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

Yahui Jiang1 | Peng Dai2 | Qindan Dai2 | Jian Ma2 | Zhisheng Wang2 | Rui Hu2 | Huawei Zou2 | Quanhui Peng2 | Lizhi Wang2 | Bai Xue2

1 College of Animal Science and Technology, Low Carbon Breeding Cattle and Safety Production University Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu, China
2 Animal Nutrition Institute, Low Carbon Breeding Cattle and Safety Production University Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu, China

Correspondence
Zhisheng Wang, Low Carbon Breeding Cattle and Safety Production University Key Laboratory of Sichuan Province, Animal Nutrition Institute, Sichuan Agricultural University, Chengdu 611130, China. Email: wangzs67@163.com

Yahui Jiang and Peng Dai contributed equally to this study.

Funding information
National Natural Science Foundation of China (NSFC), Grant/Award Number: 31802086; China Agriculture (Beef Cattle/Yak) Research System of MOF and MARA (CARS-37)

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.

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 Kjeltc digester 20 and a Kjeltc 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), 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 (NH₃-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 NH₃-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 amoeboocyte 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).

### 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 ≤ p < 0.10.

### 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

### 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 ≤ p < 0.10.

#### 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 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)       | 5.70b    | 6.94a    | 0.10     | <0.001  |
| ADG (kg)       | 0.44b    | 0.85a    | 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 ≤ 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 1000x, 100x, and 50x 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.).
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   |
| Acetate, %     | 70.78    | 67.41    | 1.67 | 0.227   |
| Propionate, %  | 9.09\(^a\) | 15.70\(^b\) | 0.82 | 0.005   |
| Isobutyrate, % | 6.11\(^a\) | 3.84\(^b\) | 0.32 | 0.008   |
| Butyrate, %    | 4.16\(^a\) | 7.08\(^b\) | 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.

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.

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.

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).

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
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, Succinivibllicum, 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., 2005, 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 lactate 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). Kaufold 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 (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.

ACKNOWLEDGEMENTS

This work was gratefully supported by the National Natural Science Foundation of China (NSFC) (No. 31802086) and China Agriculture (Beef Cattle/Yak) Research System of MOF and MARA (CARS-37). We would like to thank the experimental site of Animal Nutrition Institute, Sichuan Agricultural University (Ya’an, Sichuan, China) for providing the facilities.

AUTHOR CONTRIBUTIONS

Yahui Jiang: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, 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).

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.678

ORCID

Yahui Jiang https://orcid.org/0000-0001-8233-8299
Jian Ma https://orcid.org/0000-0002-6979-5070

REFERENCES

Aguerre, M. J., Wattiaux, M. A., Powell, J. M., Broderick, G. A., & Arndt, C. (2011). Effect of forage-to-concentrate ratio in dairy cow diets on emission of methane, carbon dioxide, and ammonia, lactation performance, and manure excretion. *Journal of Dairy Science*, 94, 3081–3093.

Ametaj, B. N., Zebeli, Q., & Iqbal, S. (2010). Nutrition, microbiota, and endotoxin-related diseases in dairy cows. *Revista Brasileira de Zootecnia*, 39, 433–444.

Bauman, D. E., Davis, C. L., & Bucholtz, H. F. (1971). Propionate production in the rumen of cows fed either a control or high-grain, low-fiber diet. *Journal of Dairy Science*, 54, 1282–1287. https://doi.org/10.3168/jds.S0022-0302(71)86021-6

Brown, M. S., Ponce, C. H., & Pulikanti, R. (2006). Adaptation of beef cattle to high-concentrate diets: Performance and ruminal metabolism. *Journal of Animal Science*, 84, E25–E33. https://doi.org/10.2527/2006.8413_suppE25x

Calsamiglia Cardozo, P. W., Ferret, A., & Bach, A. (2008). Changes in rumen microbial fermentation are due to a combined effect of type of diet and
pH. Journal of Animal Science, 86, 702–711. https://doi.org/10.2527/jas.2007-2146

Cantalapiedra-Hijar, C., Yáñez-Ruiz, D. R., Martín-García, A. I., & Molina-Alcaide, E. (2014). Effects of forage:concentrate ratio and forage type on apparent digestibility, ruminal fermentation, and microbial growth in goats. Journal of Animal Science, 87, 622–631.

Chen, G. J., Song, S. D., Wang, B. X., Zhang, Z. F., & Wang, Y. (2015). Effects of forage:concentrate ratio on growth performance, ruminal fermentation and blood metabolites in housing-feeding yaks. Asian-Australasian Journal of Animal Sciences, 28(12), 1736–1741. https://doi.org/10.5713/ajas.15.0419

Chen, Y. H., Penner, G. B., Li, M.J, Oba, M., & Guan, L. L. (2011). Changes in bacterial diversity associated with epithelial tissue in the beef cow rumen during the transition to a high-grain diet. Applied and Environmental Microbiology, 77(6), 5770. https://doi.org/10.1128/AEM.00375-11

China NY/t815-2004 (China NongYe HangYe Biaozhun/815). (2004).

Jabbar, M. A., & Anjum, M. I. (2008). Effect of diets with different forage to concentrate ratio of the diet on feeding performance, nutrient digestibility, and ruminal and fecal bacterial community between yaks and cattle-yaks raised by stall-feeding. AMB Express, 11, 98.

Desnoyers, M., Duvaux-Pontier, C., Rigalma, K., Roussel, S., Martin, O., & Giger-Reverdin, S. (2008). Effect of concentrate percentage on ruminal pH and time-budget in dairy goats. Journal of Animal Science, 2, 1802–1808.

Gäbel, G., Martens, H., Sündermann, M., & Galfi, P. (1987). The effect of diet, intraruminal pH and osmolarity on sodium, chloride and magnesium absorption from the temporally isolated and washed reticulo-rumen of sheep. Quarterly Journal of Experimental Physiology, 72, 501–511. https://doi.org/10.1113/exphphysiol.1987.sp003092

Gozho, G. N., Plaizier, J. C., Krause, D. O., Kennedy, A. D., & Wittenberg, K. M. (2005). Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. Journal of Dairy Science, 88, 1399–1403.

Gozho, G. N., Krause, D. O., & Plaizier, J. C. (2006). Rumen lipopolysaccharide and fermentation during grain adaptation and subacute ruminal acidosis in steers. Journal of Dairy Science, 89, 4404–4413. https://doi.org/10.3168/jds.S0022-0302(06)72487-0

Liu, J. H., Xu, T. T., Liu, J. Y., Zhu, W. Y., & Mao, S. Y. (2013). A high-grain diet causes massive disruption of ruminal epithelial tightjunctions in goats. American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 305(3), 232–241. https://doi.org/10.1152/ajpregu.00068.2013

Liu, J. H., Bian, G. R., Zhu, W. Y., & Mao, S. Y. (2015). High-grain feeding causes strong shifts in ruminal epithelial bacterial community and expression of Toll-like receptor genes in goats. Frontiers in Microbiology, 6, 1–10. https://doi.org/10.3389/fmicb.2015.00167

Lodemann, U., & Martens, H. (2010). Effects of diet and osmotic pressure on Na transport and tissue conductance of sheep isolated rumen epithelium. Experimental Physiology, 91, 539–550.

Ma, J., Zhu, Y., Wang, Z. S., Yu, X., Hu, R., Wang, X., Cao, G., Zou, H. W., Shah, A.M., Peng, Q. H., Xue, B., Wang, L. Z., Zhao, S., & Kong, X. (2020). Comparing the bacterial community in the gastrointestinal tracts between growth-retarded and normal yaks on the Qinghai-Tibetan Plateau. Frontiers in Microbiology, 11, 600516.

Ma, J., Shah, A. M., Shao, Y., Wang, Z. S., Zou, H. W., & Kang, K. (2020). Dietary supplementation of yeast cell wall improves the gastrointestinal development of weaned calves. Animal Nutrition, 6(4), 507–512.

Macleod, G. K., Colucci, P. E., Moore, A. D., Grieve, D. G., & Lewis, N. (1994). The effects of feeding frequency of concentrates and feeding sequence of hay on eating behavior, ruminal environment and milk production in dairy cows. Canadian Journal of Animal Science, 74(1), 103–113. https://doi.org/10.1080/00071669408417681

Mialon, M. M., Martin, C., Garcia, F., Menassol J. B., & Micol, D. (2008). Effects of the forage-to-concentrate ratio of the diet on feeding behaviour in young blond d’Aquitaine bulls. Animal, 2(11), 1682–1691.

Miettinen, H., & Huhtanen, P. (1996). Effects of the ratio of ruminal propionic acid to butyrate on milk yield and blood metabolites in dairy cows. Journal of Dairy Science, 79(5), 851–861.

Moore, J. M., Dewhurst, R. J., Evans, R. T., & Danelon, J. L. (2006). Effects of dairy cow diet forage proportion on duodenal nutrient supply and urinary purine derivative excretion. Journal of Dairy Science, 89, 3552–3562. https://doi.org/10.3168/jds.S0022-0302(06)72395-5

Plaizier, J. C., Krause, D. O., Gozho, G. N., & Micol, D. (2008). Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Veterinary Journal, 176, 21–31. https://doi.org/10.1016/j.tvjl.2007.12.016

Khafipour, E., Krause, D. O., & Plaizier, J. C. (2009). A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. Journal of Dairy Science, 92(3), 1060. https://doi.org/10.3168/jds.2008-1389

Lane, M. A., & Jesse, B. W. (1997). Effect of volatile fatty acid infusion in development of the rumen epithelium in neonatal sheep. Journal of Dairy Science, 80, 740–746.

Lechertier, C., & Peyraud, J. L. (2010). The effects of forage proportion and rapidly degradable dry matter from concentrate on ruminal digestion in dairy cows fed corn silage-based diets with fixed neutral detergent fiber and starch contents. Journal of Dairy Science, 93, 666–681. https://doi.org/10.3168/jds.2009-2349
Plaizier, J. C., Khafipour, E., Li, S., Gozh, G. N., & Krause, D. O. (2012). Subacute ruminal acidosis (SARA), endotoxins and health consequences. *Animal Feed Science and Technology*, 172(1–2), 9–21. https://doi.org/10.1016/j.anifeedsci.2011.12.004

Pu, Q. J., Wang, Z. S., Peng, Q. H., Zhang, C., Jing, X., Hu, R., & Zou, H. W. (2017). Effects of heat stress on performance, nutrient apparent digestibility and blood biochemical indices of different breeds of young beef cattle. *Chinese Journal of Animal Nutrition*, 29(9), 3120–3131.

Rodríguez-Lecompte, J. C., Kroeker, A. D., Ceballos-Márquez, A., Li, S., Plaizier, J. C., & Gomez, D. E. (2014). Evaluation of the systemic innate immune response and metabolic alterations of nonlactating cows with diet-induced subacute ruminal acidosis. *Journal of Dairy Science*, 97(12), 7777–7787. https://doi.org/10.3168/jds.2014-8319

Russell, J. B., & Rychlik, J. L. (2001). Factors that alter rumen microbial ecology. *Science*, 292, 1119–1122.

SAS Institute. (2016). *Statistical Analysis software User's guide*, version 9.4. SAS Institute Inc.

Shetaewi, M. M., & Ross, T. T. (1991). Effects of concentrate supplementation and lasalocid on serum chemistry and hormone profiles in Rambouillet ewes. *Small Ruminant Research*, 4, 365–377.

Steele, M. A., Croom, J., Kahler, M., Alzahal, O., Hook, S. E., Plaizier, K., & Mcbride, B. W. (2011). Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, 300, R1515–R1523.

Stone, W. C. (2004). Nutritional approaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. *Journal of Dairy Science*, 87, E13–E26.

Sutton, J. D., Dhanoa, M. S., Morant, S. V., France, J., Napper, D. J., & Schuller, E. (2003). Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *Journal of Dairy Science*, 86, 3620–3633. https://doi.org/10.3168/jds.S0022-0302(03)73968-X

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Verdouw, H., Van Echteld, C. J. A., & Dekkers, E. M. J. (1978). Ammonia determination based on indophenol formation with sodium salicylate. *Water Research*, 12, 399–402. https://doi.org/10.1016/0043-1354(78)90107-0

Wang, J. F., Wang, J. Q., Li, S. C., Wang, S. P., Yao, M. R., & Liu, S. J. (2005). Effects of forage to concentrate ratio on pattern of rumen fermentation and performance of lactating dairy cows. *Chinese Journal of Animal and Veterinary Sciences*, 36(6), 569–573.

Wang, Y. H., Xu, M., Wang, F. N., Yu, Z. P., Yao, J. H., Zan, L. S., & Yang, F. X. (2009). Effect of dietary starch on rumen and small intestine morphology and digesta pH in goats. *Livestock Science*, 122, 48–52.

Zebeli, Q., & Ametaj, B. N. (2009). Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. *Journal of Dairy Science*, 92, 3800–3809. https://doi.org/10.3168/jds.2009-2178

Zhao, C. X., Liu, G. W., Li, X. B., Guan, Y., Wang, Y., Yuan, X., Sun, G., Wang, Z., & Li, X. (2018). Inflammatory mechanism of rumenitis in dairy cows with subacute ruminal acidosis. *BMC Veterinary Research*, 14, 135. https://doi.org/10.1186/s12917-018-1463-7