lambs. Redden et al. (2014) showed that DMI can vary up to 30% between the low RFI and high RFI ewes. Variation in the RFI could be linked to nitrogen (N) partitioning because the latter is an important part of feed utilization efficiency in animals. It has been reported that the N utilization efficiency is higher in low RFI lambs (Gevari et al., 2020).

We hypothesized that the low RFI sheep would have higher nutrient digestibility and conversion efficiency than high RFI sheep, especially N utilization. To test this hypothesis, we investigated alterations in growth performance, feed digestibility, ruminal fermentation parameters and serum biochemical indicators along with their interactions in Hu sheep with different RFI phenotypes. This study helps to increase our knowledge of sheep productivity with different RFI and provide a reference for Hu sheep breeding with high FE.

2. Materials and methods

2.1. Animal ethics statement

The experiment was conducted in a farm located in Huzhou, Zhejiang, China, from October 4th, 2021 to December 5th, 2021. All animal processes were permitted by the Animal Care and Use Committee of Zhejiang University (Hangzhou, China) and were achieved in agreement with the university's rules for animal research.

2.2. Animals and management

Sixty-four male Hu sheep (BW = 24.39 ± 1.12 kg, postnatal days = 90 ± 7.9; mean ± standard deviation) were chosen for the experiment from the training cohort fed with the experimental diet for 20 d. Our trial lasted for 60 d, with the first 3 d dedicated to facility adaptation. All sheep were individually fed in a 200 cm × 60 cm area of the indoor pen with a slotted floor under exactly the same conditions, and experimental rations were formulated according to the feeding standard of meat-producing sheep and goats (Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2004). The ration components are shown in Table 1. All sheep were fed at 08:00 and 18:00, and had ad libitum access to fresh water. Feed was given in excess to allow 5% to 10% of daily feed refusal. From d 4, feed intake was calculated during the first 3 d of each week before morning feeding, by weighing the amount of feed offered and refused, respectively. Samples of offered and refused feed were analyzed for dry matter (DM). All sheep were weighed on d 0, 28 and 56 before morning feeding. The ADG was calculated according to the BW changes between d 0 and 56.

Table 1
Ingredients and nutrient composition of total mixed rations (% DM basis).

Ingredients	Content	Nutrient level	Content
Peanut vine	35.0	DM	55.22
Corn silage	35.0	OM	91.77
Corn grain	18.9	CP	10.76
Soybean meal	5.4	EE	2.83
Wheat bran	4.5	NDF	49.53
Limestone	0.45	ADF	32.64
$CaHPO_4$	0.15	AIA	2.09
NaCl	0.45	Starch	11.80
Premix[1]	0.15	ME, MJ/kg DM	9.82

OM = organic matter; EE = ether extract; DM = dry matter; NDF = neutral detergent fiber; CP = crude protein; ADF = acid detergent fiber; AIA = acid insoluble ash; ME = metabolizable energy.

[1] Premix, formulated to contain (per kilogram of DM total mixed rations): vitamin A ≥ 13,000 IU, vitamin D ≥ 4,000 IU, vitamin E ≥ 40 mg, Cu ≥ 15 mg, Fe ≥ 65 mg, Mn ≥ 60 mg, Zn ≥ 140 mg, I ≥ 1.5 mg, Se ≥ 0.85 mg, Co ≥ 0.05 mg.

2.3. Sample collection and measurements

Ration and fecal samples (approximately 200 g) were collected from each sheep at 07:00 and 16:00 on d 57 and 58 of the feeding period. Fecal samples were collected using plastic bags that were fixed on the buttocks of each sheep. Rations and feces were dried at 65 °C for 48 h and then smashed in a cyclone mill (Tecator 1,093; Tecator AB, Hoganas, Sweden) through a 1-mm screen. Ether extract (EE, No. 983.15), DM (No. 934.01), crude protein (CP, No. 988.05), and crude ash (No. 942.05) were analyzed by AOAC methods (AOAC, 1990). Starch was detected by a colorimetric detection kit (Nanjing Jiancheng Co. Ltd.). The neutral detergent fiber (NDF) was determined with sodium sulphite and heat-stable amylase using a fiber analyzer (A200I, USA) by the method of Van Soest et al. (1991). The ADF was measured based on the NDF residue, also conducted on the fiber analyzer (A200I, USA) system (Van Soest et al., 1991). Acid insoluble ash (AIA) was used as an internal marker to evaluate fecal excretion and nutrient apparent digestibility successfully (Vankeulen and Young, 1977). Accordingly, the content of AIA in feed and feces was first analyzed, and the nutrient apparent digestibility was calculated as follows (Li et al., 2022):

Digestibility (%) = [1 − (AIA concentration in feed/AIA concentration in feces) × (nutrient concentration in feces/nutrient concentration in feed)] × 100.

Organic matter (OM) was calculated as follows:

OM (%) = 100 − crude ash (%)

Metabolic energy was calculated as follows (Menke and Steingass, 1988):

Metabolic energy (MJ/kg DM) = 13.97 − 0.0127 × ADF (g/kg DM) + 0.0165 × EE (g/kg DM) − 0.0057 × crude ash (g/kg DM).

The chemical composition of the diet is listed in Table 1.

Urine samples were collected from each sheep at 07:00 and 16:00 on d 57 and 58 of the feeding period using a funnel-shaped latex tube tied around the sheep prepuce, allowing fluid to flow into a plastic container. A 5-mL volume of each collected sample was mixed with 20 mL of 3 mol/L H_2SO_4 to keep the urinal pH below 2.0. After that, all urine samples were immediately kept at −20 °C. Urea nitrogen and creatinine were measured by the nitrogen and creatinine urinary colorimetric detection kit (Nanjing Jiancheng Co. Ltd., Nanjing, China). Creatinine was utilized as a marker to evaluate urine volume (Leonardi et al., 2003) and was predicted at a rate of 29 mg/kg BW (Valadares et al., 1999).

Serum samples were collected from the jugular vein of each sheep into 10 mL collection tubes coated with silicone oil (free of additives) before morning feeding on d 59 of the experimental period. Next, after centrifugation at 3,000 × *g* for 15 min, serum was collected and frozen at −20 °C. The serum samples were analyzed using a 7020 auto-analyzer (Hitachi High-Technologies Corp., Tokyo, Japan) to determine the total albumin, protein, urea nitrogen, globulin, glucose, non-esterified fatty acid (NEFA), aspartate aminotransferase (AST), cholesterol, alanine aminotransferase (ALT), triglycerides, and alkaline phosphatase (ALP) contents as described by Richardson et al. (2004).

On d 60 of the experimental period, ruminal fluid was collected using an oral stomach tube before morning feeding by modifying

the method of Shen et al. (2012). Briefly, the diameter of the oral metal stomach tube used was 10.02 mm with a 12.04-mm diameter head, while the insertion depth of the tube from the sheep's incisors teeth to central rumen was about 80 cm. The ruminal fluid pH was recorded using a pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China). Samples were stored at −20 °C before volatile fatty acid (VFA), ammonia-nitrogen (NH_3–N) and microbial crude protein (MCP) concentration analysis. The VFA was measured using a gas chromatograph (GC-2011, Shimadzu, Kyoto, Japan) according to the method of Hu et al. (2005). The NH_3–N and MCP concentrations were determined using methods described by Broderick and Kang (1980) and Makkar et al. (1982), respectively.

2.4. RFI calculation

The RFI of each sheep was analyzed based on the difference between the actual and expected average daily feed intake during the experiment. A multiple linear regression model was selected to determine the regression equation:

$$Yi = \beta 0 + \beta 1 \mathrm{ADG}i + \beta 2 \mathrm{BW}^{0.75} i + ei,$$

where *Yi* is the expected average daily feed intake (kg/d) in the *i*th animal, β0 is the regression intercept, β1 is the partial regression coefficient of ADG (g/d), β2 is the partial regression coefficient of the mid-test $BW^{0.75}$ (kg), and *ei* is the random error associated with the *i*th sheep.

2.5. Statistical analysis

We used G*power (v3.1.9.7) software (gpower.hhu.de/) to calculate the power of the RFI value. Data on feed intake, growth performance, feed apparent digestibility, N partitioning, serum biochemical indices, and rumen fermentation parameters were calculated using a *t*-test model using R software (v4.1.0). Statistical significance was set at $P \leq 0.05$.

3. Results and discussion

3.1. RFI distribution and power analysis

Typically, while the RFI evaluation period for ewes was 42 d after adaptation to the diet and facilities (Snowder and Van Vleck, 2002; Cammack et al., 2005; Tortereau et al., 2020), 40 to 60 d was reported to be required for rams, though the ideal duration was slightly different between wool and meat breeds (Cockrum et al., 2013; Amarilho-Silveira et al., 2022). Reducing duration would minimize costs, but compromise accuracy. For example, reducing the evaluation period to 35 d could explain 63.6% of the feed intake in the weekly model (weekly average feed intake as a repeated measure and the weekly ADG) (Amarilho-Silveira et al., 2022). In our study, the weekly average feed intake was measured, and the mid and end ADG were calculated, removing non-genetic variation as much as possible in Hu rams, and a 56-day evaluation period was chosen. We calculated the RFI distribution of 64 sheep (Fig. 1A), and found 23 sheep with low RFI values (ranging from −0.41

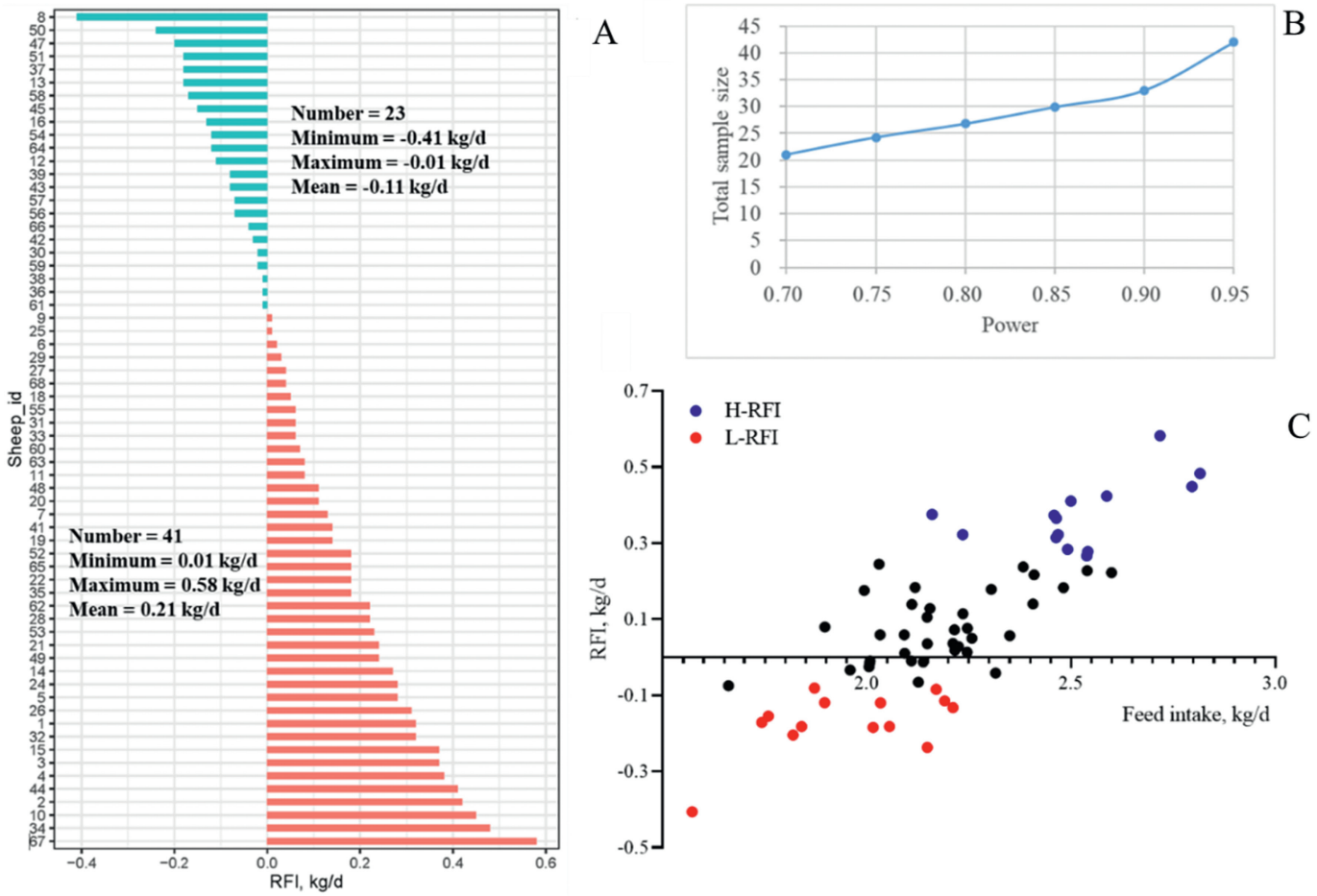

Fig. 1. The selection of the high and low residual feed intake (RFI) group. (A) The RFI distribution of all Hu sheep ($n = 64$). (B) Power analysis of RFI value in Hu sheep ($n = 64$). (C) The RFI coefficients, average daily feed intake of Hu sheep ($n = 64$), and the situation of high RFI group (H-RFI, $n = 14$) and low RFI group (L-RFI, $n = 14$) with power of 0.95.

to −0.01 kg/d) and 41 sheep with high RFI values (ranging from 0.01 to 0.58 kg/d). The difference between the mean value of high and low RFI sheep was 0.33 kg/d (0.21 vs. −0.11 kg/d).

According to the power analysis of the RFI value in 64 sheep (Fig. 1B), we found that the total sample size of the sheep ranged from 21 to 42 (equal numbers of sheep were assigned into low, middle and high RFI), with power values varying from 0.70 to 0.95. Concerning the RFI distribution and data reliability, we chose the sample size of power 0.95. Fourteen sheep with high RFI values (ranging from 0.27 to 0.58 kg/d) were categorized as low FE and defined as H-RFI group, and 14 sheep with low RFI value (ranging from −0.41 to −0.08 kg/d) were regarded as high FE and defined as the L-RFI group for later analysis (Fig. 1C). The difference between the mean values of both groups was 0.54 kg/d (0.37 kg/d of H-RFI group vs. −0.17 kg/d of L-RFI group).

3.2. Growth performance and FE

Growth performance and FE of Hu sheep are shown in Table 2. No differences were found ($P > 0.05$) in initial BW (24.46 vs. 24.39 kg), final BW (35.76 vs. 35.44 kg), metabolic BW (12.85 vs. 12.79 kg), and ADG (205.32 vs. 200.78 g/d) between the H-RFI and L-RFI groups. The L-RFI sheep consumed 1.08 kg DM/d, which was less ($P < 0.01$) than that consumed by H-RFI sheep (1.39 kg DM/d). According to the nutrient requirements of meat-type sheep and goats (Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2004), in order to have a 200 g/d ADG, the DMI are 0.94, 1.21, and 1.31 kg/d for growing male sheep at 25, 30 and 35 kg BW, respectively. Based on that, average FCR for growing male sheep from 20 to 35 kg BW was 5.77 kg DM/kg BW gain. In this study, the FCR of L-RFI sheep was 5.47 kg DM/kg BW gain, which was lower ($P < 0.01$) than that in H-RFI sheep (7.03 kg DM/kg BW gain), and lower than the standard of meat-type sheep and goats. This indicates that selection for low RFI sheep could reduce feed costs considerably. Higher FE animals were associated with lower DMI and potentially less nutrient excretion to the environment (Hernandez-Sanabria et al., 2012; Bath et al., 2013; Arndt et al., 2015). Under the present feeding conditions, the decreased DMI but similar ADG values in L-RFI sheep indicated that highly efficient sheep could reduce feed consumption without affecting the growth performance, in line with findings from previous Hu sheep studies (Zhang et al., 2017, 2021). For dairy and beef cattle, normally 3 to 5 estimates of BW are suggested to accurately estimate the ADG. For timid Hu sheep, single day weighing in the middle and end of the experiment was adopted in the present study to reduce weighing stress. Although the consistency of ADG values observed between the 2 periods, automated systems should be implemented in future trials to improve measurement accuracy.

Table 2
Characterization of intake and growth performance in Hu sheep with high and low residual feed intake (H-RFI and L-RFI, $n = 14$).

Item	H-RFI	L-RFI	SEM	*P*-value
DMI, kg/d	1.39	1.08	0.007	<0.001
Initial BW, kg	24.46	24.39	0.037	0.863
Final BW, kg	35.76	35.44	0.086	0.736
ADG, g/d	205.32	200.78	1.390	0.768
Metabolic BW ($BW^{0.75}$), kg	12.85	12.79	0.017	0.740
FCR, kg DM/kg BW gain	7.03	5.47	0.047	0.001
RFI	0.37	−0.17	0.010	<0.001

DMI = dry matter intake; ADG = average daily gain; BW = body weight; FCR = feed conversion ratio.

3.3. Apparent digestibility

Apparent digestibility of Hu sheep is listed in Table 3. NDF (62.02% vs. 55.30%), ADF (61.21% vs. 55.26%), CP (63.55% vs. 60.22%), and OM (73.39% vs. 70.06%) digestibility was higher ($P \leq 0.05$) in the L-RFI group than the H-RFI group. Several studies have suggested a negative correlation between digestibility and RFI in cattle, indicating that low RFI cattle tend to harbor higher feed digestibility (McDonald et al., 2010; Basarab et al., 2013; Johnson et al., 2019). In line with the review by Cantalapiedra-Hijar et al. (2018), a higher digestibility accompanied with low DMI in the L-RFI group was observed in the present study. Kenny et al. (2018) postulated that an increased feed consumption in high RFI cattle would reduce the ruminal residency time, hence decreasing nutrient digestibility. However, this speculation was not supported by some literature that they referenced. It was later commented that the apparently improved digestibility of low RFI cattle could be either an inherent phenomenon, or an outcome of slower passage rate of digesta through the rumen due to lower DMI. They concluded that apart from possible contributory factors associated with the nature of the diet, accurate and sensitive methods should also be employed in DM digestibility evaluation.

The volume of the rumen and ruminal microbiome should be evaluated in order to credit the apparently improved digestibility of low RFI sheep to inherency or simply a function of a slower passage rate of digesta through the rumen. In the present study, the apparent digestibility was evaluated only in the last 2 d of the experimental period with the AIA as an internal marker. Recent studies (Lawrence et al., 2011; Potts et al., 2017) using AIA and indigestible NDF as internal markers failed to find associations between nutrient digestibility and the RFI phenotype. Some probable reasons are the species of animal, diet composition, and sampling period. Furthermore, it has been indicated that the dietary AIA content should exceed 7.5 g/kg DM in order to get accurate measurements (Thonney et al., 1985). In the present experiment, with an AIA content of 20.9 g/kg DM diet, no exogenous sources of AIA, silica (Cheng and Coon, 1990), sipernat (Rodehutscord et al., 1996), or bentonite (Sales and Britz, 2001) were needed. Feeding cannulated Santa Inês ram lambs (44.3 ± 5 kg of BW) with diets of 39.5% NDF yielded 79.3%, 68.1%, and 66.5% of DM, NDF, and ADF digestibility, respectively (Ferreira et al., 2011). Feeding the 10 months old male Santa Inês crossbred sheep with a diet of roughage-to-concentrate ratio at 70:30 resulted in DM and NDF digestibility of 65.84% and 63.98%, respectively (Silva et al., 2021). However, the higher DM (85.91% and 87.78% in H-RFI and L-RFI sheep) and NDF (55.30% and 62.02% in H-RFI and L-RFI sheep) digestibility in relation to the higher NDF of the diet (49.53%) was observed in our study. The overestimation may be due to the low CP content (10.76%) in our diet. As suggested by Lee and Hristov (2013), a low CP content (less than 14%) might lead to fecal output underestimation and digestibility overestimation, respectively. Furthermore, the overestimation could also be a

Table 3
Apparent digestibility of Hu sheep with high and low residual feed intake (H-RFI and L-RFI, $n = 14$).

Item	H-RFI	L-RFI	SEM	*P*-value
DM, %	85.91	87.78	0.112	0.129
NDF, %	55.30	62.02	0.247	0.010
ADF, %	55.26	61.21	0.269	0.041
CP, %	60.22	63.55	0.143	0.027
OM, %	70.06	73.39	0.146	0.034

DM = dry matter; ADF = acid detergent fiber; NDF = neutral detergent fiber; CP = crude protein; OM = organic matter.

consequence of short sampling periods in our experiment. For digestibility determination, standard sampling periods were not unified for the AIA method, varying from one day (4 time points) to 7 d (Lawrence et al., 2011; Bonilha et al., 2017; Johnson et al., 2019). However, while one sample each on d 7, 28, 49, and 70, in pregnant beef heifers failed to find associations between nutrient digestibility and the RFI phenotype (Lawrence et al., 2011), but 3 consecutive days in Nellore bulls (Bonilha et al., 2017) and 7 consecutive days in beef cattle (Johnson et al., 2019) reported significant correlations. To minimize possible deviations in digestibility calculations, further trials by using total collection (7 consecutive days) might be a more relevant comparison for our results.

3.4. Serum biochemical indicators

Data obtained for serum biochemical indicators are listed in Table 4. It was observed that L-RFI sheep had lower ($P < 0.05$) glucose concentrations and higher ($P < 0.05$) NEFA concentrations than H-RFI sheep. No differences were found ($P > 0.05$) in cholesterol and triglyceride concentrations between the 2 groups. In addition, serum indicators reflecting liver function and protein metabolism also revealed no changes ($P > 0.05$) between H-RFI and L-RFI sheep. The content of triglycerides in blood can potentially reflect the body's energy status (Vernon et al., 2000). However, in our work, no difference was detected in the triglyceride concentration, whereas the glucose and NEFA levels were significantly changed. Glucose is an important energy source and is essential for organ function, growth, and milk production, and when animals have increased energy demands, the serum glucose concentration decreases in order to meet energy requirements (Moallem et al., 2012). According to the growth performance, apparent digestibility and N partitioning data between H-RFI and L-RFI sheep, data from our serum biochemical indicators suggested that serum-circulating glucose was effectively absorbed by body tissues and organs in L-RFI sheep to meet energy demands. Cantalapiedra-Hijar et al. (2018) suggested that low RFI animals are not only characterized by lower maintenance energy requirements, but also by higher partial efficiency of metabolizable energy utilization for growth. NEFA reflects the degree of lipid mobilization in the body under negative energy balance. When animals experience a decreased DMI, the plasma NEFA concentration gradually increases, which occurs in order to meet in the energy requirements (Kalyesubula et al., 2021). In L-RFI sheep with lower DMI, a higher NEFA concentration (285.50 vs. 253.75 μmol/L) in serum would indicate increased lipolysis to maintain body energy homeostasis. In addition, although the NEFA concentration in L-RFI sheep was higher than that in H-RFI sheep, this concentration did not lead to disease symptoms in the animals, which suggested that moderate lipolysis does negligible harm to animal production.

Table 4
Serum biochemical indicators of Hu sheep with high and low residual feed intake (H-RFI and L-RFI, $n = 14$).

Item	H-RFI	L-RFI	SEM	*P*-value
Liver function				
ALT, U/L	25.57	25.63	0.250	0.984
AST, U/L	117.14	116.58	0.589	0.931
ALP, U/L	391.55	416.41	4.470	0.616
Protein metabolism				
Total protein, g/L	61.41	61.41	0.183	0.997
Albumin (A), g/L	27.63	26.00	0.087	0.083
Globulin (G), g/L	33.79	35.41	0.157	0.346
A:G ratio	0.83	0.74	0.004	0.051
BUN, mmol/L	4.75	4.92	0.032	0.633
Energy substrates				
Glucose, mmol/L	4.34	3.92	0.017	0.014
Cholesterol, mmol/L	1.57	1.70	0.007	0.088
Triglyceride, mmol/L	0.40	0.41	0.003	0.648
NEFA, μmol/L	253.75	285.50	1.265	0.006

BUN = blood urea nitrogen; ALT = alanine aminotransferase; ALP = alkaline phosphatase; NEFA = non-esterified fatty acid; AST = aspartate aminotransferase.

3.5. Ruminal fermentation parameters

Rumen fermentation parameters of Hu sheep are listed in Table 5. No difference ($P > 0.05$) was found in ruminal pH between the L-RFI and H-RFI groups (7.10 vs. 7.08). It is possible that the rumen fluid was contaminated with saliva during sampling as the pH was slightly higher than that observed in the rumen of 10-month-old male Santa Inês crossbred sheep fed a diet with the roughage-to-concentrate ratio at 70:30 (Silva et al., 2021), and also that of rumen cannulated adult Hu sheep (Xie et al., 2018). The acetate-to-propionate concentration ratio and the molar proportion of acetate were lower ($P < 0.05$) in the L-RFI group than the H-RFI group. However, the molar proportion of propionate was higher ($P < 0.05$) in the L-RFI group than the H-RFI group. No differences ($P > 0.05$) were found in total VFA, acetate concentration, propionate concentration, butyrate concentration, and other parameters between the 2 groups. Although low RFI sheep had lower DMI, their ruminal pH and total VFA concentrations were comparable to those in the high RFI group, which are in line with previous results (Lawrence et al., 2011; Rius et al., 2012; Liang et al., 2017). The higher propionate molar proportion and lower acetate-to- propionate concentration ratio indicated that the ruminal fermentation characteristics were altered in L-RFI sheep and converted to propionate fermentation. Propionate is the primary gluconeogenic energy substrate used by the ruminant liver and accounts for 60% of the glucose used by the animal (Harfoot, 1978). With the conversion of propionate via ruminal fermentation, the methane production of L-RFI sheep was numerically lower than that of H-RFI sheep (8.40 vs. 9.11 mmol/L) following the equation established by Van Nevel and Demeyer (1995), which further confirmed that L-RFI sheep with ruminal propionate fermentation could maintain their energy

Table 5
Ruminal pH and volatile fatty acids (VFA) of Hu sheep with high and low residual feed intake (H-RFI and L-RFI, $n = 14$).

Item	H-RFI	L-RFI	SEM	*P*-value
pH	7.08	7.10	0.005	0.484
NH_3–N, mg/dL	8.29	9.71	0.065	0.164
MCP, mg/100 mL	77.94	81.80	0.123	0.002
Acetate (A), mmol/L	19.39	17.94	0.134	0.321
Propionate (P), mmol/L	3.25	3.64	0.029	0.292
Isobutyrate, mmol/L	0.33	0.36	0.002	0.201
Butyrate, mmol/L	2.99	3.10	0.025	0.479
Isovalerate, mmol/L	0.50	0.50	0.003	0.859
Valerate, mmol/L	0.41	0.36	0.002	0.072
Total VFA, mmol/L	26.80	25.85	0.180	0.633
A:P ratio	6.12	5.04	0.034	0.002
Methane[1], mmol/L	9.11	8.40	0.063	0.305
Molar proportion, mmol/100 mmol				
Acetate	72.38	69.24	0.102	0.003
Propionate	12.05	13.88	0.060	0.003
Isobutyrate	1.25	1.45	0.011	0.083
Butyrate	10.86	12.01	0.054	0.051
Isovalerate	1.91	1.96	0.013	0.691
Valerate	1.56	1.45	0.011	0.366

NH_3–N = ammonia-nitrogen; MCP = microbial crude protein.
[1] Methane production in rumen contents was calculated from the relationship between methane and VFA established by Van Nevel and Demeyer (1995): Methane (mmol/L) = 0.450 × acetate (mmol/L) − 0.250 × propionate (mmol/L) + 0.400 × butyrate (mmol/L).

requirements more effectively. Guan et al. (2008) stated that low RFI steers harbor greater butyrate and total VFA concentrations than high RFI steers. In contrast, there were no differences found in the same parameters between the 2 groups in our experiment. The NH_3–N concentration was similar ($P > 0.05$) between L-RFI and H-RFI groups (9.71 vs. 8.29 mg/dL). However, the MCP concentration in the L-RFI group was higher ($P < 0.05$) than that in the H-RFI group (81.80 vs. 77.94 mg/100 mL). The NH_3–N content reflects the degradation of feed protein in the rumen and the utilization of NH_3–N by microorganisms. In our research, CP digestibility and MCP concentration in the rumen were higher in L-RFI sheep, and there was no difference in NH_3–N concentration in the rumen between the 2 groups. These results indicated that the microbes in L-RFI sheep captured rumen NH_3–N more efficiently.

Recent studies have revealed microbial differences in the rumen between high and low RFI sheep (Perea et al., 2017; Zhang et al., 2021; Arce-Recinos et al., 2022). More importantly, a microbial profile of 6 rumen microbial species could be used to rank the FE of sheep (Ellison et al., 2019), but microbiota in both the rumen and intestinal tract should be considered, as the gastrointestinal tract microbiota are indispensable to the nutritive function of animals (Perea et al., 2017). The distinctive bacteria were different in these sheep studies, and were all amplicon sequencing data. Thus, future efforts should explain whether the microbiome in the gastrointestinal tract really contributes to sheep-to-sheep variation of FE or only co-varies with it, by using metagenomics and culturomics.

3.6. Partitioning of nitrogen

The N intake in H-RFI sheep was greater ($P < 0.05$) than that in L-RFI sheep (Table 6). The fecal N output, urinary N output, and N retention were lower ($P < 0.05$) in L-RFI sheep than that in H-RFI sheep. When converted to dietary N intake percentage, fecal N was lower ($P < 0.05$, 36.37% vs. 39.69%) and N retention was higher ($P < 0.05$, 47.09% vs. 42.83%) in L-RFI sheep than H-RFI sheep, but urinary N values were not changed ($P > 0.05$) between the 2 groups. The urinary N/N intake is often used as an indicator to reflect the efficiency of N utilization in ruminant animal production systems (Bernard et al., 2020; Khanaki et al., 2021). Urinary N is produced from the protein oxidation of tissue and the NH_3–N absorbed from the rumen (Lobley, 1992). When dietary rumen-degradable protein is present in excess of the amount required by ruminal microorganisms, the NH_3–N, is absorbed and metabolized into urea in the liver, and eventually lost in the urine. In our research, as the urinary N was lower than that reported in a previous study of Santa Inês lambs (Ferreira et al., 2011), it is possible that a higher N retention had been calculated in our experiment. No differences in ALT, AST, ALP, and BUN between the 2 groups indicated that the degree of protein oxidation of tissue was similar between the L-RFI and H-RFI sheep. In other words, sheep with high FE transfer N for tissue absorption more effectively, and possibly possess a lower protein turnover rate (Cantalapiedra-Hijar et al., 2018). Although we expect the higher CP digestibility in L-RFI sheep to exhibit enhanced feed protein degradation into NH_3–N in the rumen, the NH_3–N in turn was more efficiently captured by microbes to produce MCP in L-RFI sheep, resulting in unchanged urinary N values ($P > 0.05$) between the 2 groups. The higher CP digestibility and MCP concentration was in line with the lower fecal N output in L-RFI sheep for higher utilization efficiency of microbial protein than dietary rumen non-degradable protein.

It was observed previously that feeding highly fermentable diets increased both urea N recycling and NH_3–N capture by microbes in the rumen, resulting in lower urinary N losses and higher N retention (Stern and Hoover, 1979; Huntington, 1989; Fluharty et al., 1999). Agarwal et al. (2015) suggested that the higher N retention of feeding highly fermentable diets might be due to the higher propionate production in rumen. The role of propionate as a precursor for glucose synthesis in ruminants is well established, meanwhile propionate was shown to inhibit the synthesis of *N*-acetyl glutamate (Stewart and Walser, 1980). *N*-acetyl glutamate is an allosteric regulator of hepatic carbamoyl phosphate synthetase I. The inhibition of *N*-acetyl glutamate would result in a decrease of urea cycle activity, stimulate the release of insulin, and increase muscle protein synthesis (Abdulrazzaq and Bickerstaffe, 1989). In the present study, compared to the H-RFI sheep, L-RFI sheep had a higher molar proportion of rumen propionate, the main substrate for gluconeogenesis in ruminants. The L-RFI sheep, thus, might have higher gluconeogenesis, yielding more energy and increasing N retention by inhibiting urea cycling. Future research should include the investigation of inner mechanisms of RFI classification based on energy and N metabolism, especially the role of propionate in the distinction between L-RFI and H-RFI sheep by using metagenomics of ruminal microbiota and transcriptomic analysis of the liver.

Table 6
Nitrogen partitioning in Hu sheep with high and low residual feed intake (H-RFI and L-RFI, $n = 14$).

Item	H-RFI	L-RFI	SEM	*P*-value
N intake, g/d	23.93	18.56	0.114	<0.001
N output, g/d				
Feces	9.49	6.72	0.058	<0.001
Urine	4.19	3.06	0.035	0.001
N retention, g/d[1]	10.25	8.78	0.058	0.016
N output, % of N intake				
Feces	39.69	36.37	0.142	0.027
Urine	17.48	16.54	0.117	0.413
N retention, % of N intake	42.83	47.09	0.202	0.042

[1] N retention = N intake − fecal N − urinary N.

4. Conclusions

This study highlights that low RFI sheep exhibit lower DMI but similar ADG to high RFI sheep, which may be due to their higher nutrient apparent digestibility, changed ruminal fermentation characteristics and higher metabolizable protein efficiency and energy utilization. Importantly, selection for low RFI sheep could reduce feed costs, and improve economic benefits in the sheep industry and is of great significance to the development of feed-saving animal husbandry.

Author contributions

Hongbo Zeng: contributed to perform the experiments, data collection and analysis, and wrote the original manuscript. **Yuyang Yin**, **Lingxi Chen**, and **Zhuoxin Xu**: contributed to conduct the animal experiment. **Yang Luo**, **Qian Wang**, and **Bin Yang**: contributed to review the manuscript. **Jiakun Wang:** contributed to funding supporting, review and edit the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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