# Effects of supplementation of nonforage fiber source in diets with different starch levels on growth performance, rumen fermentation, nutrient digestion, and microbial flora of Hu lambs

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**ABSTRACT:** The objectives were to evaluate the effects of fiber source and dietary starch level on growth performance, nutrient digestion, rumen parameters, and rumen bacteria in fattening *Hu* lambs. A total of 360 *Hu* lambs (BW = 24.72 ± 0.14 kg, 2 months old) were subjected to a 2 × 3 factorial arrangement. Lambs randomly assigned 6 treatments with 6 repetitions (10 lambs per repetition) of each treatment. Six treatments were formulated to include the fiber sources with three starch levels. The experiment lasted a 63 d. The amount of feed, orts, and total feces were sampled on the 42nd day of the experiment. Rumen fluid samples were collected after 2 h of morning feeding on day 56. Rumen contents were collected last day after the selected lambs were slaughtered. Increasing the starch content decreased the digestibility of neutral detergent fiber (NDF, $P = 0.005$). Increasing the starch level increased the proportions of propionate ($P = 0.002$) and valerate ($P = 0.001$) and decreased the proportion of acetate ($P < 0.001$) and the ratio of acetate to propionate ($P = 0.005$). The abundance of *Fibrobacter succinogenes* was affected by an interaction between the fiber source and the starch level ($P < 0.001$). *Fibrobacter succinogenes* tended to be greater in lambs fed SH than in lambs fed BP ($P = 0.091$), which was greater in lambs fed high starch levels than in lambs fed low starch levels ($P = 0.014$). Increasing the starch level increased *Streptococcus bovis* abundance ($P = 0.029$) and decreased *total bacteria* ($P = 0.025$). At the genus level, increasing the starch level reduced the abundance of *Butyrivibrio_2* ($P = 0.020$). Nevertheless, the final body weight (BW) and acid detergent fiber (ADF) digestibility were greater ($P < 0.01$) in lambs fed soybean hull (SH) than in lambs fed BP. The proportion of butyrate was greater ($P = 0.005$), while the rumen pH was lower ($P = 0.001$) in lambs fed beet pulp (BP) than in those fed SH. The abundances of *Succiniclasticum*, *Candidatus_Saccharimonas*, *Ruminococcus_1*, and *Christensenellaceae_R-7* were greater in lambs fed SH than in those fed BP ($P < 0.050$), whereas the abundance of *Fibrobacter* was lower ($P = 0.011$). The predominant microbial phyla in all of the groups were *Firmicutes*, *Bacteroidetes*, and *Fibrobacteres*. Changing the starch level for fiber sources mainly changed the rumen community in terms of the phylum and genus abundances. Lambs fed SH with low starch level increased the final BW without affecting total volatile fatty acids (TVFA) concentrations.

**Key words:** growth performance, nonforage fiber sources, rumen bacteria, rumen parameters

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## INTRODUCTION

Using cereal grain as the main energy intake improved lamb performance in production systems (Papi et al., 2011; Busanello et al., 2018). However, high grain diets rapidly fermented and produced organic acids, reduced rumen pH, and increased the risks of subacute ruminal acidosis (SARA) or acute ruminal acidosis (ARA) (Penner et al., 2007; Fernando et al., 2010). Some studies suggested that starch contents typically range from 22.0% to 28.0% dry matter (DM) in the cows (Fredin et al., 2015; Ying et al., 2015). However, increasing cereal grain prices resulted in significantly higher feed costs; consequently, common strategies to reduce dietary starch that minimize the inclusion of corn and other grains may improve farm profitability, and these trends have been accompanied by an increasing supply of high-fiber byproducts (Bradford and Mullins, 2012; Dann et al., 2014). Previous studies identified the partial replacement of cereal grain with cost-effective, low-starch nonforage fiber sources (NFFS), which were a potential alternative to help overcome these issues (Ranathunga et al., 2010).

Ruminal microbes require a supply of ruminally fermentable carbohydrates for growth and protein synthesis (Dann et al., 2014). Starch provides a substrate for ruminal microbial protein synthesis and production in the host (Zebeli et al., 2010). However, sugars, digestible neutral detergent fiber (NDF), and soluble fiber ferment in the rumen also contribute to microbial protein production (Dann et al., 2014). For example, Zhao et al. (2013) observed that in vitro replacement starch with soluble fiber in the diet (from 16.4% to 23.8%) did not repress microbial synthesis. These studies show that the replacing starch with soluble fiber does not affect protein synthesis.

The rumen pH was lower when ruminants were fed higher grain diets. Increasing the starch content in diets accelerated rumen fermentation and produced more volatile fatty acids (VFAs), causing rumen pH depression (Zhang et al., 2018). Leiva et al. (2000) found that compared with corn hominy, dried citrus pulp as a source of neutral detergent soluble fiber in diets did not affect organic acid concentrations in the rumen. Voelker and Allen (2003) also found that substituting pelleted beet pulp (BP) for high-moisture corn in diets for cows did not influence total VFA (TVFA) concentrations. Iraira et al. (2013) found that feeding barley straw and BP diets for beef heifers increased the acetate proportion, whereas fed soybean hull (SH) and whole cottonseed than increased the propionate proportion. Although the VFA concentration was not different, and individual VFAs were related to the type of nonforage fiber. Based on the results in rumen fermentation, it is possible that feeding nonforage fiber with low starch in diets could maintain rumen fermentation in ruminants.

Many nonforage fibers, such as BP, SH, and citrus pulp, have the nutritional characteristics of both roughage and concentrate, containing substantial amounts of soluble fiber, protein, and energy (Salami et al., 2019). Previous studies have evaluated the partial replacement of corn grain with BP, SH, and citrus pulp (Dann et al., 2014; López et al., 2014; Maktabi et al., 2016). In these studies, reducing dietary starch using either formulation strategy consistently resulted in similar dry matter intake (DMI) with an increase or no change in fiber apparent digestibility compared with those for normal-starch diets. However, direct comparisons of the effects of feeding nonforage fiber with varying starch levels using formulation strategies within the same study are limited.

Therefore, the objectives of this experiment were to compare the effects of NFFS and starch level on the performance, nutrient digestion, rumen fermentation, and microbial populations of fattening *Hu* lambs. We hypothesized that NFFS with different starch levels would result in similar TVFA concentration and change the rumen bacterial community.

## MATERIALS AND METHODS

All procedures were approved by the Biological Studies Animal Care and Use Committee of Gansu Province, China (2005–12).

### *Animals and Management*

This experiment was conducted in Minqin, Gansu Province, China. A total of 360 weaning *Hu* lambs (BW = 24.72 ± 0.14 kg, 2 months old) were housed in individual pens with 0.75 × 1.5 × 1.0 m. All lambs were treated for internal and external parasites at the beginning of the experiment. All diets were prepared in the form of totally mixed ration (TMR) pellets. The feed was provided at 0800 h and 1800 h for ad libitum consumption, and the animals were provided free access to water.

**Table 1.** Dietary ingredients and nutrient composition (% DM)

| Fiber sources | Beet pulp | | | Soybean hulls | | |
|---|---|---|---|---|---|---|
| Starch levels (%) | 27 High | 25 Medium | 22 Low | 27 High | 25 Medium | 22 Low |
| Maize straw, % | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Sunflower seed hull, % | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Beet pulp, % | 15.00 | 15.00 | 15.00 | 0.00 | 0.00 | 0.00 |
| Soybean hull, % | 0.00 | 0.00 | 0.00 | 15.00 | 15.00 | 15.00 |
| Corn germ meal, % | 0.00 | 11.00 | 23.30 | 0.00 | 11.00 | 23.30 |
| Corn, % | 30.00 | 28.00 | 25.00 | 30.00 | 28.00 | 25.00 |
| Corn starch, % | 11.00 | 5.00 | 0.00 | 11.00 | 5.00 | 0.00 |
| Soybean meal | 4.30 | 4.30 | 3.00 | 4.20 | 4.20 | 2.90 |
| Cottonseed meal | 7.00 | 7.00 | 5.00 | 7.00 | 7.00 | 5.00 |
| Corn gluten meal | 4.00 | 1.00 | 0.00 | 4.00 | 1.00 | 0.00 |
| Limestone | 1.20 | 1.20 | 1.20 | 1.30 | 1.30 | 1.30 |
| NaCl | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 |
| Expanded urea | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Premix* | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Nutritional levels | | | | | | |
| DM, % as fed | 92.95 | 92.96 | 93.20 | 92.41 | 92.65 | 92.65 |
| Starch, % of DM | 25.99 | 24.30 | 22.88 | 27.08 | 24.25 | 22.18 |
| CP, % of DM | 17.69 | 17.20 | 17.33 | 17.43 | 17.55 | 17.02 |
| ADF, % of DM | 12.22 | 14.07 | 14.82 | 17.38 | 18.41 | 19.00 |
| NDF, % of DM | 22.48 | 27.07 | 31.53 | 28.71 | 33.24 | 35.91 |
| DE, MJ/kg | 9.91 | 9.59 | 9.35 | 9.61 | 9.26 | 9.08 |

DM = dry matter; CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; DE = digestible energy.

*The composed of mineral and vitamin premix: 70 mg/kg of Fe, 41 mg/kg of Zn, 8 mg/kg of Cu, 0.7 mg/kg of I, 24 mg/kg of Mn, 0.3 mg/kg of Se, 0.3 mg/kg of Co; 2500 UI/kg of vitamin A, 23 UI/kg of vitamin E.

Lambs were randomly assigned 6 treatments with 6 repetitions (10 lambs per repetition) of each treatment according to their BW and age. Dietary treatments were administered according to a 2 × 3 factorial arrangement including two fiber sources (BP vs. SH) with three starch levels (low = 22% DM, medium = 25% DM, and high = 27% DM). The NFFS in the diets were used as fiber sources, and dietary starch was changed by corn and corn starch. The ingredients and composition of the diet are presented in Table 1. The trial started after two weeks of diet adaption and included 63 d of data collection.

### *Measurements and Analytical Methods*

During the experimental period, all lambs were weighed, and the feed intake of each lamb was recorded. The feed efficiency was calculated as DMI/ADG. A total of 36 *Hu* lambs (6 lambs each treatment. BW = 32.72 ± 2.00 kg) were selected and moved to individual metabolism cages for a feed digestibility determination. Total tract digestibility was measured simultaneously on the 42nd day of the experiment. The amount of feed offered, orts, and total feces daily were collected and recorded for five consecutive days. A wire-screen basket placed behind each cage for feces collection. A portion of the fecal samples was treated with $H_2SO_4$ to analyze crude protein (CP). The samples of feedstuff, ration, and feces were dried in a forced-air circulation oven at 65°C for 48 h and ground through a 1-mm screen. The DM, organic matter (OM), and CP were determined according to the Association of Official Agricultural Chemists (AOAC) method (AOAC,1997). The N contents of feed and feces were measured by the Kjeldahl method, and CP was calculated as N × 6.25 (AOAC, 1997). The DM was determined by drying at 105°C in a forced air oven for 4 h. Ash concentration was determined by complete combustion in a muffle furnace at 550°C for 6 h. The NDF and ADF were measured by a fiber analyzer (A2000, Ankom Technology, Fairport, NY) according to the methods of Van Soest et al. (1991). Total starch content in the diets was determined by a commercial starch analysis kit (Megazyme, International Ireland Ltd. Bray, Ireland).

Rumen fluid samples were collected after 2 h of morning feeding on day 56 with an esophageal tube and a vacuum pump. The rumen pH was

immediately measured with a mobile pH meter (PHB-4, Shanghai Hongyi Instrument Limited, Shanghai, China). Then, samples of (5 mL) rumen fluid were preserved with 1 mL of metaphosphoric acid (25% wt/vol) and stored at −20°C for determination of VFAs. Rumen contents were collected after the selected 120 lambs (20 lambs per treatment) were slaughtered by exsanguination and then stored at −80°C until analysis of the ruminal bacterial community.

### *Rumen Fluid VFA Extraction*

The rumen fluid samples were thawed at room temperature to be centrifuged at 2,500 × *g* at 4°C for 5 min, and the supernatants were processed as described by (Liang et al., 2017). VFA concentrations were determined by gas chromatography (TRACE 1300, Thermo Scientific, Milan, Italy). The gas chromatograph was fitted with a saturated FFAP silica capillary column (DB-FFAP, 30 m × 0.32 mm × 0.25 μm, Agilent Technologies Co., Ltd., Santa Clara), and crotonic acid was used as an internal standard. The injector and detector temperatures were set at 240°C. The temperature was increased from 50°C to 190°C over 2 min at a rate of 25°C/min, and then the temperature was increased to 200°C at a rate of 10°C/min for 5 min. Finally, the temperature was increased to 220°C at a rate of 10°C/min for 5 min.

### *Extraction of DNA from Rumen Content*

DNA of rumen microorganisms was extracted by an E.Z.N.A. Bacterial DNA Kit (Omega Bio-Tek, Inc., Norcross, GA, United States) according to the manufacturer's instructions. Primer design for all microbial genes was based on published literature (Table 2). The quantity of rumen bacteria was constructed using real-time absolute quantitative PCR. The total DNA of ruminal microbial was isolated from rumen content in sheep to construct recombinant plasmid and transfected into *Escherichia coli*. Target plasmids in white colonies were selected by ampicillin screening, and their specificity was identified by direct PCR and DNA sequencing. Plasmid DNA was extracted from positive recombinant plasmids. The serial gradient concentrations of plasmid DNA were used as standard to plot standard. The quantitative real-time PCR protocol was as follows: 95°C for 10 s; 40 cycles at 95°C for 10 s, 60°C for 30 s, and 72°C for 10 s; and a final cycle at 72°C for 5 min. The reactions were performed in triplicate in 20-μL mixtures. The reaction mixture contained 1 μL of DNA, 10 μL of SYBR Green (TransGen Biotech, Beijing, China), 0.2 μL of each primer, and 8.6 μL of $ddH_2O$. Microbiota compositions were determined by using high-throughput sequencing of the 16S rRNA gene by the Illumina MiSeq 2500 platform (Lianchuan Biotechnology Co., Ltd, Hangzhou, China) according to standard protocols.

### *Statistical Analysis*

Statistical analysis was performed using the general linear model (GLM) with SPSS software version 17.0 (IBM, Armonk, NY, United States) with a 2 × 3 factorial design. The factorial arrangement incorporated the fiber sources and three starch levels. To test the effects of the fiber source and starch level, the following model was used:

**Table 2.** The sequence of primers

| Primer name | Primer sequences (5′–3′) | Reference |
|---|---|---|
| *Ruminococcus flavefaciens* | *F:5-CGAACGGAGATAATTTGAGTTTACTTAGG-3<br>†R:5-CGGTCTCTGTATGTTATGAGGTATTACC-3 | (Saminathan et al., 2016) |
| *Fibrobacter succinogenes* | F:5-GTTCGGAATTACTGGGCGTAAA-3<br>R:5-CGCCTGCCCCTGAACTATC-3 | (Denman and McSweeney, 2006) |
| *Prevotella brevis* | F:5-TAACATGAGAGTTTGATCCTGGCTC-3<br>R:5-CGTTACTCACCCGTCCCGC-3 | (Stevenson et al., 1988) |
| *Ruminococcus albus* | F:5-TTCCTAGAGATAGGAAGTTTCTTCGG-3<br>R:5-ATGATGGCAACTAACAATAGGGGT-3 | (Koike and Kobayashi, 2001) |
| *Ruminobacter amylophilus* | F:5-GCGAACTGGTTTCCTTGAGTGTATT-3<br>R:5-ACCTTCGAGCTTTAGCGTCAGTTAT-3 | (Stevenson et al., 1988) |
| *Streptococcus bovis* | F:5-CTGGGGAGCTGCCTGAATG-3<br>R:5-GCATCTGAATGCGACTGGTTG-3 | (Khafipour et al., 2009) |
| *Total bacteria* | 5-CGGCAACGAGCGCAACCC-3<br>5-CCATTGTAGCACGTGTAGCC-3 | (Denman and McSweeney, 2006) |

*F = forward.

†R = reverse.

$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_i(jk)$, where $Y_{ijk}$ = the dependent variable; $\mu$ = the overall mean; $\alpha_i$ = the effect of dietary fiber source (i = BP, SH); $\beta_j$ = the effect of dietary starch level (i = low, medium, and high); $\alpha\beta_{ij}$ = the interaction effect of fiber source and starch level, and $\varepsilon_i(jk)$ = the residual error. In the analysis of growth performance, nutrient digestion, rumen parameters, and rumen bacteria were included in the statistical model. The *P*-values characterized differences between dietary fiber sources, starch levels, and their interaction. The significant difference of data was analyzed by Duncan's multiple range tests.

The relative abundances of rumen bacteria were expressed as a proportion of total rumen bacterial 16S rRNA according to the equation: relative quantification = $2^{-(CTctarget - CTctotal\ bacteria)}$, where *C*T represents the threshold cycle (Chen et al., 2008). Before statistical analysis, the percentage of each microbe target was calculated as $(2^{-\Delta CT}) \times 100$, and the data were log10 transformed before statistical analysis (Liang et al., 2017). The level of significance was set as $P \leq 0.05$ and tendencies were considered when $0.05 < P < 0.10$.

## RESULTS

The effects of dietary fiber source and starch level on growth performance are presented in Table 3. The initial BW, ADG, and DMI ($P > 0.05$) were similar among treatments. A tendency ($P = 0.081$) for interaction between fiber source and starch level affected the ratio DMI/ADG. Nevertheless, the ratio DMI/ADG did not differ ($P > 0.05$) between fiber source and starch level. However, lambs fed SH had a greater final BW than lambs fed BP ($P = 0.004$).

The dietary fiber source × starch level interaction had no effect on apparent digestibility ($P > 0.05$, Table 4). The digestibilities of DM and OM were not different ($P > 0.05$) for different fiber sources and starch levels. The ADF digestibility ($P = 0.114$) and CP digestibility ($P = 0.154$) did not differ with starch levels. The NDF digestibility was greater ($P = 0.005$) at the low starch level than at the high starch level. However, the NDF digestibility ($P = 0.106$) was similar for both fiber sources. The ADF digestibility was greater ($P = 0.009$) in lambs fed SH than in lambs fed BP. The CP digestibility tended to be greater ($P = 0.052$) in lambs fed SH than in lambs fed BP.

The effects of fiber sources and starch levels on rumen fermentation are presented in Table 5. The dietary fiber source × starch level interaction had an effect on TVFA concentration ($P = 0.082$). The acetate/propionate ratio and the proportions of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and lactate concentration ($P > 0.05$) were not affected by interactions between the fiber source and starch level. An interaction between fiber source and starch level affected the rumen pH ($P < 0.001$); lambs fed SH had greater rumen pH than lambs fed BP ($P = 0.001$), but increasing the starch level tended to decrease rumen pH ($P = 0.080$). The TVFA concentration and proportions of isobutyrate, butyrate, isovalerate, and lactate ($P > 0.05$) did not differ by starch level. Increasing the starch level decreased the proportion of acetate ($P < 0.001$) and the acetate/propionate ratio ($P = 0.005$) and increased the proportions of propionate ($P = 0.002$) and valerate ($P = 0.001$). The fiber source had no effect on TVFA concentration; proportions of acetate, propionate, isobutyrate, isovalerate, and lactate or the acetate/propionate ratio ($P > 0.05$). The proportion of butyrate ($P = 0.005$) was greater in lambs fed BP than in those fed SH.

The effects of dietary fiber source and starch level on the amount of rumen bacteria are shown in Table 6. There was no significant ($P > 0.05$) interaction between fiber source and starch level for most bacterial proportions, and *Fibrobacter succinogenes* was an exception ($P < 0.001$). *Fibrobacter succinogenes* tended to be greater in lambs fed SH than in lambs fed BP ($P = 0.091$), and was also

**Table 3.** Effects of dietary fiber source and starch level on growth performance

| Fiber sources | Beet pulp | | | Soybean hulls | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Starch levels (%) | 27 High | 25 Medium | 22 Low | 27 High | 25 Medium | 22 Low | SEM | Fiber sources | Starch level | Interaction |
| Initial BW, kg | 29.90 | 29.90 | 29.86 | 30.36 | 29.69 | 30.28 | 0.131 | 0.429 | 0.580 | 0.551 |
| Final BW, kg | 47.25 | 46.93 | 46.54 | 48.44 | 47.57 | 48.42 | 0.216 | 0.004 | 0.527 | 0.490 |
| ADG, kg/d | 0.28 | 0.27 | 0.26 | 0.29 | 0.28 | 0.29 | 0.003 | 0.169 | 0.106 | 0.147 |
| DMI, kg/d | 1.67 | 1.68 | 1.66 | 1.75 | 1.68 | 1.71 | 0.013 | 0.109 | 0.594 | 0.443 |
| F/G | 5.95 | 6.23 | 6.38 | 6.05 | 6.01 | 5.89 | 0.055 | 0.382 | 0.132 | 0.081 |

SEM = standard error of the sample mean; BW = body weight; ADG = average daily gain; DMI = dry matter intake; F/G = DMI/ADG.

**Table 4.** Effects of dietary fiber source and starch level on apparent digestibility

| Fiber sources | Beet pulp | | | Soybean hulls | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 27 | 25 | 22 | 27 | 25 | 22 | | | | |
| Starch levels (%) | High | Medium | Low | High | Medium | Low | SEM | Fiber sources | Starch level | Interaction |
| Apparent digestibility, % | | | | | | | | | | |
| DM | 59.7 | 59.5 | 59.2 | 57.2 | 57.4 | 56.8 | 0.008 | 0.168 | 0.971 | 0.997 |
| OM | 63.1 | 63.4 | 63.1 | 62.8 | 62.4 | 61.9 | 0.007 | 0.599 | 0.967 | 0.970 |
| CP | 58.3 | 58.7 | 56.0 | 60.1 | 62.7 | 58.3 | 0.007 | 0.052 | 0.114 | 0.767 |
| NDF | 36.5 | 44.4 | 50.4 | 41.9 | 51.6 | 52.2 | 0.017 | 0.106 | 0.005 | 0.746 |
| ADF | 28.8 | 32.9 | 37.4 | 37.7 | 45.0 | 45.4 | 0.019 | 0.009 | 0.154 | 0.873 |

SEM = standard error of the sample mean; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.

**Table 5.** Effects of dietary fiber source and starch level on rumen fermentation parameters

| Fiber sources | Beet pulp | | | Soybean hulls | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 27 | 25 | 22 | 27 | 25 | 22 | | | | |
| Starch levels (%) | High | Medium | Low | High | Medium | Low | | Fiber sources | Starch level | Interaction |
| TVFA, mmol/L | 85.61 | 76.95 | 77.04 | 64.49 | 70.64 | 87.09 | 2.849 | 0.304 | 0.433 | 0.082 |
| VFA molar ratios, mol/100 mol | | | | | | | | | | |
| Acetate, % | 55.19 | 59.63 | 59.93 | 55.45 | 60.76 | 60.14 | 0.589 | 0.631 | <0.001 | 0.928 |
| Propionate, % | 30.02 | 26.09 | 25.45 | 32.31 | 26.38 | 26.90 | 0.681 | 0.301 | 0.002 | 0.817 |
| Isobutyrate, % | 0.40 | 0.75 | 0.59 | 0.55 | 0.56 | 0.70 | 0.041 | 0.751 | 0.128 | 0.158 |
| Butyrate, % | 11.58 | 11.23 | 11.62 | 8.76 | 9.91 | 10.18 | 0.333 | 0.005 | 0.656 | 0.576 |
| Isovalerate, % | 0.54 | 0.85 | 0.88 | 1.13 | 1.05 | 0.95 | 0.087 | 0.102 | 0.861 | 0.446 |
| Valerate, % | 2.27 | 1.46 | 1.53 | 1.80 | 1.36 | 1.14 | 0.088 | 0.052 | 0.001 | 0.629 |
| A/P | 2.00 | 2.40 | 2.45 | 1.80 | 2.46 | 2.38 | 0.076 | 0.644 | 0.005 | 0.788 |
| Rumen pH | $6.18^{c}$ | $6.77^{ab}$ | $6.57^{b}$ | $6.89^{a}$ | $6.72^{ab}$ | $6.64^{ab}$ | 0.044 | 0.001 | 0.080 | <0.001 |
| Lactate, mmol/L | 0.67 | 0.61 | 1.94 | 1.32 | 0.72 | 0.72 | 0.140 | 0.379 | 0.423 | 0.536 |

SEM = standard error of the sample mean; TVFA = total volatile fatty acids; A/P = Acetate/Propionate.

[a,b]Mean in the same row with different superscripts are different ($P < 0.05$).

greater at the high starch level than at the low starch level ($P = 0.014$). Compared with lambs fed BP, lambs fed SH exhibited increased *Streptococcus bovis* abundance ($P < 0.001$). However, increasing the starch level decreased the abundance of *S. bovis* ($P = 0.029$). The *Prevotella brevis* abundance was greater ($P = 0.001$) in lambs fed BP than in lambs fed SH, whereas the *Ruminobacter amylophilus* abundance was lower ($P = 0.009$). The *total* amount of *bacteria* was greater at the high starch level than at the low-starch level ($P = 0.025$).

No interaction was found between fiber source and starch level for the abundance of rumen bacteria at the phylum level ($P > 0.05$, Fig. 1, Table 7). The predominant microbial phyla were *Firmicutes*, *Bacteroidetes*, and *Fibrobacteres*, which was consistent among all fiber sources and starch levels. An increase in the starch level increased the abundances of *Proteobacteria* ($P = 0.005$) and *Tenericutes* ($P = 0.001$) and decreased the abundance of *Verrucomicrobia* ($P = 0.003$). Lambs fed SH exhibited a greater abundance of *Firmicutes* ($P = 0.001$), while *Bacteroidetes* ($P = 0.019$), *Fibrobacteres* ($P = 0.011$) and *Proteobacteria* ($P = 0.018$) were more abundant in lambs fed BP.

No interaction was observed between fiber source and starch level for the abundances of rumen bacteria at the genus level ($P > 0.05$, Fig. 2, Table 8). The abundance of *Lachnospiraceae_NK3A20* was affected by the starch level ($P = 0.042$) and was greatest at the medium starch level. Meanwhile, the abundance of *Butyrivibrio_2* decreased with increasing starch level ($P = 0.020$). The abundance of *Fibrobacter* was greater ($P = 0.023$) in lambs fed BP, whereas the abundances of *Candidatus_Saccharimonas* ($P = 0.011$), *Christensenellaceae_R-7* ($P = 0.020$), *Ruminococcus_1* ($P = 0.013$), and *Succiniclasticum*

**Table 6.** Effects of dietary fiber source and starch level on the amount of rumen bacteria

| Fiber sources | Beet pulp | | | Soybean hulls | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Starch levels (%) | 27 High | 25 Medium | 22 Low | 27 High | 25 Medium | 22 Low | SEM | Fiber sources | Starch level | Interaction |
| Bacteria, Log10 16S rRNA copy number/mL rumen fluid | | | | | | | | | | |
| *Ruminococcus flavefaciens* | 13.51 | 13.71 | 13.83 | 13.72 | 13.85 | 13.43 | 0.091 | 0.914 | 0.715 | 0.357 |
| *Fibrobacter succinogenes* | 12.30[a] | 11.98[ab] | 11.56[c] | 11.48[c] | 12.08[ab] | 11.77[ab] | 0.059 | 0.091 | 0.014 | <0.001 |
| *Prevotella brevis* | 10.54 | 10.11 | 10.77 | 9.92 | 9.87 | 9.22 | 0.123 | 0.001 | 0.606 | 0.061 |
| *Ruminococcus albus* | 9.60 | 9.35 | 9.39 | 9.73 | 9.84 | 9.33 | 0.075 | 0.214 | 0.229 | 0.317 |
| *Ruminobacter amylophilus* | 8.29 | 7.87 | 8.50 | 8.19 | 9.08 | 9.42 | 0.136 | 0.009 | 0.067 | 0.085 |
| *Streptococcus bovis* | 8.02 | 7.97 | 8.13 | 8.19 | 8.29 | 8.37 | 0.027 | <0.001 | 0.029 | 0.332 |
| *Total bacteria* | 15.50 | 14.95 | 15.48 | 15.47 | 15.14 | 15.01 | 0.068 | 0.423 | 0.025 | 0.119 |

SEM = standard error of the sample mean.

[a,b]Mean in the same row with different superscripts are different ($P < 0.05$).

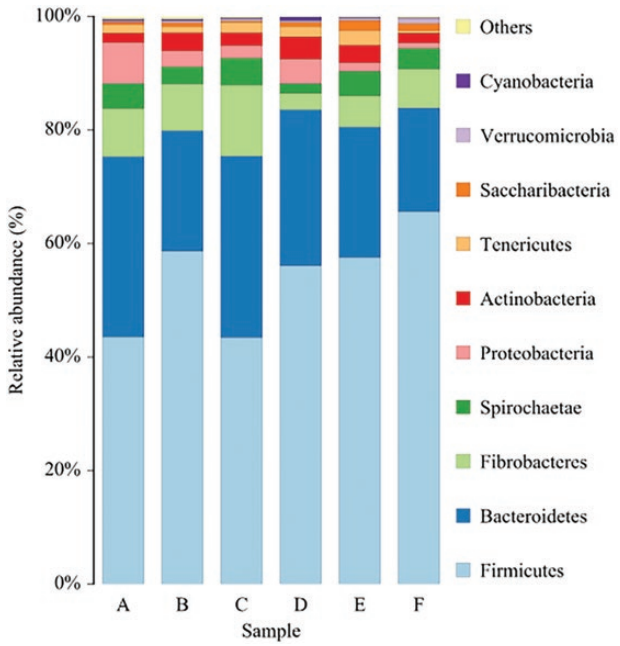


**Figure 1.** The distribution histogram of the rumen bacteria by fiber source and starch level at the phylum level (%). Note: A = 27 % starch and beet pulp group; B = 25% starch and beet pulp group; C = 22% starch and beet pulp group; D = 27% starch and soybean hulls group; E = 25% starch and soybean hulls group; F = 22% starch and soybean hulls group.

($P$ = 0.028) were greater in lambs fed SH. In addition, the abundance of *Ruminococcaceae_NK4A214* tended to be greater ($P$ = 0.051) in lambs fed SH than in lambs fed BP.

## DISCUSSION

In the present study, we found that lambs fed SH had greater final BW than those fed BP, which is different from the results of Santos-Silva (2018), who found that lambs fed NDF contents of different origin (ground alfalfa or soybean hulls) did not affect growth performance. Although NDF digestibility was not different between lambs fed SH and BP, lambs fed SH increased ADF digestibility. Meanwhile, the greater fiber in SH than in BP increased the ruminal mean retention time, which also increased the roughage digestibility (Salinas-Chavira et al., 2013). The dietary starch level did not affect DMI, ADG, or the ratio of DMI to ADG in our experiment, which was in agreement with the results of Neto et al. (2015). The growth performance of lambs did not differ by starch level due to their similar nutrient intake, OM digestibility, and digestible energy (Berry et al., 2004). However, the NDF digestibility decreased with increasing starch level in our study. This result indicates that excessive starch fermentation could inhibit fiber digestibility (Allen, 2012), which contributed to high levels of starch reducing the ruminal pH and impairing the activity of fibrous bacteria in the rumen (Calsamiglia et al., 2002; Fernando et al., 2010). As reported earlier, *F. succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* play a key role in fiber degradation in the rumen (Wanapat and Cherdthong, 2009). We found that the amount of *F. succinogenes* (cellulolytic bacteria) was affected by the interaction between fiber source and starch level. *Fibrobacter succinogenes* tended to be greater in lambs fed SH than in lambs fed BP, and it was greater in lambs fed the high starch than in lambs fed the low starch level. Previous studies showed that replacing barley silage with dried distiller triticale grains in feedlot cattle increased the amount of *F. succinogenes* (Wu et al., 2011). These findings agreed with the understanding that *F. succinogenes* abundance is related to the type of forage diet (Tajima et al., 2001). However, Zhang et al. (2010) found that *F. succinogenes* decreased with increasing corn starch, and the rumen pH may affect the

**Table 7.** Effects of dietary fiber source and starch level on rumen bacteria at the phylum level (%)

| Fiber sources | Beet pulp | | | Soybean hulls | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Starch levels (%) | 27 High | 25 Medium | 22 Low | 27 High | 25 Medium | 22 Low | SEM | Fiber sources | Starch level | Interaction |
| *Firmicutes* | 45.92 | 56.25 | 51.05 | 57.47 | 62.83 | 68.69 | 1.921 | 0.001 | 0.111 | 0.464 |
| *Bacteroidetes* | 31.06 | 23.15 | 29.47 | 26.15 | 20.76 | 17.89 | 1.364 | 0.019 | 0.097 | 0.352 |
| *Fibrobacteres* | 9.60 | 9.07 | 9.77 | 6.40 | 6.65 | 5.74 | 0.622 | 0.011 | 0.987 | 0.871 |
| *Spirochaetes* | 3.62 | 3.00 | 3.31 | 2.48 | 3.24 | 2.90 | 0.203 | 0.295 | 0.988 | 0.380 |
| *Proteobacteria* | 5.84 | 2.50 | 2.43 | 3.19 | 1.10 | 0.74 | 0.427 | 0.018 | 0.005 | 0.791 |
| *Actinobacteria* | 2.21 | 4.01 | 2.52 | 2.36 | 3.29 | 2.05 | 0.270 | 0.516 | 0.059 | 0.787 |
| *Candidatus Sacchari bacteria* | 0.60 | 0.74 | 0.37 | 0.70 | 1.10 | 0.89 | 0.101 | 0.109 | 0.423 | 0.694 |
| *Bacteria unclassified* | 0.59 | 0.60 | 0.41 | 0.53 | 0.42 | 0.24 | 0.046 | 0.134 | 0.100 | 0.820 |
| *Verrucomicrobia* | 0.09 | 0.31 | 0.39 | 0.24 | 0.34 | 0.66 | 0.044 | 0.066 | 0.003 | 0.490 |
| *Synergistetes* | 0.12 | 0.21 | 0.11 | 0.07 | 0.08 | 0.10 | 0.026 | 0.211 | 0.657 | 0.650 |
| *Tenericutes* | 0.16 | 0.06 | 0.09 | 0.18 | 0.09 | 0.05 | 0.013 | 0.748 | 0.001 | 0.490 |
| *Cyanobacteria* | 0.03 | 0.03 | 0.02 | 0.19 | 0.04 | 0.02 | 0.026 | 0.253 | 0.336 | 0.369 |
| *SR1* | 0.11 | 0.04 | 0.02 | 0.01 | 0.01 | 0.02 | 0.016 | 0.156 | 0.516 | 0.397 |
| *Candidatus Saccharibacteria* | 0.02 | 0.02 | 0.04 | 0.03 | 0.04 | 0.00 | 0.006 | 0.863 | 0.793 | 0.105 |
| *Elusimicrobia* | 0.04 | 0.01 | 0.03 | 0.01 | 0.00 | 0.00 | 0.005 | 0.082 | 0.237 | 0.582 |

SEM = standard error of the sample mean.

activity of *F. succinogenes*. In our studies, this difference may be explained by the fact that the effect of fiber source in diets obscured the starch influence. Overall, *F. succinogenes* was more sensitive to grain challenge than *R. albus* and *R. flavefaciens* (Pourazad et al., 2017). In the present study, the abundance of ruminal *R. flavefaciens* and *R. albus* was not influenced by starch level. It is possible that a moderate level of feeding and the fibrous nature of concentrate prevent rapid decreases in pH (Martinez et al., 2010), and *R. albus* and *R. flavefaciens* could benefit from mild pH depression (Pourazad et al., 2017).

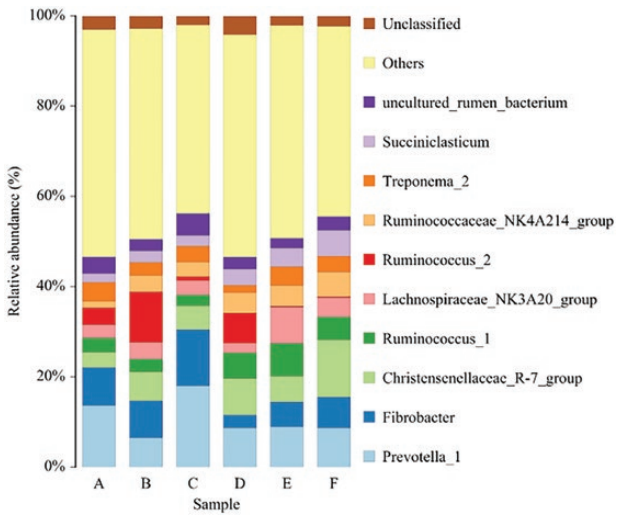


**Figure 2.** The distribution histogram of the rumen bacteria by fiber source and starch level at the genus level (%). Note: A = 27 % starch and beet pulp group; B = 25% starch and beet pulp group; C = 22% starch and beet pulp group; D = 27% starch and soybean hulls group; E = 25% starch and soybean hulls group; F = 22% starch and soybean hulls group.

The rumen pH decreases when VFAs and lactate accumulate, and buffers in saliva are overcome by the accumulation of these acids (Plaizier et al., 2008). In our study, the rumen pH was greater in lambs fed SH than in lambs fed BP, which is consistent with the results of Ferreira (2011), who found that the rumen pH increased with increasing SH for ram lambs. The higher rumen pH in lambs fed SH was likely a result of the NDF of SH stimulating the chewing activities of ruminants, which promoted saliva secretion, stabilized the rumen environment, and caused salivation that increased the rumen pH. The concentrations of TVFAs were not different among treatments, which could be partially explained by the lack of treatment effects on DM and OM digestibility (Dai et al., 2019). However, the concentrations of TVFAs were affected by the interaction of fiber sources and starch level. Because the TVFA was related to the ratio of NFC/NDF. The proportions of acetate and propionate and the ratio of acetate to propionate were similar in lambs fed SH and BP. Ferreira et al. (2011) found that increasing SH contents in high-concentrate diets for ram lambs affected rumen fermentation; SH addition increased the acetate concentration and the ratio of acetate to propionate, whereas quadratic effects were observed on the propionate concentration. Asadollahi et al. (2018) found that lambs fed sugar BP increased the proportion of acetate and the ratio of acetate to propionate. It is clear that no change was found in the proportions of acetate and

**Table 8.** Effects of dietary fiber source and starch level on rumen bacteria at the genus level (%)

| Fiber sources | Beet pulp | | | Soybean hulls | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Starch levels (%) | 27 High | 25 Medium | 22 Low | 27 High | 25 Medium | 22 Low | SEM | Fiber sources | Starch levels | Interaction |
| *Acetitomaculum* | 0.57 | 0.66 | 0.29 | 0.29 | 0.54 | 0.53 | 0.094 | 0.779 | 0.686 | 0.537 |
| *Butyrivibrio_2* | 0.32 | 0.85 | 1.58 | 0.58 | 1.10 | 1.83 | 0.181 | 0.479 | 0.020 | 1.000 |
| *Candidatus_Saccharimonas* | 0.42 | 0.69 | 0.24 | 0.79 | 1.71 | 1.25 | 0.158 | 0.011 | 0.248 | 0.606 |
| *Christensenellaceae_R-7* | 3.37 | 6.42 | 5.31 | 8.11 | 5.74 | 12.68 | 0.843 | 0.020 | 0.212 | 0.121 |
| *Erysipelotrichaceae_UCG-009* | 0.14 | 0.63 | 1.14 | 0.74 | 0.91 | 0.55 | 0.130 | 0.710 | 0.393 | 0.172 |
| *Fibrobacter* | 8.44 | 8.20 | 12.47 | 2.87 | 5.48 | 6.86 | 1.011 | 0.023 | 0.259 | 0.791 |
| *Lachnospiraceae_ND3007* | 0.79 | 0.16 | 0.08 | 0.27 | 0.21 | 0.13 | 0.077 | 0.344 | 0.053 | 0.201 |
| *Lachnospiraceae_NK3A20* | 2.88 | 3.64 | 3.20 | 2.15 | 7.98 | 4.19 | 0.564 | 0.162 | 0.042 | 0.146 |
| *Olsenella* | 1.19 | 2.04 | 1.29 | 1.32 | 2.07 | 0.35 | 0.227 | 0.568 | 0.086 | 0.586 |
| *Prevotella_1* | 13.65 | 6.52 | 17.99 | 8.67 | 8.93 | 8.66 | 1.172 | 0.085 | 0.138 | 0.112 |
| *Prevotellaceae_UCG-001* | 2.08 | 1.22 | 1.99 | 1.42 | 1.18 | 1.27 | 0.171 | 0.170 | 0.385 | 0.660 |
| *Rikenellaceae_RC9* | 3.37 | 2.11 | 2.69 | 1.80 | 3.24 | 2.09 | 0.389 | 0.669 | 0.957 | 0.362 |
| *Ruminococcaceae_NK4A214* | 1.41 | 3.58 | 3.13 | 4.51 | 4.52 | 5.43 | 0.536 | 0.051 | 0.561 | 0.697 |
| *Ruminococcaceae_UCG-014* | 3.56 | 1.92 | 3.59 | 3.20 | 3.12 | 1.15 | 0.409 | 0.518 | 0.556 | 0.206 |
| *Ruminococcus_1* | 3.21 | 2.89 | 2.39 | 5.71 | 7.30 | 5.17 | 0.640 | 0.013 | 0.705 | 0.796 |
| *Succiniclasticum* | 1.87 | 2.53 | 2.35 | 3.55 | 4.09 | 5.70 | 0.494 | 0.028 | 0.559 | 0.716 |
| *Treponema_2* | 4.26 | 2.94 | 3.59 | 1.67 | 4.23 | 3.57 | 0.350 | 0.524 | 0.698 | 0.067 |
| *Unclassified* | 3.00 | 2.80 | 1.97 | 4.16 | 2.09 | 2.31 | 0.336 | 0.698 | 0.190 | 0.515 |

SEM = standard error of the sample mean.

propionate and the ratio of acetate to propionate in our study. The soluble NDF was high in SH and BP, and NDF digestibility was similar between fiber sources. The proportion of butyrate was greater in lambs fed BP than in lambs fed SH. Abo-Zeid (2017) also found similar results; substituting sugar BP for maize grains increased the molar proportion of butyrate in growing buffalo calves. However, the abundance of *Butyrivibrio_2*, which was the main producer produced butyrate, was not affected by fiber source in our study. An explanation that pectin of BP could stimulate butyrate-producing *P. brevis* activity and protozoan activity (Nograsek et al., 2015).

In this experiment, a high starch level increased the concentrations of propionate and valerate and decreased the concentration of acetate and the ratio of acetate to propionate. The results from the present study are in accordance with those of previous studies by Lechartier and Peyraud. (2011). The molar proportion of propionate increased when lambs were fed higher starch contents due to the increased activity of amylolytic bacteria. In our study, the populations of amylolytic bacteria (*R. amylophilus* and *S. bovis*) increased with increasing starch level. However, De Souza (2017) found that increasing the concentrate in the diets of beef cattle decreased *S. bovis* abundance and increased *R. amylophilus* abundance. Others reported that *R. amylophilus* and *S. bovis* have a preference for the degradation of NFC and starch (de Souza et al., 2017). This difference suggested that the *S. bovis* abundance was related to NFC sources other than starch. The concentration of acetate decreased when lambs were fed more starch, because higher starch improved the efficiency of fermentation and reduced the activity of cellulolytic bacteria (Hernandez et al., 2017). Feeding lambs with the high starch level decreased the ratio of acetate to propionate. This is generally observed when sheep are shifted from a high-forage to a high-grain diet or increasing grain diets (Christophersen et al., 2008).

As expected, the *P. brevis* was greater in the BP group than in the SH group. An explanation was that *Prevotella* spp. were the principal rumen pectin-utilizing bacteria and that the higher pectin in BP than in SH enhanced the biomass and activity of *P. brevis* (Marounek and Dušková, 1999).

In the present study, *Firmicutes* was the most abundant phylum, followed by *Bacteroidetes* and *Fibrobacteres*, which was consistent with the results of previous studies (Guo et al., 2019). *Firmicutes* abundance was increased in lambs fed SH, whereas *Bacteroidetes* abundance was increased in lambs fed BP. Wang et al. (2018) found that the replacement of forage fiber with nonforage fiber sources in vitro decreased *Firmicutes* and increased *Bacteroidetes*. Castillo-Lopez et al. (2017) reported

that feeding distiller grains to dairy cattle reduced *Bacteroidetes* and tended to increase *Firmicutes*. These differences due to *Firmicutes* were associated with the plant-adherent fraction and the degradation of starch and were also affected by the choice of forage source (Brulc et al., 2009; Witzig et al., 2010; El Kaoutari et al., 2013). *Bacteroidetes* abundance was greater in lambs fed BP because *Bacteroidetes* prefer noncellulosic plant constituent degraders and xylan degradation (Dodd et al., 2011; Thoetkiattikul et al., 2013). *Fibrobacteres* are dynamic and vary according to the fiber content of the diet (Thoetkiattikul et al., 2013), and Sun et al. (2008) reported that *Fibrobacteres* (such as *F. succinogenes*) are the major agents of cell-wall pectin degradation in the rumen. These findings may be explained by the observation that *Fibrobacteres* was increased in lambs fed BP in the present study. *Proteobacteria* include diverse bacterial taxa capable of catabolizing a wide range of dietary components (Evans et al., 2011). Auffret et al. (2017) found that a concentrate-based diet increased *Proteobacteria*. We also found the feeding a high starch level and BP increased *Proteobacteria*. This means that higher starch content and digestible fiber (e.g., pectin) had a tendency to increase *Proteobacteria.*

In this study, at the genus level, *Butyrivibrio_2* declined with increasing starch level. *Butyrivibrio* species mainly produce butyrate, and they are as effective hemicellulose degraders (Meehan and Beiko, 2014). However, this finding was not consistent with the proportion of butyrate and abundance of *Butyrivibrio_2*. These differences may be explained by the low rumen pH at high starch levels inhibiting *Butyrivibrio_2* in our study. Previous studies reported that some genera of *Lachnospiraceae* are associated with starch-degrading alpha-glucosidases and phosphorylases and produce short-chain fatty acids (Biddle et al., 2013; Meehan and Beiko, 2014). In the present study, *Lachnospiraceae_ND3007* increased with increasing starch level, while in contrast, the abundance of *Lachnospiraceae_NK3A20* declined with increasing starch level. The results indicated that *Lachnospiraceae_ND3007* and *Lachnospiraceae_NK3A20* were related to starch content. Compared with lambs fed BP, lambs fed SH increased in *Candidatus_saccharimonas* and *Christensenellaceae R-7*. Other studies have shown that *Candidatus_saccharimonas* and *Christensenellaceae R-7* had positive correlations with the metabolites involved in amino acid biosynthesis and metabolism of energy substrates (Ogunade et al., 2019). This result may be explained by the fact that lambs fed SH tended to have greater CP digestibility than lambs fed BP in our study. Previous studies showed that various microbes in the family *Ruminococcaceae* are typical fiber degrading bacteria (Kang et al., 2017), and *Succiniclasticum* can degrade succinic acid produced by rumen fiber decomposition into propionic acid, thus promoting the decomposition of fiber (Wang et al., 2019). *Succiniclasticum* and *Ruminococcus_1* were greater in lambs fed SH than in lambs fed BP in our study. This result may be explained by the fact that lambs fed SH had increased the NDF digestibility than lambs fed BP, which increased *Succiniclasticum* and *Ruminococcus_1*.

## CONCLUSIONS

In this study, feeding the high starch diets increased the proportions of propionate and valerate and decreased the proportion of acetate and the ratio of acetate to propionate. Lambs fed SH increased in ADF digestibility and the rumen pH. In addition, the fiber sources mainly changed the abundances of *P. brevis*, *R. amylophilus*, and *S. bovis* in the rumen community, whereas high starch levels increased the abundance of *S. bovis*. Fiber sources with different starch levels mainly changed the rumen community in terms of the phylum and genus abundances. Lambs fed SH with low starch level increased the final BW without affecting TVFA concentrations. Partial replacement forage for SH in low-starch diets can increase lambs' performances.

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*Conflict of interest statement*. We declare that we have no financial and personal relationships with other people or organizations.

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