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Effects of the higher concentrate ratio on the production performance, ruminal fermentation, and morphological structure in male cattle-yaks

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Abstract

Background: The present study evaluated the effects of the different concentrate-to-forage ratio on the parameters of production, ruminal fermentation, blood biochemical indices, and ruminal epithelial morphological structure of the male cattle-yaks.

Methods: Eight male cattle-yaks (280 ± 10 kg of body weight) were randomly divided into the high concentrate (HighC, 70% concentrate feeds on a dry matter basis) and low concentrate (LowC, 50% concentrate feeds on a dry matter basis) groups. All the animals were regularly provided rations twice a day at 08:00 and 16:00 h and had free access to water. The experiment lasted for 37 days.

Results: The dry matter intake and average daily gain of the HighC group were higher ($p < 0.05$) than those of LowC group. Moreover, a high concentrate diet was found to significantly increase ($p < 0.05$) the total volatile fatty acid (TVFA) production, and the ratio of propionate and butyrate in TVFA. On the contrary, the ruminal pH, the ratio of isobutyrate and isovalerate, and the acetate-to-propionate were significantly decreased ($p < 0.05$) after high concentrate feeding. The lipopolysaccharide concentrations of the ruminal fluid and plasma in the HighC group were higher ($p < 0.05$) than those of the LowC group. The results of the ruminal histomorphology showed the rumen to possess an inflammatory reaction.

Conclusion: These findings revealed that upon higher dry matter intake and average daily gain, high concentrate feeding altered the rumen fermentation and morphology, inducing the ruminal inflammation of the cattle-yak.

KEYWORDS
cattle-yak, high concentrate, morphological structure, production performance, ruminal fermentation

1 | INTRODUCTION

Yaks (*Bos grunniens*) are irreplaceable domestic animals of the Qinghai-Tibetan Plateau with a vital ecological niche in the Qinghai-Tibetan Plateau ecosystem (Ma, Zhu, et al., 2020). However, due to the harsh environment of the plateau, the forage grass tends to become extremely scarce in the cold season. Generally, the production performance of yaks cannot catch up with the other breeds of beef cattle,

resulting in a lower economical income (Dai et al., 2021). Thus, to promote the production performance of yaks, the cattle-yak is bred to combine the traits such as cold tolerance along with the excellent productivity of the other cattle. Compared to yak, the cattle-yak is characterized by bigger size, better heat tolerance, a larger quantity of milk production, and a stronger labour force owing to heterosis. In China, there is an increasing demand for beef every year. However, because of the limited resource availability, more beef is being imported annually (Liao et al., 2018). Therefore, to meet the shortage in beef production, the yak and other beef cattle are fattened. To date, most yaks and cattle-yaks are slaughtered without fattening decreasing the productivity of the animals.

In the development process of farming modern large-scale ruminants, the animals are commonly fed diets containing high grain. The ruminants can utilize the high grain diets to maximize the energy intake in response to meet the requirements of the increased feed intake. High grain diets are commonly fed to increase the deposition of fat in the meat-producing ruminants (Janes et al., 1985). However, the ruminants fed high grain diets cause ruminal or metabolic acidosis, severely damaging feed conversion, the gastrointestinal function, and health and welfare of animals (Ma et al., 2021). Subacute ruminal acidosis is one of the common nutritional diseases, causing the death of microbes to release the endotoxins, resulting in ruminal dysfunction (Plaizier et al., 2008). The changes in the ruminal histomorphology are another consequence associated with high grain diets (Ma et al., 2021).

The growth performance of the cattle-yaks can be improved by stall-feeding with mixed ration (Dai et al., 2021). In dairy cows (Lechartier & Peyraud, 2010), cattle (Brown et al., 2006), and yaks (G. J. Chen et al., 2015), a high concentrate feeding model is conducive for improving the performance of the production. However, the effects of a high concentrate diet on the production performance of the cattle-yaks remain limited; besides, there is a paucity of literature dealing with the rearing of cattle-yaks on similar diets and under the same environmental conditions. Therefore, the present study aimed to investigate the effects of high dietary concentrate levels on the productive parameters, ruminal fermentation, blood biochemistry, and ruminal epithelial morphological structure of cattle-yaks.

2 | MATERIALS AND METHODS

2.1 | Animals, diets, and experimental design

This study involved eight male cattle-yaks [280 ± 10 kg of initial body weight (BW) and 3-year-old of age]. The animals were marked with ear tags and randomly assigned to two groups: the high concentrate group (HighC, the ratio of concentrate to roughage was 70:30) and the low concentrate group (LowC, the ratio of concentrate to roughage was 50:50). The animals in the two groups were housed in individual tie-stalls (2 × 2.5 m). All the animals were provided with total mixed rations twice daily at 08:00 and 16:00 h and were provided free access to water during the experiment. A 7-day adaptive phase was followed by a 30-day experimental period.

TABLE 1 Ingredient and the nutrient composition of the basal diets

Items	The ratio of concentrate to forage[1]	
	LowC	HighC
Ingredient (% of DM)		
Corn grain	29.31	41.01
Wheat bran	7.58	10.60
Soybean meal	6.06	8.48
Rapeseed meal	5.05	7.07
Sodium bicarbonate	0.75	1.06
Salt	0.50	0.70
Calcium carbonate	0.50	0.70
Vinasse	17.70	12.40
Oat grass	32.30	17.63
Premix[2]	0.25	0.35
Total	100	100
Nutrient composition (% of DM)		
NEmf (MJ/kg)[3]	7.62	7.81
CP	11.33	13.20
EE	10.20	16.20
NDF	32.10	24.44
ADF	13.6	11.28
Ash	8.63	9.29
Ca	1.32	1.50
P	1.04	1.26

Abbreviations: ADF, acid detergent fibre; CP, crude protein; DM, dry matter; EE, ether extract; NDF, neutral detergent fibre.

[1]LowC = low-concentrate (50%, DM basis) diet (control); HighC = high-concentrate (70%, DM basis) diet.

[2]Contained (per kilogram):5–20 IU of vitamin A, 0.5–2.5 IU of vitamin D_3, 500–2000 mg of vitamin E, 1200–3500 mg of Fe, 100–500 mg of Cu, 800–3000 mg of Mn, 1000–3500 mg of Zn.

[3]Estimated based on the Feeding Standards of Cattle, China Nongye HangYe Biaozhun/815 (People's Republic of China, 2004).

The basal diets were formulated to meet or exceed the nutrient demand according to the Chinese Beef Cattle Raising Standard (NY/T815-2004). The diet comprised chopped oat grass (appropriately 5 cm size) and concentrate, and all the components of the diet were mixed according to the formula every morning during the experiment. The composition and nutrient concentrations of the basal diets are shown in Table 1. All the animals were weighed before and after the experiment. After the experiment, all the cattle-yaks were immediately slaughtered.

2.2 | Measurements, sample collection, and analyses

The dry matter intake (DMI) for the individual cattle-yak was determined by recording the quantity of daily feed offered and refused. Each

cattle-yak was weighed before feeding in the morning on 2 consecutive days in the beginning and at the end of the study, and subsequently the average daily gain (ADG) was determined. The gain-to-feed ratio (G:F) was calculated as the ratio between ADG and DMI.

Samples of the total mixed ration were collected weekly and frozen at −20°C before subsequent analysis. A total of 100 g mixed feed samples were collected and dried in a forced-air oven at 65°C to a constant weight and ground through a 1-mm sieve before analysis. The crude protein (CP) in the diet was determined as described in AOAC (Horwitz & Latimer, 2005) with a Kjeltec digester 20 and a Kjeltec System 1026 distilling unit (Tecator AB). The contents of the neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured by the method of Van Soest et al. (1991) using heat-stable amylase (type XI-A of Bacillus subtilis; Sigma-Aldrich, St. Louis, MO, USA). The ash (method 942.05), ether extract (EE) (method 920.39), calcium (method 978.02), and phosphorous (method 946.06) in the diet were analyzed as per the description mentioned in AOAC (Horwitz & Latimer, 2005).

Ruminal pH was measured with a pH meter. The samples were harvested 2 h after the morning feeding on days 10, 20, and 30 of the total experiment period using the gastric tube type rumen fluid sampler (Anscitech A1141K; Anscitech Animal Husbandry Technology Co., Ltd.) inserted in the rumen. At the end of the experiment, all the cattle-yaks were slaughtered, and 100 ml samples of the ruminal fluid were rapidly collected for determining the volatile fatty acid (VFA), ammonia N (NH_3-N), and lipopolysaccharide (LPS). The ruminal content was squeezed through four layers of cheesecloth, and the supernatant fluid was stored at −20°C until analysis. The samples for the VFAs assays were pre-processed using 25% (w/v) metaphosphoric acid. The samples with metaphosphoric acid were thawed at room temperature, and centrifuged (10,000 × *g* for 15 min at 4°C). Then, the supernatant was used to measure the VFAs. The VFA concentrations were determined by gas chromatography using a Thermon-3000 5% Shincarbon A column (1.6 3.2 mm 60–80 mesh, Shinwakako, Japan) at 190°C. Nitrogen was used as the carrier gas. The NH_3-N concentration was determined by the spectrophotometric method as described by Verdouw et al. (1978).

Approximately 15 ml of the blood samples from each animal were collected from the jugular vein before feeding in the morning on the last day of the experiment using a heparinized syringe. The samples were immediately centrifuged at 3000 × *g* for 15 min at 4°C for separation of the serum. The concentrations of glucose (GLU), total cholesterol (TCLO), urea, total protein (TP), globulin, albumin, and serum total bilirubin in the serum samples were measured using an automatic biochemical analyzer (Autolab PM-4000; AMS & Alliance company, Italy). The activities of the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using commercial kits (Wuhan MSK Biological Technology Co., Ltd.) according to the manufacturer's instructions. The concentration of the cell-free LPS in the rumen fluid and blood plasma was determined by the Limulus amoebocyte lysate assay (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China). The plasma and ruminal samples used to determine the free LPS were initially treated as recommended by Khafipour et al. (2009).

TABLE 2 Effect of the dietary concentrate on the dry matter intake (DMI), average daily gain (ADG), DMI per body weight and G:F in the cattle-yak

Items	LowC	HighC	SEM	*p*-Value
DMI (kg)	5.70[b]	6.94[a]	0.10	<0.001
ADG (kg)	0.44[b]	0.85[a]	0.09	0.033
DMI (% of BW)	1.95	2.23	0.21	0.378
G:F	0.08	0.14	0.02	0.164

Abbreviations: BW, body weight; G:F, gain to feed ratio; HighC, high concentrate; LowC, low concentrate; SEM, standard error of the mean.
a, b Different superscripts in the same row indicate significant differences ($P \leq 0.05$).

The segments of the ruminal tissue (4 × 5 cm) were collected from the ventral sac of each cattle-yak shortly after slaughter and fixed in a 4% formalin solution for subsequent assessment of histomorphology. The ruminal tissue was treated with haematoxylin and eosin as described by Ma, Shah, et al. (2020). Briefly, after rinsing with water, the samples were dehydrated in a graded series of absolute alcohol, made transparent in xylene, embedded in paraffin. Sections of 6 μm thickness were stained with haematoxylin/eosin and observed under a light microscope under 1000×, 100×, and 50× magnification. The length and width of all the rumen papillae from a slide were determined by the computer-operated Image C picture analysis programme (Nikon Eclipse E100, Japan). The surface of papillae was calculated as height × width. To determine the papillae health, the pathological changes in ruminal tissue were analyzed using an eyepiece camera mounted on the light microscope (Nikon Eclipse Ci-L) equipped with the scanning software (CaseViewer 2.4; 3DHISTECH Ltd., Hungary) and panoramic scan (Pannoramic Desk/Midi/250/1000; 3DHISTECH Ltd.).

2.3 | Statistical analysis

Data were based on each pen as the experimental unit, and the normality and homogeneity of data were tested first. Then, all the experimental data were analyzed by the independent sample *t* test of the SAS statistical software (version 9.4; SAS Institute, 2016). Data were shown as means and standard error of the mean (SEM). A significance level was indicated at $p < 0.05$, and a trend was declared at $0.05 \leq p < 0.10$.

3 | RESULTS

3.1 | The body weight gain and productive parameters

The data of DMI, ADG, DMI per BW, and G:F are presented in Table 2. The DMI and ADG of the HighC group were higher ($p < 0.05$) than those of the LowC group, which were increased by 21.75% and 93.18%, respectively. No significant difference ($p > 0.05$) of DMI per BW was observed between the two groups although improvement of DMI per

TABLE 3 Effect of the dietary concentrate on the ruminal fermentation in the cattle-yak

Items	LowC	HighC	SEM	*p*-Value
pH	6.81[a]	5.86[b]	0.19	0.036
TVFA, mmol/L	33.29[b]	66.68[a]	4.60	0.006
Aceate, %	70.78	67.41	1.67	0.227
Propionate, %	9.09[b]	15.70[a]	0.82	0.005
Isobutyrate, %	6.11[a]	3.84[b]	0.32	0.008
Butyrate, %	4.16[b]	7.08[a]	0.54	0.019
Isovalerate, %	8.82[a]	4.98[b]	0.62	0.012
Valerate, %	1.05	0.99	0.12	0.773
NH_3-N, mg/dl	20.57	34.87	6.68	0.205
Acetate:propionate	7.74[a]	4.35[b]	0.43	0.005

Note: a, b, c means in a row without a common superscript letter differ within a subclass as noted *p*-value.
Abbreviations: HighC, high concentrate; LowC, low concentrate; SEM, standard error of the mean; TVFA, total volatile fatty acid.

body weight was observed in the HighC (14.4%) group. The G:F data showed no difference ($p > 0.05$) between the two groups, but the value of G:F in the HighC group was higher by 75% than that in the LowC group.

3.2 | Ruminal fermentation parameters

The analysis of in vivo fermentation is depicted in Table 3. The ruminal pH showed a significant decrease with the increase in the dietary concentrate level ($p < 0.05$), while the content of TVFA, propionate, and butyrate significantly increased ($p < 0.05$). In contrast, the concentrations of isobutyrate and isovalerate of the HighC group were higher than those of the LowC group ($p < 0.05$). However, there was no significant ($p > 0.05$) variation in the acetate and valerate between the two groups. The ratio of acetate and propionate was significantly ($p < 0.05$) higher in the LowC group than that of the HighC group, whereas the NH_3-N concentration did not differ significantly ($p > 0.05$) between the two groups.

3.3 | The concentration of LPS in the ruminal fluid and plasma and blood biochemical indices

The LPS concentrations in the rumen and plasma are shown in Figure 1. The concentrations of LPS in the rumen and plasma of the HighC group were higher ($p < 0.05$) than those of the LowC group, which was increased by 77.5% and 41.3%, respectively.

Effects of the dietary concentrate level on the blood biochemical indices are presented in Table 4. There was no significant change ($p > 0.05$) in the concentration of GLU, total cholesterol, urea, TP, albumin, AST, and ALT. On the contrary, the content of globulin in the LowC group was higher ($p < 0.05$) than that in the HighC group.

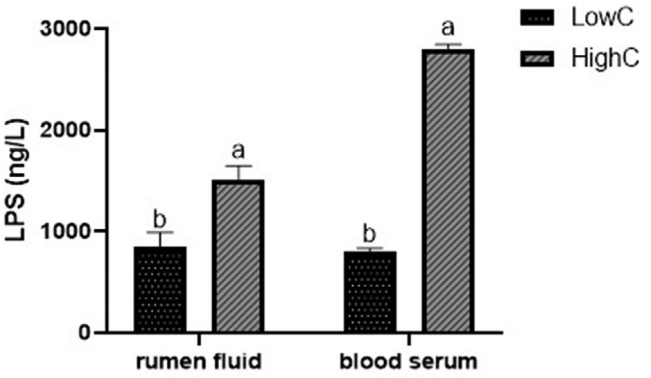

FIGURE 1 Effect of dietary concentrate on lipopolysaccharide (LPS) in the ruminal fluid and blood of the cattle-yak. a and b in the column chart of different fillers means that there was a significant difference between the two treatments in the ruminal fluid or blood

TABLE 4 Effect of dietary concentrate on the blood biochemical indices in the cattle-yak

Items	LowC	HighC	SEM	*p*-Value
GLU, mmol/L	4.48	4.94	0.21	0.193
TCLO, mmol/L	1.95	2.22	0.36	0.635
Urea, mmol/L	4.01	4.51	0.44	0.471
TP, g/L	80.78	72.96	2.85	0.109
Globulin, g/L	40.75[a]	31.62[b]	1.79	0.011
Albumin, g/L	40.03	39.90	0.67	0.900
AST, U/L	70.60	63.78	9.61	0.650
ALT, U/L	19.53	21.14	1.70	0.548
AST/ALT	3.59	2.99	0.27	0.196

Note: AST/ALT means the ratio of means aspartate aminotransferase and serum alanine aminotransferase.
Abbreviations: ALT, serum alanine aminotransferase; AST, aspartate aminotransferase; GLU, glucose; HighC, high concentrate; LowC, low concentrate; SEM, standard error of the mean; TCLO, total cholesterol; TP, total protein.
a, b Different superscripts in the same row indicate significant differences ($P \leq 0.05$).

TABLE 5 Effect of dietary concentrate on the ruminal histology of the cattle-yak

Items	LowC	HighC	SEM	*p*-Value
Papillae length, mm	0.66	1.20	0.12	0.022
Papillae width, mm	0.36	0.34	0.05	0.864
Papillae area, mm^2	0.25	0.43	0.07	0.178

Abbreviations: HighC, high concentrate; LowC, low concentrate; SEM, standard error of the mean.

3.4 | Ruminal histomorphology

The rumen tissue histology is presented in Table 5. The papillae length of the HighC group was significantly higher ($p < 0.05$) than that of the lowC group. There were no significant changes ($p > 0.05$) in the papillae width and area between the two groups. Representative light

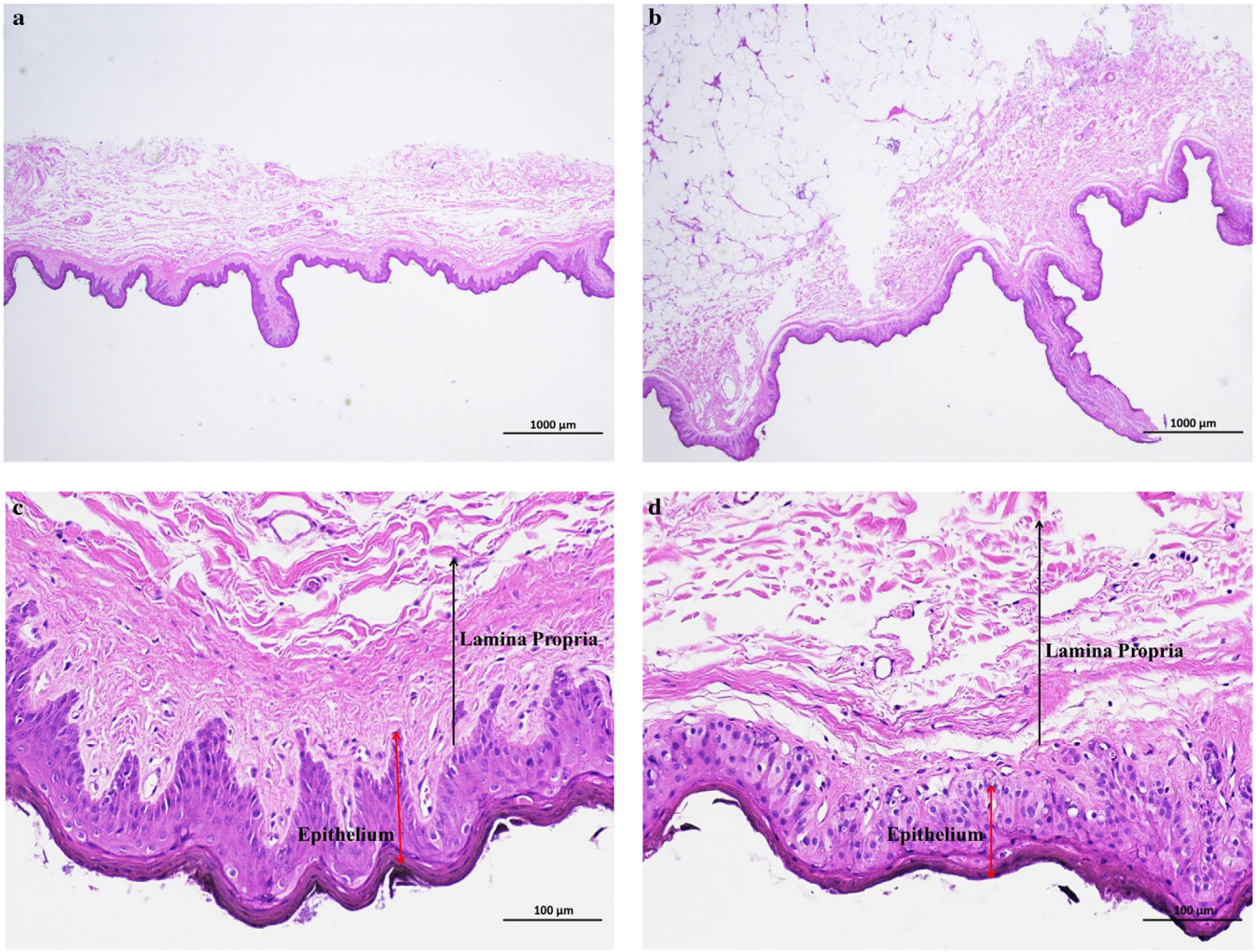

FIGURE 2 Effect of dietary concentrate on the histomorphology of the ruminal papillae in the cattle-yak. Light micrograph of the papillae cross-section of the low concentrate (LowC) group (a, scale bar = 1000 μm; c, scale bar = 100 μm; e, scale bar = 100 μm; g, scale bar = 50 μm) and high concentrate (HighC) group (b, scale bar = 1000 μm; d, scale bar = 100 μm; f, scale bar = 100 μm; h, scale bar = 50 μm). The black arrow indicates the lamina propria, and the red arrow indicates the epithelium in (c) and (d). Arrow points to the inflammatory cell infiltration in (f). Ruminal papillae consisted of stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), and stratum basale (SB) are shown in (g) and (h)

micrographs of the rumen papillae cross-sections from two the groups are shown in Figure 2. The colour of the lamina propria in the ruminal epithelium of the LowC group was stained deeper than that of the HighC group (Figure 2a,b). The morphology and structure of the lamina propria of the LowC group were normal and tightly arranged (Figure 2c). On the contrary, the lamina propria of the HighC group were found to have a loose structure and cellular swelling, and the cells of the lamina propria showed dissociation in the HighC group (Figure 2d). A small amount of inflammatory cell infiltration was seen locally in the epithelial papilla of the rumen of the HighC group (Figure 2f). The mucosa of rumen was found to be linked with highly keratinized stratified squamous epithelium consisted of four layers: basal, spinous, granular, and keratin (Figure 2g,h) between the two groups. However, the cells of stratum spinosum and stratum basale were arranged disorderly in the HighC group.

4 | DISCUSSION

4.1 | The gain in the bodyweight and productive parameters

To date, very few studies have been performed to investigate the effects of forage-to-concentrate ratio on the DMI and growth performance in cattle-yaks, but it has been well-studied in other ruminants. Some studies have reported the DMI to be unaffected by the increase in the percentage of concentrate in the diet of goats (Cantalapiedra-Hijar et al., 2014) and Holstein cows (Aguerre et al., 2011). On the contrary, Chen et al. (2015) found that the DMI of yaks increased (from 5.33 to 5.63 kg/day) with the increased percentage of concentrate in the diet from 30% to 60%. Similarly, Desnoyers et al. (2008) observed that the DMI was increased from 30% to 60% after an increase in

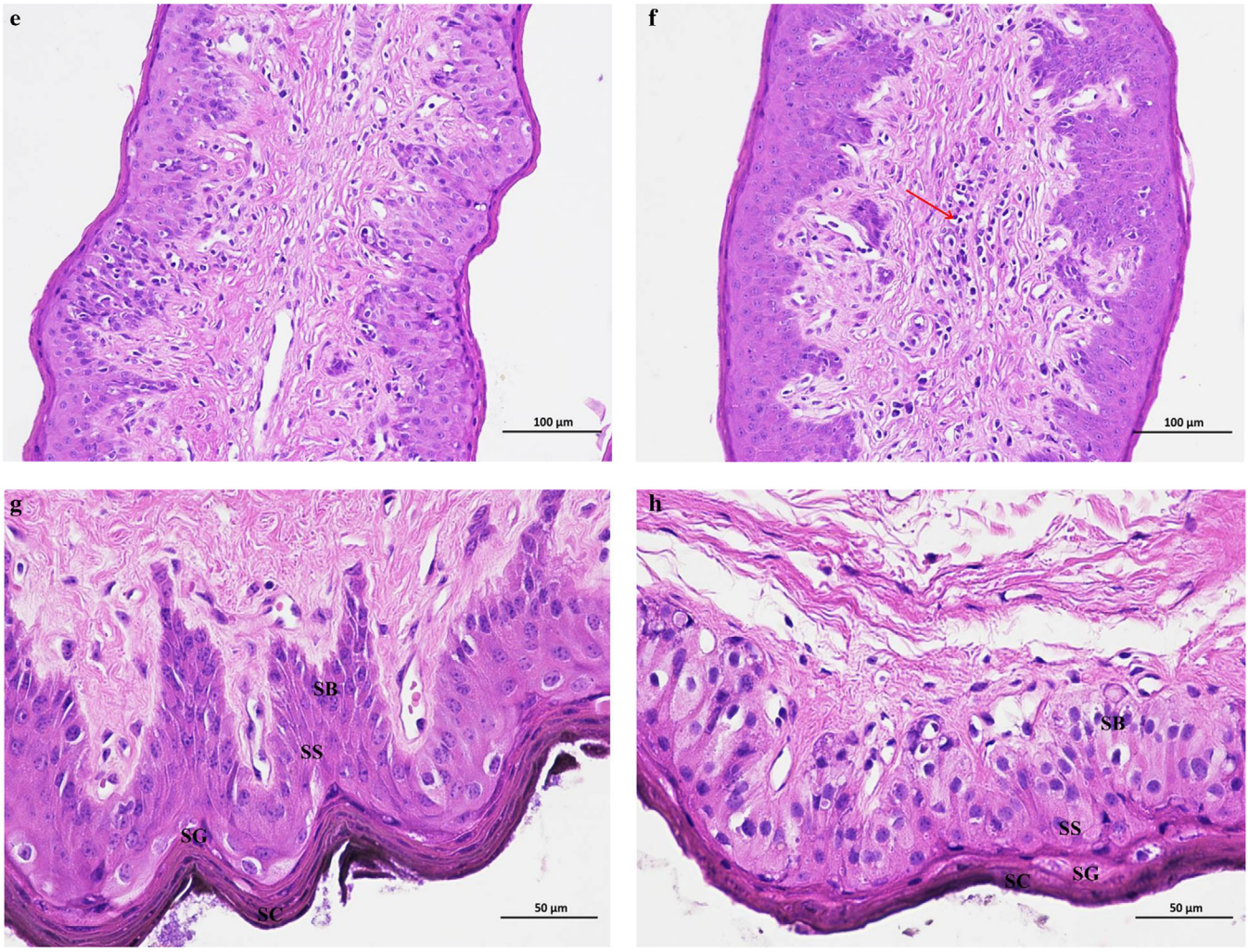

FIGURE 2 Continued

the percentage of concentrate in the diet of dairy goats. In line with these results, our present study indicated that the diet with a high concentrate-to-forage ratio can increase the DMI of cattle-yaks (from 6.94 to 5.70 kg/day). The percentage of concentrate was possibly under the level of concentrate affecting the ruminal fermentation and might be related to the low rumen fill effect of concentrate (Jarrige et al., 1995). Many studies demonstrated that high concentration in diet can increase the available energy and improve the production performance of the ruminants (Jabbar & Anjum, 2008; Mialon et al., 2008; J. F. Wang et al., 2005). Mialon et al. (2008) revealed that the ADG of the blond d'Aquitaine bulls during the finishing period was 1860, 1490, and 1710 g/day among straw-concentrate (8/92), hay-concentrate (44/56), and maize silage-concentrate (57/43) diets, respectively. In agreement with the above results, this study was in agreement with the above result indicating that diets with a low forage-to-concentrate ratio increased the ADG of the cattle-yaks. Furthermore, the changes in the ADG in the HighC group were likely attributable to an increase in the DMI because increasing the feed intake can improve the production efficiency of the ruminants (Brown et al., 2006).

4.2 | Ruminal fermentation parameters

The pH value of the rumen is mainly affected by the dietary composition. In this experiment, the HighC group containing a high proportion of concentrate was rich in rapidly fermentable carbohydrates, resulting in a low ruminal pH. There is a decrease in the pH due to an increase in the total VFA concentration in the rumen (Stone, 2004). The low ruminal pH is an important indicator for defining subacute ruminal acidosis (SARA), but there is no general agreement on the pH threshold that defines SARA. Gozho et al. (2006) suggested that the decrease in the ruminal pH below 5.8 for longer than 3 h/day indicated SARA. Although no continuous ruminal pH was obtained in this experiment due to severe stress for the cattle-yak when the ruminal fluid was collected through the oral cavity of the cattle-yak, it imposes severe stress. As a result, there was no continuous ruminal pH, and the ruminal pH of the cattle-yak was compared to the recommended pH values of the SARA cattle (Gozho et al., 2006), indicating the risk of SARA in cattle-yaks. pH is an important but not the only factor driving the onset of SARA (Calsamiglia et al., 2008). Animal health can be further assessed by other physiological indicators.

Although the effects of the different concentrate-to-forage ratio have been widely studied on the concentration of NH_3-N, TVFA, and individual VFA in the rumen; however, there were inconsistencies. Although enhanced propionate concentration has been reported in the rumen of the cows fed high-grain compared to the cows fed high-forage diets, the concentration of TVFA and the molar proportions of acetate has not been altered (Bauman et al., 1971; Sutton et al., 2003). Moorby et al. (2006) reported the linear increase in the TVFA and butyrate concentrations and a decrease in the acetate with an increasing proportion of concentrate in the dietary DM, without affecting the concentration of propionate. Miettinen and Huhtanen (1996) also demonstrated that a higher proportion of dietary concentrate can increase the higher propionate in the rumen and reduced the ratio of acetate acid and propionate. Similar results have been obtained in the present study. Macleod et al. (1994) reported that hay decreased the ruminal molar proportions of isobutyrate and isovalerate. Y. H. Chen et al. (2011) also demonstrated that the proportion of isovalerate was also increased by increasing the concentration in the diet. The present study revealed that the molar proportion of butyrate was increased in the ruminal fluid of the cattle-yaks fed a high concentrate diet, and decreased the molar proportions of isobutyrate and isovalerate. This could be explained by possibilities. The rumen fermentation changes from the fermentation of structural carbohydrates to non-structural forms, and the effect of high concentrations in the diet on the rumen fermentation may be influenced by the rumen microbes. The rumen microbial composition is altered by the ruminal pH and the low pH in the rumen showing a higher proportion of members of several genera such as *Butyrivibrio*, *Succiniclasticum*, *Mogibacterium*, *Ruminococcus*, and *Butyrivibrio* in the rumen epithelium, with the increase in the production of butyrate in the rumen (Liu et al., 2015). Although there was no statistical difference in NH_3-N concentration between the treated groups, the NH_3-N concentration was found to be higher in the HighC group. This might be due to the increase in the nitrogen intake by the animals with an increase in the proportion of concentrate in the diet.

4.3 | LPS in the ruminal fluid and plasma

Feeding high concentrate can increase the availability of energy to the animals. The ruminal LPS concentration increases with the increase in the proportions of the dietary concentrate (Gozho et al., 2006; Zebeli & Ametaj, 2009). The free rumen LPS concentration has been found to increase following grain engorgement, but the reported range of the free LPS in the ruminal fluid varied substantially in most studies. More specifically, as the proportion of barley grain increased by 15% from 0% to 45% (on a DM basis), the concentration of LPS in the rumen increased quadratically from 781 to 8890 ng/ml (Zebeli & Ametaj, 2009). Khafipour et al. (2009) reported that the concentration of LPS varied from 2818 ng/ml in the control group (C:F = 50:50) to 1,0715 ng/ml in the SARA cows (C:F = 60:40). Gozho et al. (2007) demonstrated an LPS range of 2454–12,882 ng/ml in the Holstein dairy cows during periods of control and grain-induced SARA. The results of the present study were in concert with the experimental findings of Gozho et al. (2005, 2006), which have reported a lower range of LPS in steers. Besides, there was an increase in the concentration of LPS from 375 to 887 ng/ml with an abrupt induction of SARA and from 631 to 871 ng/ml with gradual adaptation to 61% wheat-barley pellet in the diet, respectively. The discrepancy among the above studies is probably due to the difference in the body condition, nutrient composition, and the method of LPS determination in different animals (Zhao et al., 2018). LPS can translocate through the ruminal epithelium into the blood, and the damage to the ruminal wall is associated with the low ruminal pH and leads to the further increase in the ruminal LPS translocation into the bloodstream (Khafipour et al., 2009; Plaizier et al., 2012). Khafipour et al. (2009) reported that a grain-based SARA challenge to increase the LPS from <0.05 (Control) to 0.52 EU/ml (SARA). In addition, Jin et al. (2016) also reported the plasma LPS concentration of lacteal artery and vein in cows with a long-term high-concentrate diet feeding to be 0.86 and 0.27 EU/ml, respectively. The present study observed that low ruminal pH leads to a rise in blood LPS concentrations. Although some studies did not determine the LPS in the peripheral circulation during experimentally induced ruminal acidosis (Li et al., 2012; Rodríguez-Lecompte et al., 2014;), the ruminal pH modulates the release and accumulation of LPS affecting the metabolic processes and changes in the cell membrane of the ruminal bacteria (Russell & Rychlik, 2001). There is a strong negative relationship between the ruminal pH and concentration of LPS in the ruminal fluid (Ametaj et al., 2010).

4.4 | Blood parameters

Rumen fermentation products such as propionate and endogenous lactic acid are the main precursors of GLU in blood. There were no differences between the two groups. The cattle-yak fed high concentrate diet showed higher blood concentrations of GLU and TCLO, inferring the level of energy metabolism activity. In the present study, the concentration of GLU in both groups was higher than that of the previous study (Pu et al., 2017) because the low concentrate-to-forage ratio (30:70) was used in the previous experiment. The serum TP concentrations reflect the conjugate changes in the albumin and globulins. The serum albumins did not change markedly as the globulin levels. The LowC group had a higher concentration of globulins than that of the HighC group. Therefore, it can be inferred that these proteins play crucial roles in the immunological defence of the organism (Shetaewi & Ross, 1991).

4.5 | Rumen histomorphology

The cellular structure of the rumen epithelium consists of several distinct cellular layers which are not easily permeable to the endotoxin and bacteria unless the ruminal epithelium structure is disrupted to a great extent (Liu et al., 2013). The papillae length the HighC group was increased by 81.94% than that of the LowC group. Lane and Jesse (1997) demonstrated that infusing 50% of net energy requirement

in the form of short-chain fatty acids at physiological concentrations increased the papillae length. Wang et al. (2009) also reported that the ruminal papillae height and surface were impaired in the goats fed high starch diets although there were no differences in the width and surface area of the ruminal epithelium between the two groups. This is probably due to the stimulatory effect of a diet rich in carbohydrates which increased the production of the short-chain fatty acids in the rumen (Gäbel et al., 1987). Kauffold and Voigt&Herrendoerfer et al. (1977) found the propionate and butyrate to be the main short-chain fatty acid promoting the ruminal epithelium proliferation. According to the present study, the length of the rumen papillae was increased by the increase in the proportion of propionate and butyrate in the rumen. The higher production of short-chain fatty acids requires a stronger absorption capacity in the rumen. The proliferation of the ruminal epithelium led to a strong increase in the size and surface area of the ruminal papillae. On the other hand, the excessive short-chain fatty acids resulted in the low ruminal pH, damaging the morphology of the ruminal epithelium. In the present study, feeding a high concentrate diet was found to induce the loose structure of the lamina propria, cellular swelling, and dissociation, indicating that there was an inflammatory cell infiltration in the lamina propria of the epithelial papilla with a small area. Steele et al. (2011) reported that feeding high-energy diets would result in a deterioration of the cellular junctions and large spaces between the cells of the ruminal epithelium in the non-lactating Holstein dairy cows. On the contrary, Lodemann and Martens (2010) demonstrated high concentrate diets to exert a positive effect on the barrier function of the ruminal epithelium. The difference could be explained by the exposure time of the ruminal epithelium under low ruminal pH and an abnormal metabolic environment.

5 | CONCLUSION

A higher concentrate-to-forage ratio (70:30) improved the DMI and growth performance of the cattle-yak. Therefore, diets with a high concentrate ratio could change the ruminal fermentation, reduce the ruminal pH, producing more LPS both in the rumen and plasma, and change the morphology of the ruminal epithelium. The proportion of propionate and butyrate in the rumen of the HighC group was increased, and the papillae length of the HighC group was also found to increase although there was inflammation in the ruminal epithelium. Based on the results of the present study, it can be concluded that the high concentrate-to-forage ratio (not higher than 70:30) might increase the production of cattle-yak, but it is fraught with a risk of inflammation in the animals. Further studies should be carried out to investigate the relationship between the short fatty acids such as propionate and butyrate and the proliferation and inflammation of the ruminal epithelium.

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AUTHOR CONTRIBUTIONS

Yahui Jiang: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Software, Supervision, Writing original draft, Writing-review & editing; Peng Dai: Data curation, Formal analysis, Investigation, Methodology, Software, Writing-original draft; Qindan Dai: Data curation, Investigation; Jian Ma: Formal analysis, Methodology, Software, Writing-review & editing; Zhisheng Wang: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing-review & editing; Rui Hu: Methodology, Project administration, Supervision; Zou Wei: Project administration, Validation; Peng Hui: Project administration, Supervision; Lizhi Wang: Project administration, Supervision; Bai Xue: Project administration, Supervision.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The experiments involving animals in this study were performed according to the guidelines and regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China). All experimental protocols were approved by the Institutional Animal Care and Use Committee of the College of Animal Science and Technology, Sichuan Agricultural University (No. DKYB20081003).

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