Effects of forage rape at various levels on growth performance, carcass traits, meat quality, rumen fermentation and rumen microbiota of Hu lambs

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Research

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Effects of forage rape at various levels on growth performance, carcass traits, meat quality, rumen fermentation and rumen microbiota of Hu lambs

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Abstract

Background: The objective of this study was to investigate the effects of forage rape (*Brassica napus*) in total mixed ration (TMR)-based diet on growth performance, carcass traits, meat quality, rumen fermentation and rumen microbiota of Hu lambs.

Methods: A total of 50 Hu lambs (20.86 ± 3.00 kg, three-month-old) were randomly allocated into five dietary treatments: Ctrl, T1, T2, T3 and T4 with 0, 10%, 20%, 30% and 40% of forage rape, respectively. Each treatment had 10 replicates of one lamb each and the study lasted for 60 days.

Results: The results showed that T2, T3 and T4 increased the average daily gain (ADG) and decreased the feed conversion ratio (FCR) significantly compared with Ctrl and T1 ($P < 0.05$). Moreover, the final body weight and ADG were increased, and the FCR were decreased linearly ($P < 0.05$) along with increasing forage rape levels. The relative weight of liver was increased in T3 and T4 compared with Ctrl ($P < 0.05$). With increasing forage rape levels, the relative content of intramuscular heptadecenoic acid and α-linolenic acid, and the composition of various amino acids in the muscle of lambs were increased linearly ($P < 0.05$), while ruminal concentration of ammonia nitrogen was decreased linearly ($P < 0.05$). No difference on carcass traits, meat quality or ruminal profile of short-chain fatty acids were observed among groups ($P > 0.05$). In addition, the inclusion of forage rape altered the rumen microbial community, and increased the relative abundance of cellulolytic bacteria and short-chain fatty acid producers, including genera *Family_XIII_AD3011_group* and *Anaerovorax* in T1,

Succiniclasticum and *Fibrobacter* in T2, *Ruminiclostridium_5* in T3, and members of family *Lachnospiraceae* and genus *Shuttleworthia* in T4.

Conclusion: TMR included with forage rape could improve growth performance, meat nutritional value and rumen microbial community of Hu lambs.

Keywords: forage rape, lamb, growth performance, meat quality, rumen, microbiota

1. Background

Forage rape (*Brassica napus*) is an oil crop grown worldwide. Apart from being used as an oil crop, forage rape has become part of the forage system for ruminants grazing on pasture in Europe, Australia and New Zealand [1]. In southern China, there is a long-term shortage of high-quality forage, which challenges the development of ruminant breeding. The high humidity climatic conditions and acidic soil conditions are extremely detrimental to the growth of alfalfa, but it is conducive to the growth of forage rape. According to previous studies, the content of crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) based on dry matter (DM) of forage rape was 17~36%, 13~16%, 54~57% and 32~35%, respectively during the flowering period [2]. In addition, the rumen degradation rate of the main nutrients is quite high [3-5]. Therefore, forage rape is gradually being used as a high-quality forage for ruminants in Southern China in recent years [2].

Actually, forage rape has been found to support the rapid growth of ruminants. When fed as the sole diet, the average growth of young sheep on forage rape was 225 g/d, which was much higher than on kale and swedes (120 g/d and 95 g/d, respectively) [6]. As a supplementation, forage rape was reported to reduce the acetate-to-propionate ratio and energy losses in the rumen, mainly methane emissions, resulting in improved feed efficiency [7].

However, to the best of our knowledge, no study has been conducted to explore the utilization of forage rape in the form of total mixed ration (TMR) pellets, and little data

58 are available describing the effect of forage rape on meat quality, meat nutritional level
59 or rumen microbial communities. Therefore, the objective of the present study was to
60 investigate the effects forage rape at various levels on growth performance, carcass
61 traits, meat quality and nutritional level, rumen fermentation and rumen microbiota of
62 Hu lambs, which will provide scientific basis for the efficient use of forage rape.

63 **2. Materials and methods**

64 **2.1 Animals, diets and management**

65 A total of 50 healthy Hu lambs (three-month-old; castrated male lambs; average
66 bodyweight = 20.86 ± 3.00 kg) were used in the feeding trial. The trial was conducted
67 at Tianyao Animal Husbandry Company in Hubei Province. The lambs were randomly
68 divided into five groups (Ctrl, T1, T2, T3 and T4) and fed diets with 0, 10%, 20%, 30%
69 and 40% of forage rape, respectively. The forage rape used in the present study were
70 harvested at the stage of full bloom, and chopped to 2-cm pieces and air-dried. The
71 nutritional composition of forage rape hay was 17.30% CP, 4.24% ether extract, 40.1%
72 NDF, 37.8% ADF and 14.3% crude ash. Glucosinolates, the anti-nutritional factors of
73 forage rape were 10.17 μmol/g based on DM.

74 The diets were processed into pellets with a particle size of 5 mm using a flat diet
75 pelletizer (DL-150, LongChang, China). The composition of the diets and nutritional
76 values are listed in Table 1.

77 Before starting the trial, 8-day adaptation period was given to get animals adjusted
78 to various diets. The feeding trial lasted for 60 days. Thereafter, all lambs were housed

in individually pens, fed three times per day at 08:00, 14:00 and 20:00 and had free access to feed and water. The ambient temperature range was 21° C to 38° C during the experiment. Feed intake was recorded daily. Body weight (BW) was recorded before morning feeding in the beginning of the formal trial (initial BW) and at the end of the trial before sacrifice (final BW). Average daily gain (ADG), dry matter intake (DMI) and feed conversion ratio (FCR) were calculated.

2.2 Carcass traits and sample collection

At the end of the experiment, lambs were fasted for 24 h before sacrifice. Then five lambs with similar BW per group were randomly selected and sacrificed. At slaughter, hot carcass weight (HCW) was recorded after evisceration, which was used to assess the dressing percentage (HCW × 100/slaughter BW). Weights of liver, kidney and thyroid were also recorded to calculate their relative organ indexes. The outline of the *longissimus dorsi* (LD) muscle between the 12th and 13th rib was traced onto acetate paper to calculate the area. Tissue samples of LD muscle and *biceps femoris* muscle were collected from the left side of the carcass and stored at 0-4° C to measure the meat quality (pH, cooked meat rate and water loss rate) and meat nutritional value.

In addition, 100 mL of ruminal content samples were sterilely collected immediately after slaughter and squeezed through four layers of cheesecloth to remove particulate matter. Half of the remaining ruminal fluid was stored at -80° C for DNA extraction. Two drops of saturated mercury dichloride ($HgCl_2$) solution were added to the other half of the ruminal fluid to inactivate the rumen microorganisms, and supernatant was

collected after centrifugation at 15000 × g/min at 4° C for the analysis of ruminal fermentation parameters.

2.3 Meat quality measurements and nutritional analysis

The pH of the LD muscles was determined 45 min and 24 h after slaughter (maintained at 0-4° C) using a portable pH meter equipped with penetrating electrode (D-51, Horiba Ltd., Kyoto, Japan). Samples of *biceps femoris* muscle (100 g ± 5 g) were weighted and cooked in boiling water for 30 min. After cooling for 30 min, the cooked samples were blotted dry and weighed to calculate the cooked meat rate (post-cooking weight/pre-cooking weight × 100%). Filter paper press method was applied to determine the water loss rate. Briefly, a slice of LD muscle was sampled using a sampler with a diameter of 2.5 cm, and the thickness of the slice was 1 cm. After weighing (W1), the slice was placed on 18 pieces of medium-speed qualitative filter paper which was placed between glass plates and subjected to a fixed weight of 35 kg for 5 min. Then the slice was weighed again (W2) to calculate the water loss rate ((W1 – W2)/W1 × 100%).

One portion of LD muscles was used for proximate analysis including moisture, CP, crude fat and crude ash according to Association of Analytical Chemists methods (AOAC, 2000). The other portion of LD muscles were freeze-dried for the evaluation of intramuscular fatty acid composition and amino acid composition. Fatty acids were converted to methyl esters by transesterification and then analyzed by gas chromatography (Agilent 7890A FID, Agilent Technologies, CA, US) on a fused silica capillary column (Agilent, DB-23, 30 m × 0.32 m). Injector and detector temperatures

were of 180° C and 280° C, respectively. For each fatty acid, the results were expressed as a percentage of the total fatty acids. The amino acid composition was analyzed following the instruction of GB/T5009.124–2016 (Standardization Administration of the People's Republic of China). Briefly, crushed samples were added to 10-15 mL 6 M HCl with 3–4 drops of phenol. After hydrolyzation for 22 h at 110 ± 1° C under nitrogen, the samples were filtrated and 1 ml of supernatant was evaporated in a vacuum drying oven at 40–50° C, and re-dissolved in 1 ml of saline sodium citrate (pH = 2.2). Then, the amino acid composition was determined using an automatic amino acid analyzer (L-8800, HITACHI Ltd., Tokyo, Japan). The standard column was 4.6 mm × 60 mm, and the temperatures of the reactive column and reactor part were 57° C and 136° C, respectively.

2.4 Ruminal fermentation parameters

The pH of the ruminal fluid supernatant was determined with the pH meter mentioned above. The content of ammonia nitrogen was analyzed with the method of phenol-sodium hypochlorite colorimetric [8]. For short-chain fatty acid (SCFA) analysis, gas chromatography was applied using Dionex ICS-3000 ion chromatograph, and the analytical column was IonPac AS-HC separation column (4 mm × 250 mm) with IonPac AG 11-HC guard column (4 mm × 50 mm).

2.5 DNA extraction, PCR amplification of 16S rRNA gene and sequencing

16S rRNA gene of rumen microbiota was sequenced and analyzed as described by Du et al. [9] earlier. Briefly, microbial genomic DNA was extracted from five randomly

selected ruminal samples each group using the QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, CA, US) according to the manufacturer's instructions. Then, the concentration of DNA was determined using spectrophotometry and its quality was evaluated using 2% agarose gel electrophoresis. The microbial 16S rRNA gene was amplified using the universal primer sets 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with a unique error-correcting barcode for each sample. The barcoded amplicons were visualized using 2% agarose gel electrophoresis and purified using the AxyPrep DNA Gel Extraction kit (Axygen Inc., CA, US). The purified amplicons were pooled in equal concentrations and sequenced using the pair-end method on Illumina Miseq250 platform.

For 16S rRNA gene analyzing, the obtained raw reads were firstly merged into sequences based on the relationship among their overlaps, and poor or low-quality sequences were discarded. Then the obtained sequences were aligned into operational taxonomic units (OTUs) analysis using the software VSEARCH (version 1.9.6) based on 97% sequence similarity. The alpha diversity of the rumen microbiota was estimated using the number of Chao1, Ace, Shannon and Simpson indices implemented in QIIME (version 1.9.1). The principal coordinate analysis (PCoA) based on weighted unifrac distance was performed to represent the beta diversity, which illuminate the species complexity of rumen microbial community. The representative OTU sequences were then compared with the Silva_132 16S rRNA database (http://www.arb-silva.de/) using

RDP Classifier for taxonomic classification (at 80% confidence threshold) at the kingdom, phylum, class, order, family, and genus levels.

2.6 Statistical Analysis

Results were expressed as treatment means and pooled standard error of the mean (SEM). Phenotypic data (growth performance, carcass traits, meat quality and nutritional value, and ruminal fermentation parameters) and the alpha diversity indexes of the rumen microbiota were analyzed using one-way ANOVA and Duncan's multiple comparison test by SPSS 21.0 software (IBM Inc., NY, US). Polynomial contrasts were performed to determine the linear and quadratic effects of increasing dietary forage rape on the measured traits. $P \leq .05$ was considered significant. For the relative abundances of rumen bacteria, LEfSe analysis was utilized to determine the difference, and a significant change was observed with an LDA (linear discriminant analysis) score > 2.0 calculated by LEfSe.

3. Results

3.1 Growth performance and carcass traits

The dietary inclusion level of forage rape had no influence on the DMI of lambs (P = 0.855), but group T2, T3 and T4 increased the final BW and ADG significantly compared with Ctrl ($P < 0.05$), resulting in remarkably decreased FCR ($P < 0.05$) as shown in Table 2. In addition, the final BW ($P = 0.005$) and ADG ($P < 0.001$) increased linearly with increasing forage rape levels, and FCR decreased both linearly ($P < 0.001$) and quadratically ($P < 0.001$) with increasing forage rape levels.

There was no difference in the dressing percentage or LD area among the five dietary treatment groups ($P > 0.10$). However, the liver index was increased significantly in group T3 and T4 ($P < 0.05$), and the liver index, the slaughter weight and HCW of the lambs exhibited a linear increase with increasing forage rape levels ($P = 0.003$, 0.012 and 0.036, respectively). A quadratic effect of forage rape levels was found on the index of kidney ($P = 0.029$), and group T2 and T3 showed the highest kidney index.

3.2 Meat quality and nutritional value

For lambs fed diets with various levels of forage rape, no difference was observed in the pH value, water loss rate, cooked meat rate or proximate nutrition (moisture, CP, crude fat and crude ash) of the meat ($P > 0.10$, Table 3).

Compared with Ctrl and T1, the relative content of intramuscular heptadecenoic acid (C17:1n-7) in the LD muscle was increased in T3 and T4 ($P < 0.05$, Table 4), and the relative content of α-linolenic acid (C18:3n-3) was increased in T2, T3 and T4 ($P < 0.05$). In addition, the relative content of heptadecenoic acid and α-linolenic acid showed a linear increase with increasing forage rape levels ($P < 0.001$), while a quadratic effect of forage rape levels was found on the relative content of heptadecenoic acid ($P = 0.047$). Overall intramuscular SFAs (saturated fatty acids), MUFAs (monounsaturated fatty acids) and PUFAs (polyunsaturated fatty acids) contents were not affected by feed ($P > 0.10$). In the current study, the contents of two long-chain omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, were too low to be detected.

No difference in the composition of amino acid was found in the LD muscle among the five dietary treatment groups ($P > 0.05$, Table 5). However, with increasing forage rape levels, the contents of valine, threonine, leucine, isoleucine, methionine, arginine, and umami amino acids (aspartic acid and glutamic acid) were increased linearly ($P < 0.05$).

3.3 Ruminal fermentation parameters

The ruminal fluid maintained a normal pH value (6.8 to 7.2, Table 6) for lambs fed different diets, and the level of forage rape did not influence the ruminal pH value, the concentration of total SCFA or the profile of SCFA ($P > 0.10$). However, the concentration of ammonia nitrogen was found to be decreased linearly with increasing forage rape levels ($P = 0.019$).

3.4 Rumen microbiota

In the present study, an average of 176777 raw reads were obtained from the rumen microbiota, and an average of 88388 clean reads were remained. The rarefaction curves of all the samples were nearly asymptotic (Supplementary Figure S1), indicating that the depth of sequencing covered most of the microorganisms in the sample. In regard to the alpha diversity, no difference was observed among the five groups except that Chao1 and Ace indices were decreased in T1 compared with Ctrl ($P < 0.05$, Figure 1). In addition, a quadratic effect of forage rape levels was found on Chao1 and Ace indices ($P = 0.048$ and 0.024, respectively). According to the PCoA analysis (Figure 2), the rumen microbial communities were closely clustered according to different dietary

225 treatments, and the principal coordinates 1, 2 and 3 accounted for 14.23%、10.36 and
226 8.01% of the total variation, respectively.

227 Overall, 17 phyla were detected, with 10 of them identified in the rumen of all the
228 lambs (Supplementary Figure S2). *Bacteroidetes* and *Firmicutes* accounted for
229 approximately 92.5% of total sequences, dominating across all rumen microbial
230 communities. *Proteobacteria*, *Patescibacteria*, *Spirochaetes*, *Tenericutes* and
231 *Euryarchaeota* were less abundant, accounting for 0.1–10% of the total sequences. A
232 total of 170 genera were detected at the genus level, with the top 30 most abundant
233 genera shown in Supplementary Figure S3. The most abundant genus was
234 *Rikenellaceae_RC9_gut_group* (13.2-18.2%), *Prevotella_1* (10.0-22.2%) and
235 unclassified genus (7.5-14.8%), followed by *Succiniclasticum* (3.2-6.4%),
236 *f__F082_Unclassified* (3.1-8.7%) and *Christensenellaceae_R-7_group* (2.5-3.9%),
237 while the specific rank of each genus varied across different groups.

238 Based on the LEfSe analysis (Figure 3), the most differentially abundant bacterial
239 taxa in Ctrl belong to the genera *Quinella* and *Anaeroplasma*, while genera
240 *Family_XIII_AD3011_group*, *Anaerovorax*, *Clostridium_sensu_stricto_1*, *Olsenella*
241 and *Straphylococcus* were more abundant in T1, genera *Succiniclasticum*, *Fibrobacter*,
242 *Lachnospiraceae_FCS020_group*, *Eubacterium_uniforme* and *Pyramidobacter* were
243 more abundant in T2, genera *Ruminiclostridium_5* was more abundant in T3, and
244 genera *Schwartzia*, *Lachnospiraceae_UCG_008*, *Lachnospiraceae_AC2044_group*,
245 *Blautia*, *Shuttleworthia* were mostly relatively abundant in T4. The genera *Quinella*,

246 *Family_XIII_AD3011_group* and *Succiniclasticum* were the taxa that weighted most to
247 the differences among the communities, with an absolute LDA score more than 3.

248 **4. Discussion**

249 Owing to the fast growth rate and high nutritional value of forage rape, it has been
250 proposed as a promising feed crop for ruminants, which partially alleviates the lack of
251 high-quality forage in Southern China [2, 3]. To our knowledge, this was the first study
252 to systemically evaluate the application effect of forage rape at various levels on sheep
253 in the form of TMR pellets.

254 Under the conditions of this study, Hu lambs fed diets with 20-40% of forage rape
255 had greater final BW, ADG and feed conversion efficiency than lambs fed diets without
256 or with 10% of forage rape. Since DMI was not affected, the result implied greater feed
257 efficiency of forage rape than peanut vine, which promoted the growth of lambs. Apart
258 from high rumen degradation rate of the main nutrients [3, 10], sheep and cattle fed
259 forage rape were found to emit less enteric methane, resulting in less energy loss [7].

260 Glucosinolates are main anti-nutritional factors in forage rape and rapeseed, which
261 restrict their utilization as feed resources. Compared with rapeseed, the content of
262 glucosinolates is lower in vegetative tissues, like root, stem and leaf [11]. Besides,
263 ruminants have relatively stronger tolerance capability to glucosinolates [12]. In our
264 study, the glucosinolates concentration of diet included with 40% of forage rape was
265 4.07 μmol/g, within the tolerance threshold of lambs. Therefore, up to 40% of forage
266 rape in TMR had no adverse effect on the growth performance, carcass traits or the

267 index of thyroid of Hu lambs in the present study. However, the index of liver was
268 elevated when 30-40% of forage rape was included in TMR, which might be mild
269 hepatic edema caused by glucosinolates metabolites, like nitriles [12].

270 Nowadays there is increasing consumer demanding for high-quality meat products
271 [13]. Although animal genetics is an important determinant of meat quality and flavor,
272 the diet can have direct and indirect effects on meat sensory properties. The ultimate
273 pH and water holding capacity affect the tenderness of meat. According to the present
274 study, up to 40% of forage rape did not affect the pH value, water loss rate, cooked
275 meat rate or the proximate nutrition of lamb.

276 Fatty acids and amino acids are considered key factors to determine the nutritional
277 value and flavor of lamb, so we further investigated the effect of forage rape on the
278 composition of intramuscular fatty acids and amino acids. According to Frank et al.
279 [14], the content of α-linolenic acid in the grilled loins was increased when forage rape
280 was used as finishing feed for lambs compared with ryegrass. In the current study, the
281 relative content of α-linolenic and heptadecenoic acid were increased when lambs were
282 fed 20-40% of forage rape. Despite that, the predominant SFAs were palmitic (C16:0)
283 and stearic (C18:0) acids, and the predominant MUFA was oleic acid (C18:1n-9c) for
284 lambs fed different diets, consistent with previous results [14, 15]. And the predominant
285 fatty acids and overall SFAs, MUFAs and PUFAs were not influenced by diets. In the
286 present study, the amino acid composition was not affected by the dietary treatments.
287 However, five kinds of essential amino acid (EAA) and three kinds of non-essential

amino acid (NEAA) showed a linear increase with increasing forage rape levels. The percentage of amino acids producing the tastes of umami was also increased linearly. The EAA and NEAA requirements of an adult man are 0.18 g/kg per day and 0.48 g/kg per day, respectively, which equals EAA/NEAA = 37.5%. In this study, the mean ratios of EAA/NEAA of the LD muscle were 74-76%, which were much higher than those recommended by FAO/WHO/UNU [16], therefore lamb appears to be an excellent source of protein.

Generally, the ruminal concentration of ammonia nitrogen is a reflection of the balance statues of protein degradation and microbial protein synthesis [17]. When lambs were fed different diets, their ruminal ammonia nitrogen remained the optimal concentration (6 to 21 mmol/L, i.e. 8.4 to 29.4 mg/100 mL) as proposed by McDonald et al. [18]. Interestingly, the concentration of ruminal ammonia nitrogen was decreased linearly with increasing forage rape levels, implying a possible linear increase in nitrogen utilization, which is in accordance with the linear increase in the growth performance of lambs. According to the present study, increasing forage rape levels from 0 to 40% did not affect the profile of SCFA, suggesting that the ruminal fermentation pattern of the diets was unaffected by the inclusion of forage rape.

Although a few investigations have evaluated the nutritional properties of forage rape used in ruminants [3, 4, 6, 7], less information is available on the rumen microbiota. In the current study, the divergence of the rumen microbial communities of Hu lambs fed different diets confirmed that rumen microbiota could response to changes in the diets.

When lambs were fed diets with 10% of forage rape, the community richness of rumen microbiota decreased since Chao1 and Ace indices were reduced, but the community diversity remained unchanged since similar Shannon and Simpson indices were observed compared with group Ctrl.

Out of the eighteen significantly abundant genera with LDA score larger than two, *Quinella* is a propionate-producing bacterium [19]. Some members of the genus *Quinella* were found in the rumen of sheep and steers fed diets with molasses, and the presence of *Quinella* was increased in growing lambs with ruminal acidosis caused by smaller grinded grains [20]. The functional role of *Quinella* in the rumen fermentation of group Ctrl are yet to be explored. Compared with low-production dairy cows, the rumen fluid of high-production dairy cows was depleted of *Anaeroplasma* in *Tenericutes* [21]. As an opportunistic pathogen, the relative absence of *Anaeroplasma* in lambs fed forage rape might reduce the probability of inflammation in the rumen epithelium [22].

Dietary inclusion of 10% of forage rape promoted the abundance of *Family_XIII* (from family to genus) and several strict anaerobes. *Family_XIII* was commonly found in gastrointestinal tract of animals, and many strains have been implicated in the production of butyrate that exerts important pleiotropic functions [23]. *Anaerovorax*, *Clostridium_sensu_stricto_1* and *Olsenella* are anaerobes producing acetate, butyrate and lactic acid [24-26]. The genera *Succiniclasticum*, *Fibrobacter*, *Lachnospiraceae_FCS020_group* and *Eubacterium_uniforme* were more abundant in

lambs fed 20% of forage rape. *Succiniclasticum* is a ruminal bacterium converting succinate to propionate as the sole energy-yielding mechanism， therefore it cooperates with cellulolytic bacteria in the rumen. *Fibrobacter* (from phylum to genus) is highly efficient in degrading crystalline cellulose, and shows a high ability to solubilize plant cell wall polysaccharides [27]. Members in the family *Lachnospiraceae* and the genus *Eubacterium_uniforme* are cellulolytic bacteria capable of producing butyrate. When 30% of forage rape was included in the diet of lambs, the genus *Ruminiclostridium_5* was found to be abundant in the rumen. *Ruminiclostridium* can effectively utilize cellulose, cell wall polysaccharides and raw lignocellulose feedstocks to improve the digestibility of feed nutrition [28]. Similar with *Succiniclasticum*, the genus *Schwartzia* is another succinate-specific bacteria and propionate producer [29]. In addition, 40% of forage rape promoted the abundance of several butyrate-producing bacteria, including *Lachnospiraceae UCG 008*, *Lachnospiraceae AC2044 group*, *Blautia (also from* the family *Lachnospiraceae)* and *Shuttleworthia.* Since the rumen fluid was collected after 24 hours of fasting, we did not observe a higher butyrate or propionate concentration in the current study. However, it seemed that feeding of animals with forage rape facilitated the growth of SCFA producers in the rumen and enhance the ruminal fibrolytic function, thus elevated the ADG and feed conversion efficiency of lambs.

5. Conclusion

In conclusion, increasing dietary inclusion of forage rape from 0 to 40% improved the growth performance and increased the content of α-linolenic acid and a variety of amino

acids in the muscle of Hu lambs linearly, while no detrimental impact on carcass traits, meat quality or ruminal fermentation parameters was observed. Though limitations of our study included that the maximum inclusion level of forage rape was 40%, the linear effects indicate that better results could be expected with higher levels of forage rape. Furthermore, this study also provides the first evidence that the inclusion of forage rape altered the rumen microbial community, and increased the relative abundance of cellulolytic bacteria and SCFA producers, including *Family_XIII*, *Lachnospiraceae* and genera *Succiniclasticum*, *Fibrobacter*, *Ruminiclostridium_5*, etc. Taken together, our findings suggest that TMR pellets included with forage rape are recommended for lambs to improve growth performance, meat nutritional value and rumen microbial community.

Abbreviations

ADF: Acid detergent fiber; ADG: Average daily gain; BW: Body weight; CP: Crude protein; DM: Dry matter; DMI: Dry matter intake; EAA: Essential amino acid; FCR: Feed conversion ratio; HCW: Hot carcass weight; LD: *Longissimus dorsi*; LDA: Linear discriminant analysis；MUFA: Monounsaturated fatty acids; NDF: Neutral detergent fiber; NEAA: Non-essential amino acid; OTU: Operational taxonomic unit; PCoA: Principal coordinate analysis; PUFA: Polyunsaturated fatty acids; SCFA: Short-chain fatty acid; SEM: Pooled standard error of the mean; SFA: Saturated fatty acids； TMR: Total mixed ration.

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Authors' contributions

ED, WG and JW designed the research; ED, WG, NZ, FC, QF and JW performed the research and analyzed the data; ED wrote the manuscript; WZ, SH, GZ and TD participated in the sample collection and revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author by request. The datasets supporting the conclusions of this article are included in the article.

Ethics approval and consent to participate

The use of animals, including welfare, husbandry, experimental procedures, and the collection of samples used for this study, were conducted according to the principles of

the Animal Care and Use Committee of the Hubei Academy of Agricultural Sciences (Hubei, China), which approved the study protocol.

Consent for publication

Not applicable.

Competing interests

The authors declare that no competing interests exist. The manuscript has not been published previously.

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Figure 1. Alpha diversity of the rumen microbial community of lambs. **(a)** Chao1 and Ace indices. **(b)** Shannon and Simpson indices.

* Bars with asterisk differ significantly ($P < 0.05$).

Figure 2. Principal coordinate (PCoA) analysis based on weighted unifrac distance of the rumen microbial community.

Figure 3. Differences in the relative abundances of rumen microorganisms. **(a)** LEfSe cladogram of the microbial communities. Differences are represented in the color of the group, where taxa are most abundant. **(b)** Histogram of linear discriminant analysis (LDA) scores computed for each taxon ranging from phylum to genus.

Supplementary Figure S1. Rarefaction analysis of 16S rRNA gene sequences from the rumen microbiota of lambs.

Supplementary Figure S2. Relative abundance of rumen microorganisms at phylum level.

Supplementary Figure S3. Relative abundance of rumen microorganisms at genus level.

502 **Table 1.** Diet composition and nutrient levels (based on air-dried material)

Item (%, unless otherwise indicated)	Ctrl	T1	T2	T3	T4
Corn	30.9	33.5	35.2	36.9	39.6
Soybean meal	14.0	11.4	8.8	6.2	3.6
Forage rape	0.0	10.0	20.0	30.0	40.0
Peanut vine	52.0	42.0	33.0	24.0	14.0
Premix [1]	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	1.5	1.5	1.4	1.3	1.2
Salt	0.6	0.6	0.6	0.6	0.6
Total	100.0	100.0	100.0	100.0	100.0
Nutrient levels [2]					
Digestible Energy (MJ/kg)	10.86	10.90	10.90	10.89	10.94
Crude protein	12.70	12.70	12.71	12.70	12.71
Calcium	1.65	1.60	1.57	1.53	1.46
Total phosphorus	0.50	0.51	0.51	0.51	0.51
Neutral detergent fiber	27.29	26.74	26.56	26.38	25.84
Acid detergent fiber	16.25	17.14	18.27	19.40	20.29

503 [1] The premix provided the following (per kilogram of diet): iron, 80 mg; copper, 10 mg;

504 zinc, 50 mg; manganese, 30 mg; selenium, 0.30 mg; iodine, 0.80 mg; cobalt, 0.80 mg;

505 vitamin A, 10000 IU; vitamin D_3, 3000 IU; vitamin E, 50 mg.

506 [2] Calculated value for digestible energy, measured values for the rest.

507 **Table 2.** Effects of dietary forage rape levels on the growth performance and carcass
508 traits of lambs

Item	Ctrl	T1	T2	T3	T4	SEM	*P* value		
							ANOVA	Linear	Quadratic
Initial BW (kg)	20.65	20.98	20.95	20.95	20.76	0.43	0.999	0.952	0.787
Final BW (kg)	27.87^{b}	29.10^{ab}	32.34^{a}	33.17^{a}	32.35^{a}	0.67	0.036	0.005	0.217
ADG (g)	120^{b}	135^{b}	190^{a}	204^{a}	193^{a}	8	< 0.001	< 0.001	0.104
DMI (g)	1188	1332	1316	1364	1255	53	0.855	0.669	0.335
FCR	9.89^{a}	9.85^{a}	6.97^{b}	6.70^{bc}	6.51^{c}	0.23	< 0.001	< 0.001	< 0.001
Slaughter weight (kg)	29.30^{b}	29.50^{b}	33.18^{ab}	33.66^{a}	32.70^{ab}	0.64	0.052	0.012	0.251
HCW (kg)	14.01^{b}	14.73^{ab}	16.61^{a}	16.27^{a}	15.68^{ab}	0.34	0.077	0.036	0.073
Dressing percentage (%)	47.97	49.95	50.05	48.31	47.94	0.57	0.623	0.684	0.196
LD area (cm^2)	11.19	13.17	10.92	10.25	11.4	0.44	0.304	0.407	0.164
Liver index (%)	1.77^{c}	1.81^{bc}	2.04^{abc}	2.21^{a}	2.08^{ab}	0.05	0.019	0.003	0.259
Kidney index (%)	0.28	0.31	0.37	0.36	0.30	0.01	0.165	0.381	0.029
Thyroid index (%)	9.83	8.67	8.70	8.56	8.34	0.34	0.711	0.234	0.568

509 $^{a\text{-}c}$ Within a row, values with different superscript letters differ significantly ($P < 0.05$).
510 BW: body weight; ADG: average daily gain; DMI: average daily dry matter intake;
511 FCR: feed conversion ratio; HCW: hot carcass weight; LD: *longissimus dorsi*; SEM:
512 pooled standard error of the mean.

513 **Table 3.** Effects of dietary forage rape levels on the meat quality and nutritional value

514 of lambs

Item	Ctrl	T1	T2	T3	T4	SEM	*P* value		
							ANOVA	Linear	Quadratic
pH_{45min}	6.51	6.5	6.43	6.57	6.43	0.024	0.267	0.202	0.719
pH_{24h}	5.88	5.99	5.94	5.91	5.91	0.025	0.702	0.727	0.505
Water loss rate (%)	27.11	27.17	28.14	26.44	24.53	0.581	0.379	0.248	0.128
Cooked meat rate (%)	57.27	54.48	60	53.44	55.76	0.795	0.244	0.705	0.199
Moisture (%)	73.45	72.80	73.87	73.29	73.99	0.29	0.737	0.470	0.686
CP (%)	21.22	21.89	21.38	20.94	21.44	0.15	0.380	0.628	0.826
Crude fat (%)	4.24	4.46	3.67	4.93	3.62	0.33	0.730	0.758	0.730
Crude ash (%)	0.98	0.99	1.01	1.03	1.02	0.01	0.248	0.035	0.644

515 DM: dry matter; CP: crude protein; SEM: pooled standard error of the mean.

516 **Table 4.** Intramuscular fatty acid composition and groups of fatty acids of *longissimus*

517 *dorsi* (% of total fatty acids)

Item	Ctrl	T1	T2	T3	T4	SEM	*P* value		
							ANOVA	Linear	Quadratic
C10:0	0.18	0.14	0.13	0.15	0.15	0.01	0.204	0.423	0.047
C12:0	0.28	0.13	0.21	0.22	0.20	0.02	0.266	0.569	0.277
C14:0	3.44	2.75	2.77	3.21	2.80	0.15	0.524	0.462	0.458
C14:1n5	0.12	0.10	0.11	0.12	0.10	0.01	0.838	0.637	0.891
C15:0	0.41	0.32	0.42	0.45	0.43	0.02	0.300	0.238	0.721
C16:0	27.16	26.94	25.68	25.90	25.58	0.33	0.409	0.082	0.642
C16:1n-7	0.31	0.36	0.29	0.45	0.21	0.06	0.784	0.311	0.642
C17:0	1.13	1.02	1.24	1.23	1.23	0.03	0.132	0.064	0.965
C17:1n-7	0.56^{c}	0.54^{c}	0.62^{bc}	0.68^{ab}	0.75^{a}	0.02	0.003	< 0.001	0.226
C18:0	17.32	16.44	17.42	17.68	16.76	0.45	0.922	0.972	0.845
C18:1n-9c	38.42	39.92	38.54	39.94	40.20	0.48	0.676	0.321	0.943
C18:2n-6c	5.64	6.41	7.15	5.31	6.15	0.32	0.428	0.975	0.368
C20:0	0.12	0.11	0.13	0.14	0.12	0.01	0.376	0.527	0.376
C20:1	0.12	0.14	0.14	0.14	0.12	0.00	0.561	0.943	0.122
C18:3n-3	0.25^{c}	0.31^{c}	0.39^{b}	0.38^{b}	0.48^{a}	0.02	< 0.001	< 0.001	0.901
C22:0	0.44	0.39	0.49	0.44	0.45	0.03	0.896	0.735	0.913
C20:3n-6	0.18	0.19	0.25	0.16	0.18	0.02	0.517	0.809	0.349
C20:4n-6	1.99	2.00	2.20	1.53	1.73	0.15	0.719	0.394	0.732
SFAs	52.74	50.28	50.98	51.88	50.18	0.51	0.478	0.343	0.693
MUFAs	41.46	42.85	41.61	43.24	43.54	0.52	0.646	0.243	0.881
PUFAs	8.06	8.91	9.99	7.38	8.54	0.48	0.531	0.869	0.457

518 $^{a\text{-}c}$ Within a row, values with different superscript letters differ significantly ($P < 0.05$).

519 SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated

520 fatty acid; SEM: pooled standard error of the mean.

521 **Table 5.** Amino acid composition and groups of amino acids of *longissimus dorsi* (%

522 based on DM)

Item	Ctrl	T1	T2	T3	T4	SEM	*P* value		
							ANOVA	Linear	Quadratic
Essential AA									
Lysine	3.38	3.84	3.67	3.63	4.04	0.08	0.136	0.056	0.950
Valine [3]	1.99	2.25	2.17	2.13	2.41	0.05	0.095	0.040	0.786
Threonine [2]	1.75	1.97	1.88	1.86	2.10	0.04	0.097	0.042	0.731
Histidine [3]	1.22	1.49	1.3	1.28	1.47	0.04	0.103	0.305	0.979
Leucine [3]	3.24	3.63	3.48	3.43	3.89	0.08	0.105	0.047	0.678
Isoleucine [3]	1.92	2.16	2.08	2.05	2.32	0.05	0.108	0.042	0.792
Methionine [3]	1.07	1.2	1.14	1.14	1.29	0.03	0.112	0.047	0.670
Phenylalanine [3]	1.94	2.23	2.04	2.02	2.29	0.05	0.140	0.154	0.777
Total essential AA	16.51	18.78	17.75	17.54	19.82	0.42	0.111	0.061	0.798
Non-essential AA									
Proline [2]	1.57	1.68	1.6	1.57	1.78	0.03	0.126	0.128	0.32
Alanine [2]	2.29	2.53	2.41	2.38	2.67	0.05	0.124	0.073	0.657
Cystine	0.34	0.34	0.35	0.33	0.38	0.01	0.557	0.322	0.453
Tyrosine [3]	1.47	1.6	1.5	1.52	1.71	0.04	0.257	0.134	0.451
Arginine [3]	2.62	2.91	2.81	2.76	3.12	0.06	0.086	0.035	0.69
Aspartic acid [1]	3.60	4.06	3.84	3.82	4.33	0.09	0.095	0.046	0.666
Serine [2]	1.41	1.58	1.5	1.49	1.67	0.03	0.103	0.053	0.688
Glutamic acid [1]	7.14	7.88	7.6	7.54	8.51	0.17	0.113	0.040	0.605
Glycine [2]	1.9	2.05	1.92	1.88	2.14	0.04	0.168	0.254	0.371
Total non-essentail AA	22.32	24.63	23.54	23.31	26.32	0.50	0.104	0.053	0.560
Essential/Non-essentail AA	73.89	76.18	75.43	75.21	75.29	0.40	0.525	0.533	0.268
Total AA	38.84	43.41	41.29	40.85	46.14	0.92	0.104	0.055	0.662
Umami AA	10.74	11.94	11.44	11.37	12.84	0.26	0.106	0.042	0.625
Sweet AA	8.91	9.82	9.32	9.19	10.37	0.19	0.106	0.078	0.536
Bitter AA	15.47	17.48	16.51	16.33	18.50	0.39	0.110	0.063	0.715

523 AA: amino acid; [1] Umami AA; [2] Sweet AA; [3] Bitter AA; SEM: pooled standard error

524 of the mean.

525 **Table 6.** Effects of dietary forage rape levels on the ruminal fermentation parameters
526 of lambs

Item	Ctrl	T1	T2	T3	T4	SEM	*P* value		
							ANOVA	Linear	Quadratic
NH_3-N (mg/100 mL)	21.33^{ab}	26.88^{a}	17.40^{ab}	17.65^{ab}	10.92^{b}	1.85	0.076	0.019	0.298
Ruminal pH	6.93	7.16	7.14	6.84	7.12	0.06	0.299	0.929	0.719
Total SCFA (mmol/L)	36.53	30.41	23.12	25.81	24.82	2.19	0.312	0.079	0.272
SCFA profile (mmol/L)									
Acetic acid/Acetate	23.57	16.21	13.33	15.59	15.12	1.35	0.133	0.061	0.085
Propionic acid/Propionate	5.79	5.83	4.47	4.53	4.70	0.44	0.778	0.300	0.670
Butyric acid/Butyrate	4.43	3.84	2.60	3.57	2.51	0.33	0.295	0.084	0.644
Isobutyric acid/Isobutyrate	0.84	1.47	1.03	0.85	0.95	0.10	0.426	0.549	0.297
Valeric acid/Valerate	0.44	0.59	0.33	0.31	0.29	0.05	0.182	0.069	0.786
Isovaleric acid/Isovalerate	1.40	2.46	1.36	1.20	1.25	0.17	0.605	0.175	0.419
Acetate/Propionate	4.12	2.91	3.29	3.45	3.52	0.15	0.139	0.507	0.068

527 $^{a-b}$ Within a row, values with different superscript letters differ significantly ($P < 0.05$).

528 SCFA: short-chain fatty acids; SEM: pooled standard error of the mean.

Figures

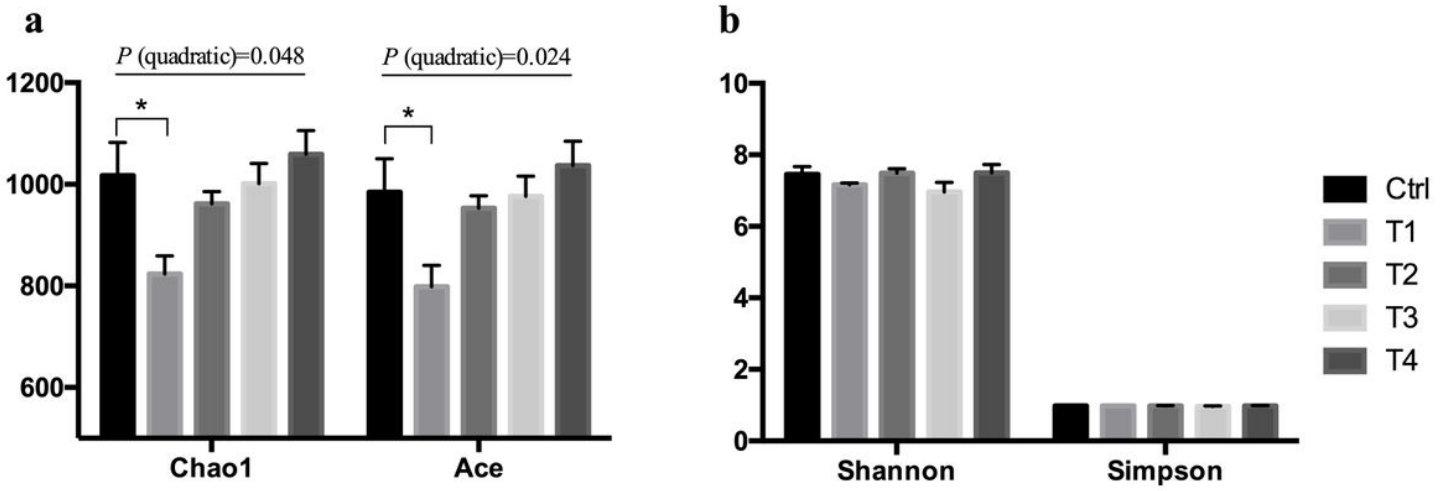

Figure 1

Alpha diversity of the rumen microbial community of lambs. (a) Chao1 and Ace indices. (b) Shannon and Simpson indices. * Bars with asterisk differ significantly ($P < 0.05$).

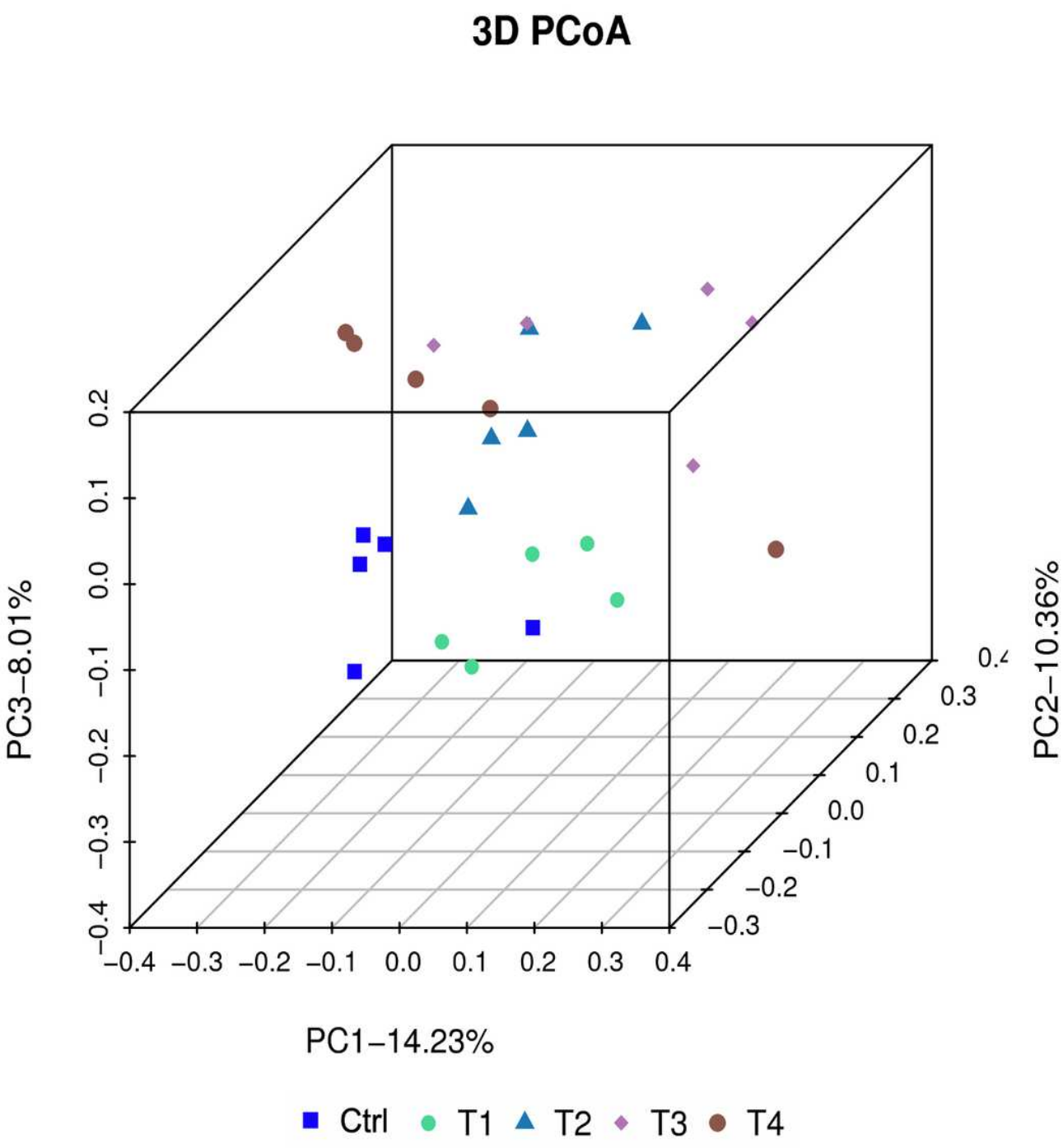

Figure 2

Principal coordinate (PCoA) analysis based on weighted unifrac distance of the rumen microbial community.

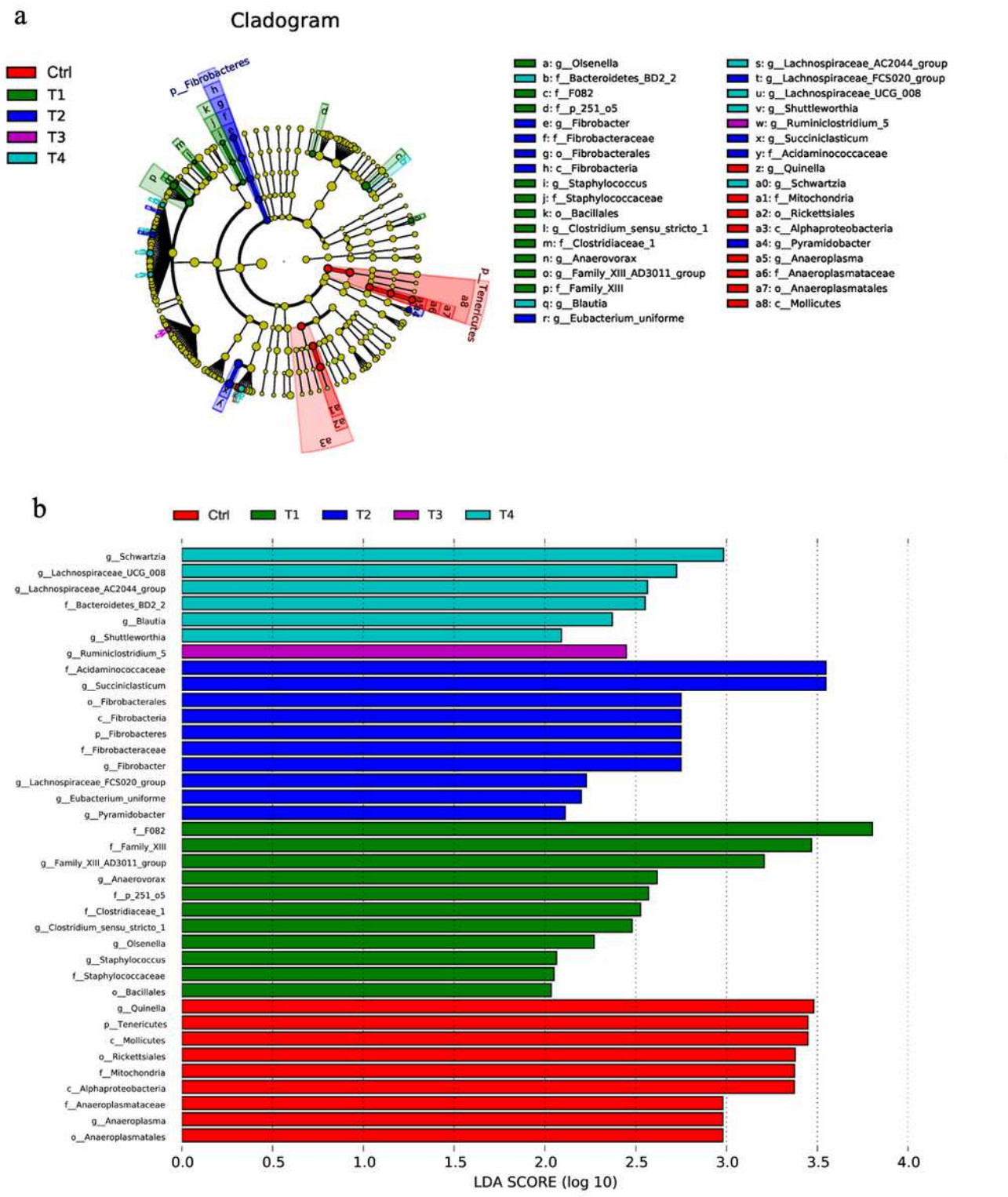

Figure 3

Differences in the relative abundances of rumen microorganisms. (a) LEfSe cladogram of the microbial communities. Differences are represented in the color of the group, where taxa are most abundant. (b) Histogram of linear discriminant analysis (LDA) scores computed for each taxon ranging from phylum to genus.