Effects of Dietary Non-Fibrous Carbohydrate to Neutral Detergent Fiber Ratio on Rumen Fermentation Parameters, Plasma Biochemical Indices, and Nutrient Digestibility in Qianbei Ma Goats: Postprint

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Abstract

This experiment aimed to investigate the effects of different dietary non-fibrous carbohydrate (NFC) to neutral detergent fiber (NDF) ratios on rumen fermentation parameters, plasma biochemical indices, and nutrient digestibility in Qianbei Ma goats. A 3$×$3 Latin square experimental design was adopted, with 6 healthy adult Qianbei Ma goats selected as experimental animals, divided into 3 groups with 2 replicates per group and 1 goat per replicate. The dietary NFC/NDF ratios for Group , Group , and Group were 2.14:1.00, 1.05:1.00, and 0.40:1.00, respectively. The experiment consisted of 3 periods, each lasting 15 days, including a 10-day preliminary period and a 5-day formal collection period. Measured indices included: dietary nutrient digestibility, rumen fermentation parameters (pH, buffering capacity, ammonia nitrogen and volatile fatty acid concentrations, cellulase activity), and plasma biochemical indices (lipopolysaccharide, albumin, urea nitrogen, glucose concentrations and catalase, aspartate aminotransferase, superoxide dismutase, glutathione peroxidase activities). The results showed: 1) Dry matter intake did not differ significantly among the three experimental groups ($P>0.05$), while all nutrient digestibility coefficients in Group were significantly lower than those in Group and Group ($P<0.05$). 2) Rumen fluid pH in Group was significantly lower than that in Group and Group ($P<0.05$); rumen fluid buffering capacity in Group was significantly higher than that in Group ($P<0.05$), while cellobiase activity and acetate concentration were significantly lower than those in Group ($P<0.05$), and total volatile fatty acid and butyrate concentrations were significantly lower than those in the other two groups ($P<0.05$); the acetate to propionate ratio in rumen fluid increased sequentially, while propionate concentration decreased

sequentially, in Groups , , and , with significant differences among groups (P<0.05); no significant differences were observed among the three groups in rumen fluid carboxymethyl cellulase, xylanase, or microcrystalline cellulase activities (P>0.05). 3) Plasma lipopolysaccharide content decreased sequentially and significantly in Groups , , and (P<0.05), while no significant differences were observed in other plasma biochemical indices (P>0.05). In summary, excessively high NFC/NDF ratio had adverse effects on rumen fermentation parameters and plasma biochemical indices in Qianbei Ma goats; under the conditions of this experiment, a dietary NFC/NDF ratio of 1.05:1.00 is recommended.

Full Text

Abstract

This study investigated the effects of dietary non-fiber carbohydrate to neutral detergent fiber ratio (NFC/NDF) on rumen fermentation parameters, plasma biochemical indexes, and nutrient digestibility in Qianbeima goats. Six healthy adult Qianbeima goats were selected and allocated to three groups using a 3$×$3 Latin square design, with two replicates per group and one goat per replicate. Trial groups , , and were fed diets with NFC/NDF ratios of 2.14:1.00, 1.05:1.00, and 0.40:1.00, respectively. The experiment consisted of three periods, each lasting 15 days (10-day pre-trial period and 5-day trial period). Measured parameters included: dietary nutrient digestibility, rumen fermentation parameters (pH, buffer capacity, ammonia nitrogen and volatile fatty acid concentrations, cellulase activity), and plasma biochemical indexes [lipopolysaccharide, albumin, urea nitrogen, glucose content, and activities of catalase, glutamic-oxalacetic transaminase, superoxide dismutase, and glutathione peroxidase]. The results showed: 1) No significant differences in dry matter intake were observed among the three groups (P>0.05), while trial group exhibited significantly lower nutrient digestibility compared to groups and (P<0.05). 2) Rumen fluid pH in trial group was significantly lower than in groups and (P<0.05). Trial group showed significantly higher rumen buffer capacity than group (P<0.05), but significantly lower cellobiase activity and acetic acid concentration than group (P<0.05), and significantly lower total volatile fatty acids and butyrate concentrations than the other two groups (P<0.05). The acetate to propionate ratio increased progressively while propionate concentration decreased across groups , , and , with significant differences among groups (P<0.05). No significant differences were observed in carboxymethyl cellulase, xylanase, or microcrystalline cellulase activities among the three groups (P>0.05). 3) Plasma lipopolysaccharide content decreased progressively and significantly from group to group (P<0.05), while other plasma biochemical indexes showed no significant differences (P>0.05). In conclusion, excessively high NFC/NDF ratios adversely affected rumen fermentation parameters and plasma biochemical indexes in Qianbeima goats. Under the conditions of this experiment, an NFC/NDF ratio of 1.05:1.00 was optimal.

Key words: non-fiber carbohydrate to neutral detergent fiber ratio; rumen fermentation parameter; plasma biochemical index; nutrient digestibility; Qianbeima goat

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Carbohydrates are the primary chemical components of goat diets and are degraded by rumen microorganisms into volatile fatty acids (VFAs) such as acetate, propionate, and butyrate, providing energy for both the animal and microbial proliferation. Carbohydrates are classified as either fibrous carbohydrates (FC) or non-fiber carbohydrates (NFC), with NFC comprising carbohydrate components other than neutral detergent fiber (NDF). Previous studies commonly used the concentrate-to-forage ratio to reflect dietary carbohydrate structure and nutritional level. Recent research suggests that NFC/NDF more accurately represents the relationship between dietary carbohydrates and has become a recognized indicator for measuring dietary fermentation extent. Literature reports indicate that different NFC/NDF ratios can affect rumen fermentation patterns [1], nutrient digestibility [2], and microbial flora [3]. An appropriate NFC/NDF ratio is a prerequisite for ensuring animal health and an effective measure for improving growth performance [4]. Studies have also shown that NFC/NDF is associated with subacute ruminal acidosis [5] and can regulate the populations of total rumen bacteria and lactobacilli [6]. Zhang Litao et al. [7] reported that the appropriate dietary NFC/NDF ratio for meat sheep is 0.82:1.00, or an appropriate dietary NDF level of 42.21%.

Qianbeima goat is a unique breed in Guizhou, mainly produced in Xishui, Renhuai, and other areas of Guizhou Province. It is one of the three excellent local goat breeds in Guizhou, characterized by roughage tolerance, strong disease resistance, wide adaptability, good meat quality, and high economic value, receiving significant attention from local government departments and farms. For various reasons, Qianbeima goat was not recognized as a new goat genetic resource by the Ministry of Agriculture until December 2009, making it a newly discovered breed with unique academic research value. Currently, re-

search reports on nutritional regulation of Qianbeima goats are relatively scarce, primarily focusing on rumen-protected choline, distiller' s grains, and dietary cation-anion difference conducted by our research group, with no reports on the effects of dietary NFC/NDF on rumen fermentation. Rumen fermentation is an important area of goat diet research and is closely related to healthy goat farming. Therefore, this study focused on rumen fermentation, supplemented with plasma biochemical indexes and nutrient digestibility, to investigate the effects of dietary NFC/NDF on Qianbeima goats, providing technical accumulation and reference for improving the farming efficiency of Qianbeima goats.

1.1 Experimental Animals and Design

A 3$\times$3*Latinsquaredesignwasemployed*, *usingsixadultQianbeimagoatsofconsistentage*(4*years*)*andbodyweig* kg] as experimental animals. The goats were divided into three groups with two replicates per group and one goat per replicate. Trial groups , , and were formulated with dietary NFC/NDF ratios of 2.00:1.00, 1.00:1.00, and 0.50:1.00, respectively. To prevent ruminal acidosis and potential trial failure in group with the highest NFC/NDF ratio, a small amount of sodium bicarbonate was added to their diet. Experimental diets were formulated according to reference [8], with composition and nutrient levels shown in Table 1 . The measured NFC/NDF values showed slight variations from the designed levels.

Table 1 Composition and nutrient levels of experimental diets (DM basis) %

Items	Trial group	Trial group	Trial group
Ingredients			
Chinese wildrye	10.00	30.00	50.00
Corn	48.00	34.00	20.00
Wheat bran	15.00	12.00	10.00
Soybean meal	10.00	8.00	5.00
Rapeseed meal	5.00	4.00	3.00
$CaCO_3$	0.50	0.50	0.50
NaCl	0.50	0.50	0.50
Premix[1]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Nutrient levels[2]			
ME/(MJ/kg)	11.12	10.03	8.94
CP	15.78	14.69	13.59
EE	3.21	3.02	2.83
Ash	6.25	6.54	6.83
NDF	24.37	36.98	52.79
ADF	12.50	19.00	25.50
NFC	52.39	38.77	21.76
NFC/NDF	2.14:1.00	1.05:1.00	0.40:1.00

[1] One kg of premix contained: VA 300–500 IU, VD_3 150–200 IU, VE 850 IU, Fe 1,500–5,000 mg, Cu 500–620 mg, Mn 1,500–4,000 mg, Zn 2,000–3,500 mg, I 50–200 mg, Se 10–20 mg, Co 20–40 mg, Lys 10 g.

[2] Nutrient levels were measured values except that NFC and ME were calculated values. NFC (%) = 100 –NDF –CP –EE –ash.

Experimental goats were individually housed in metabolic cages. Prior to the trial, health status was observed for 10 days, followed by a 45-day experimental period. The trial consisted of three periods, each comprising a 10-day pre-trial period for dietary adaptation and gradual adjustment to the target NFC/NDF, and a 5-day trial period (sampling period). All goats underwent unified management including deworming and disinfection. They were fed three times daily at 09:00, 13:00, and 18:00, with free access to water, and maintained under adequate lighting, dry and ventilated conditions.

1.2.1 Dietary Nutrient Levels and Digestibility

During the trial period, residual feed from the previous day was weighed accurately before morning feeding each day to calculate dry matter intake (DMI), and diet samples were collected and mixed to prepare analytical samples. Fresh fecal samples were collected daily at 10:00 and 17:00 during the 5-day trial period, mixed after 5 consecutive days, and treated with 10% hydrochloric acid for nitrogen fixation. Samples were taken using the quartering method, dried in a 65°C oven, equilibrated to room temperature, ground, and prepared as analytical samples. Contents of dry matter (DM), crude protein (CP), ether extract (EE), ash, NDF, acid detergent fiber (ADF), and acid-insoluble ash (AIA) were determined according to *Feed Analysis and Feed Quality Detection Technology* [9].

The acid-insoluble ash (AIA) method was used as an internal marker to calculate nutrient digestibility using the following formula:

Nutrient digestibility (%) = 100 –[100 × (AIA content in diet / AIA content in feces) × (nutrient content in feces / nutrient content in diet)]

1.2.2 Rumen Fermentation Parameters

On the final day of each trial period, sufficient rumen fluid was collected via oral intubation using a stomach tube sampler before morning feeding. After filtration through four layers of gauze, pH was measured using a portable pH meter (FG2-ELK, METTLER-TOLEDO, Switzerland). Rumen fluid buffer capacity was determined using the method of Tucker et al. [10]. The remaining rumen fluid was centrifuged at 3,000 r/min, and the supernatant was used to determine ammonia nitrogen (NH_3-N) concentration by the method of Feng Zongci et al. [11], cellulase activity by the method of Wang Jiaqi [12], and VFA concentration by the method of Wang Hongrong et al. [13]. VFA concentration was measured using a gas chromatograph (GC-9A, Shimadzu, Japan) with the

following conditions: column CP-WAX (30.00 m × 0.53 mm × 1.00 m); flame ionization detector (FID) and vaporization chamber temperature both at 200°C; column temperature programmed from 100°C to 150°C at 3°C/min; sensitivity 10^1, attenuation 52; crotonic acid as internal standard.

1.2.3 Plasma Biochemical Indexes

On day 4 of the trial period, 10 mL of blood was collected from the jugular vein using heparinized tubes before morning feeding, centrifuged at 3,000 r/min for 15 min, and plasma was collected in 1.5 mL centrifuge tubes for analysis of lipopolysaccharide (LPS), glucose (Glu), urea nitrogen (UN), and albumin (Alb) contents, as well as catalase (CAT), glutamic-oxalacetic transaminase (AST), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities. Detection kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.3 Statistical Analysis

Data were analyzed using SPSS 17.0 software for one-way ANOVA, with means compared using the LSD method. Significance level was set at $P<0.05$, and results are expressed as mean ± standard deviation (mean±SD).

2 Results and Analysis

2.1 Nutrient Intake and Digestibility

Observation revealed that goats in trial group exhibited faster feeding rates and higher DMI in the early stage, which declined in the later stage. Groups and remained relatively stable without significant fluctuations. As shown in Table 2 , different NFC/NDF ratios had minimal impact on overall DMI in Qianbeima goats, with no significant differences among the three groups ($P>0.05$).

For DM digestibility, groups and were 18.90% and 13.95% higher than group , respectively. For organic matter (OM) digestibility, groups and were 13.39% and 10.05% higher than group . For CP digestibility, groups and were 19.91% and 16.44% higher than group . For EE digestibility, groups and were 23.54% and 18.00% higher than group . For NDF digestibility, groups and were 16.99% and 18.42% higher than group . For ADF digestibility, groups and were 13.42% and 17.30% higher than group .

All nutrient digestibility values were significantly higher in groups and compared to group ($P<0.05$), with no significant differences between groups and ($P>0.05$).

Table 2 Effects of different NFC/NDF on nutrient intakes and digestibility of Qianbeima goats %

Items	Trial group	Trial group	Trial group
DM			
Intake/(g/d)	1,207.56$±95.14	1, 261.67±105.60	1, 243.11±102.42
	$*OM*$		
	$*$	$Intake/(g/d)$	1, 147.30±90.39
	$*CP*$		
	$*$	$Intake/(g/d)$	181.62±14.31
	$*EE*$		
	$*$	$Intake/(g/d)$	$41.18±3.24^{a}$
	$*NDF*$		
	$*$	$Intake/(g/d)$	$294.28±23.19^{c}$
	$*ADF*$		
	$*$	$Intake/(g/d)$	$150.95±11.89^{c}$

Items	Trial group	Trial group
DM		
Intake/(g/d)	$Digestibility/±2.31^{a}$	61.91±2.53^{a}
	$Digestibility/$	60.91±2.
	$Digestibility/±1.91^{a}$	45.68±2.55^{a}
	$Digestibility/±2.74^{a}$	60.11±2.55^{a}
	$Digestibility/±2.68^{a}$	43.14±2.63^{a}
	$Digestibility/±2.42^{a}$	39.84±2.58^{a}

Items	Trial group	Trial group
DM		
Intake/(g/d)	1, 261.67±105.60	1, 243.11±102.42
	1, 184.35±99.14	1, 137.82±93.75
	188.73±15.95	184.46±15.03
	$39.24±3.28^{b}$	$34.81±2.87^{c}$
	$459.70±37.88^{b}$	$666.03±55.75^{a}$
	$239.69±19.76^{b}$	$316.99±26.55^{a}$

In the same row, values with no letter or the same letter superscripts mean no significant difference (P>0.05), while different letter superscripts mean significant difference (P<0.05). The same as below.

2.2 Rumen Fermentation Parameters

As shown in Table 3 , trial group with the highest NFC/NDF ratio exhibited the lowest rumen fluid pH, which was significantly lower than groups and (P<0.05). Rumen fluid buffer capacity was significantly higher in group than in group (P<0.05). For NH_3-N concentration, group was significantly higher than group (P<0.05). No significant differences were observed in carboxymethyl cellulase, microcrystalline cellulase, or xylanase activities among groups (P>0.05), while cellobiase activity was highest in group , significantly higher than group (P<0.05).

Total volatile fatty acids (TVFA) concentration in groups and was 5.03% and 5.30% higher than group , respectively (P<0.05). Acetate concentration increased progressively from group to group , with group significantly lower than group (P<0.05). Propionate concentration showed the opposite trend, decreasing progressively from group to group , with significant differences among all three groups (P<0.05). Butyrate concentration was lowest in group , significantly lower than groups and (P<0.05). The acetate to propionate ratio increased progressively and significantly across groups , , and (P<0.05).

Table 3 Effects of different NFC/NDF on rumen fermentation parameters of Qianbeima goats

Items	Trial group	Trial group	Trial group
pH	6.31$±0.27^b\|6.68±0.21^a\|6.76±0.20^a\|\|$Buffercapacity/(mL/L)$\|27.79±3.39^b\|31.79±3.65^{ab}|34.25±2.
	$mL)$]|1.09±0.16|0.97±0.21|0.92±0.24||$Xylanase/[mol/(min\cdot$		
	$mL)$]|8.37±1.94|7.81±1.55|6.65±1.21||$Microcrystallinecellulose/[mol/(min\cdot$		
	$mL)$]|0.73±0.09|0.82±0.16|0.93±0.20||$Cellobiase/[mol/(min\cdot$		
	$mL)$]|2.21±0.52^b|2.42±0.39^{ab}|2.98±0.60^a||$TVFA/(mmol/L)$|60.82±2.15^a|60.98±1.35^a|57.91±1.51

2.3 Plasma Biochemical Indexes

As shown in Table 4 , plasma LPS content decreased progressively with decreasing NFC/NDF, with significant differences among all three groups (P<0.05). No significant differences were observed in other plasma biochemical indexes (Alb, UN, Glu content and CAT, AST, SOD, GSH-Px activities) among the three groups (P>0.05).

Table 4 Effects of different NFC/NDF on plasma biochemical indexes of Qianbeima goats

Items	Trial group	Trial group	Trial group
LPS/(EU/mL)	0.87$±0.04^a\|0.79±0.49^b\|0.73±0.04^c\|\|$Alb/(g/L)$\|29.28±2.47\|30.49±2.01\|27.94±2.98\|\|$UN/(mmol/L$		
	$Px/(U/mL)$|242.13±23.10|233.34±16.22|229.48±$21.71		

3 Discussion

3.1 Effects of NFC/NDF on Nutrient Intake and Digestibility

Feed intake reflects animal preference for diets and is an indicator ensuring nutrient intake. Both excessively high and low NFC levels in diets can affect feed intake. Xu Zhijun et al. [14] reported that when feeding Small-tailed Han sheep lambs with four diets containing *Caragana* silage as roughage (concentrate-to-roughage ratios of 70:30, 60:40, 50:50, 40:60), DMI was highest in the 50:50 group and lowest in the 70:30 group. Tang Zhigao et al. [15] found that when feeding pregnant Small-tailed Han sheep with corn straw as roughage (concentrate-to-roughage ratios of 20:80, 30:70, 40:60), DMI was highest in the 40:60 group during both early and late pregnancy, with no significant differences between the other two groups. The present results showed no significant differences in DMI among the three groups. According to observations, during the early stage of each period, group exhibited faster feeding rates and higher DMI than groups and due to the high-concentrate diet aligning with goats' natural dietary preferences. However, in the later half of each period, feeding rate and DMI declined, likely because prolonged consumption of the high-concentrate diet (80% concentrate) led to continuous lactic acid accumulation from NFC fermentation by rumen microorganisms, which decreased rumen fluid pH, inhibited fibrolytic enzyme activity, disrupted microbial flora, and consequently

reduced DMI in the later stage, resulting in no significant differences among groups across the entire period. This inference is supported by results for other nutrient intake and digestibility parameters and rumen fermentation parameters. In contrast, group showed higher DMI than group , suggesting that an NFC/NDF ratio of 1.05:1.00 was appropriate for DMI under the conditions of this experiment.

Maintaining a high NFC/NDF ratio can promote rumen microbial proliferation and protein utilization while improving DM and organic matter digestibility [16]. Wang Wenqi et al. [4] reported that DM and OM digestibility in ewes (Suffolk × Altay) increased significantly with increasing dietary NFC levels (33.96%, 37.78%, 45.80%, 49.40%, 50.03%, 53.29%), while NDF digestibility in the first four NFC level groups was significantly lower than in the last two groups. Valdés et al. [17] found in a free-choice feeding experiment with sheep (NDF levels of 33.2%, 28.7%, 24.1%, 19.5%) that OM and DM digestibility increased gradually with decreasing dietary NDF level, while ADF and NDF digestibility were highest in the 24.1% NDF group. The present results showed that nutrient digestibility decreased progressively with decreasing NFC/NDF, with groups and significantly higher than group . These findings align with the DMI discussion above, as feces collection occurred during the last 5 days of each period, and the adult goats, despite reduced DMI during the trial period, maintained high utilization efficiency by reducing fecal excretion to meet nutritional requirements. Zhang Litao et al. [7] reported consistent results in Dorper × Small-tailed Han F1 crossbred meat sheep. Overall, however, combined with rumen fermentation parameters and plasma biochemical indexes, excessively high NFC levels should be avoided in production practice.

3.2 Effects of NFC/NDF on Rumen Fermentation Parameters

Rumen fluid pH is a core indicator reflecting normal rumen fermentation, primarily influenced by dietary composition and saliva buffer secretion [18]. For ruminants, the appropriate pH range is 6.2–6.8, which is a prerequisite for normal rumen fermentation. In practice, excessively low pH (pH<5.5) caused by high concentrate levels can induce ruminal acidosis, a major factor harming goat rumen health. The present results showed that rumen fluid pH decreased progressively and significantly with increasing NFC/NDF across groups , , and . This occurs because higher dietary NFC levels provide more readily fermentable carbohydrates that are preferentially fermented by rumen microorganisms to produce lactic acid; when lactic acid accumulation exceeds a certain level, pH decreases, inducing ruminal acidosis (pH<5.5). These findings are supported by previous in vivo [19] and in vitro [20] studies. Considering that the highest NFC/NDF ratio (2.14:1.00) might cause ruminal acidosis, a small amount of sodium bicarbonate was added to the diet of group , limiting the pH reduction (minimum 6.31). Although group pH was statistically lower than groups and , it remained within the normal rumen range and did not affect normal fermentation. Liu Jie et al. [21] found that meat sheep fed a diet with NFC/NDF of

2.17:1.00 had rumen fluid pH of 6.31 at 08:00, consistent with our results.

Stable and strong buffer capacity is a major physiological characteristic distinguishing ruminants from monogastric animals. Rumen fluid buffer capacity is closely related to dietary composition, saliva secretion, and rumen wall secretion, and is affected by pH and TVFA concentration. Generally, high NDF levels more effectively stimulate chewing and saliva secretion into the rumen, increasing buffer capacity. Miller et al. [22] and Tucker et al. [10] reported that higher dietary NFC levels result in lower rumen fluid buffer capacity. In this experiment, rumen fluid buffer capacity increased progressively with decreasing dietary NFC level, with group significantly lower than group , consistent with the above findings. Zhang Xiandong [23] reported that adding 1.5% and 3.0% buffer (sodium bicarbonate:magnesium oxide = 2:1) to a diet with a 60:40 concentrate-to-roughage ratio (using Chinese wildrye as roughage) significantly increased rumen fluid buffer capacity in the 3.0% group compared to the control. In the present study, even with added sodium bicarbonate in group , rumen fluid buffer capacity remained significantly lower than group , further indicating that dietary NFC/NDF should not be excessively high.

As a degradation product of dietary protein, endogenous protein, and other non-protein nitrogen compounds in the rumen, NH_3-N is the primary raw material for rumen microorganisms to synthesize microbial protein when energy and carbon skeletons are available [24]. NH_3-N concentration reflects rumen microbial utilization of dietary nitrogen to some extent. Excessively high NH_3-N concentration indicates that the rate of ammonia release from nitrogen source degradation exceeds the rate of microbial protein synthesis, increasing nitrogen loss in the nitrogen cycle; conversely, it limits microbial protein synthesis [25]. This experiment found that NH_3-N concentration decreased progressively with decreasing NFC/NDF across groups , , and , supported by numerous literature reports. Zhao Guoqi et al. [26] fed Xu-Huai white goats diets with NDF levels of 60.47%, 52.05%, 43.64%, and 35.23%, finding that NH_3-N concentration in the 60.47% NDF group was significantly lower than other groups, concluding that high dietary NDF (NDF>60.47%) was the main factor limiting microbial NH_3-N utilization, and that appropriately reducing dietary NDF level could promote NH_3-N utilization by rumen microorganisms. Similar reports have been documented in dairy cows by Cai Jingjing et al. [27] and Agle et al. [28].

Carboxymethyl cellulase, xylanase, microcrystalline cellulase, and cellobiase are cellulolytic enzymes that degrade fiber in the rumen, with activity primarily affected by rumen fluid pH. Enzyme activity decreases when pH<6 [29]. Wang Shuiping et al. [30] fed lactating dairy cows diets with NDF levels of 50.22%, 44.89%, 35.53%, and 28.63%, finding that dietary concentrate-to-roughage ratio had no significant effect on rumen fluid carboxymethyl cellulase, microcrystalline cellulase, xylanase, or cellobiase activities. Wang Hairong [31] reported that feeding Sunite sheep diets with concentrate-to-roughage ratios of 10:90, 30:70, and 50:50 (NDF levels of 64.38%, 54.59%, and 42.71%) did not significantly affect rumen fluid cellulase activity. The present results showed that

cellobiase activity in group was significantly lower than group , indicating that reducing NFC level (i.e., decreasing NFC/NDF) enhanced rumen microbial fiber-degrading capacity. This finding differs somewhat from previous reports, possibly due to variations in dietary composition and feeding management.

Volatile fatty acids produced by rumen microbial fermentation are the main energy source for ruminants. Dietary composition is the primary factor affecting VFA composition and determines rumen fermentation type. Diets with high NFC/NDF undergo propionic acid-type fermentation with high propionate concentration, while the opposite results in acetic acid-type fermentation with high acetate concentration. In this experiment, decreasing NFC/NDF resulted in progressively increased acetate concentration, decreased propionate concentration, and increased acetate/propionate ratio, consistent with this pattern. Similar reports exist in the literature. Zhang Yingying et al. [32] fed Jinnan cattle diets with NDF levels of 77.03%, 63.75%, 50.39%, and 37.29%, reporting that decreasing dietary NDF level (i.e., increasing NFC/NDF) resulted in gradually decreased acetate and butyrate concentrations and increased propionate concentration. Zhu Dan et al. [33] obtained similar results in an in vitro rumen fermentation study feeding dairy cows diets with NDF/starch ratios of 0.83, 1.13, 1.56, and 2.38. Wei Deyong et al. [6] used a self-control method to feed castrated white goats from the Yangtze River Delta region diets with NFC/NDF ratios of 0.42:1.00, 1.04:1.00, and 2.73:1.00, finding that increasing NFC/NDF resulted in progressively decreased acetate/propionate ratio, indicating a shift from acetic acid-type to propionic acid-type fermentation. All these results are consistent with our findings.

3.3 Effects of NFC/NDF on Plasma Biochemical Indexes

Endotoxin is a complex primarily containing LPS in the outer membrane of Gram-negative bacterial cell walls, with LPS being its main antigenic and pathogenic component. Decreased rumen fluid pH can cause death and lysis of Gram-negative bacteria, increasing free LPS content in the rumen and enhancing intestinal permeability to LPS [34], leading to increased blood LPS content, liver damage, and metabolic diseases. Wang Linfeng et al. [35] demonstrated that exogenous LPS injection could cause liver injury and affect hepatic nutrient metabolism in goats. Hu Honglian et al. [36] fed dairy goats diets with NFC/NDF ratios of 1.02:1.00, 1.24:1.00, 1.63:1.00, and 2.58:1.00, reporting that blood LPS content in the high NFC/NDF group (2.58:1.00) was significantly higher than in the low NFC/NDF group (1.02:1.00). Zhang Sen et al. [37] reported similar conclusions in dairy cows. The present results showed that NFC/NDF and plasma LPS content exhibited the same numerical trend, with LPS content increasing progressively from group to group . Since the minimum rumen fluid pH (6.31) remained within the normal range of 6.2–6.8, although plasma LPS content showed statistical differences, the numerical variation was small. This suggests that NFC/NDF should be controlled within an appropriate range in production practice.

Alb, UN, and Glu are important indicators reflecting immune function, protein metabolism, and glucose metabolism, respectively, and are significant for understanding animal condition. Cui Xiaopeng et al. [38] supplemented pregnant Tibetan ewes with diets containing different NDF levels (37.55%, 33.61%, 29.67%), finding that plasma Glu content in the 29.67% group was significantly higher than in the 37.55% group, with no significant differences in Alb or Glu content among other groups. Men Xiaoming [39] fed non-pregnant Small-tailed Han sheep ewes diets with concentrate-to-roughage ratios of 20:80, 30:70, and 40:60, reporting that Glu content increased with increasing concentrate-to-roughage ratio, while plasma UN and Alb content showed the opposite trend. Yan Wenping et al. [40] reported that when Jinnan cattle consumed diets with different NDF levels (77.03%, 63.75%, 50.39%, 37.29%), serum UN content was highest in the 37.29% NDF group, and serum Alb content in the 50.39% and 37.29% groups was significantly higher than in the 77.03% and 63.75% groups. The present results showed no significant differences in plasma Alb, UN, or Glu content among groups, differing from previous studies, possibly due to variations in animal breed, sex, physiological stage, and feeding management.

AST is an important indicator of liver function. When liver function is affected, blood transaminase activity increases. In this experiment, no statistical differences in AST activity were observed, indicating that liver function was not affected under the experimental conditions. Similar reports have been documented in Dorper sheep by Xu Xiangting et al. [41].

CAT, SOD, and GSH-Px constitute the enzymatic system for scavenging free radicals and are indicators of antioxidant stress capacity. Shi Liguang et al. [42] fed Hainan black goats diets with concentrate-to-roughage ratios of 50:50, 20:80, and 80:20 (NDF levels of 44.05%, 54.46%, and 34.23%), finding no significant differences in plasma CAT, SOD, or GSH-Px activities. In the present experiment, NDF levels in the three groups were 24.37%, 36.98%, and 52.79%, with no significant differences in plasma CAT, SOD, or GSH-Px activities among groups, consistent with the above conclusion. However, different results have been reported: feeding 45 growing Guizhou black goats diets with different NDF levels showed that plasma SOD and GSH-Px activities increased with decreasing dietary NDF level, with the 34.7% and 25.8% NDF groups significantly higher than the 44.4% group [43]. Sgorlon et al. [44] reported similar results in lactating sheep. These discrepancies with our results may be attributed to differences in animal species, physiological stages, and feeding management.

High NFC/NDF (2.14:1.00) was beneficial for nutrient digestibility in Qianbeima goats but had no significant effect on feed intake and adversely affected rumen fermentation parameters and plasma biochemical indexes. In production practice, NFC/NDF should be controlled at an appropriate level. Under the conditions of this experiment, an NFC/NDF ratio of 1.05:1.00 was recommended.

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Note: Figure translations are in progress. See original paper for figures.