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Effects of chop lengths of ramie silage on ruminal fermentation, free amino acid content, and cellulase activity in goats

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Abstract: Twelve Liuyang black goats were selected and divided into three groups in a randomized design and they were provided one of the three treatment diets with different chop lengths of ramie silage [1 cm (RS1), 2 cm (RS2), and 3 cm (RS3)]. The length had no effect on pH or the content of acetic acid, butyric acid, and valeric acid. In contrast, NH_3–N decreased (P = 0.024) and the ratio of acetate to propionate (P = 0.083) and total volatile fatty acid (TVFA) content (P = 0.087) tended to decrease, and the proportion of propionic acid tended to increase (P = 0.096) with an increase in the chop length of ramie. The increasing lengths of ramie silage decreased the content of total essential amino acids (AA) (P = 0.001), total non-essential AA (P = 0.003), and total AA (P = 0.001), and decreased the concentration of aspartic acid (P = 0.076) in the ruminal fluid. Ramie silage affected xylanase activity (P = 0.043), with greater activity in RS1 than in RS2 and RS3. The recommended chop length of ramie silage is 1 cm because of increasing TVFA, amino acid concentration, and xylanase activity in the ruminal fluid of goats.

Keywords: black goats; fiber degradation; forage ramie; nutritional assessment; rumen microbes

Ramie (*Boehmeria nivea*), known as "China grass", has been traditionally grown for several years mainly as a fibre crop in China and it is also used as feedstuff for domestic livestock as it is palatable and nutrient-rich (Dai et al. 2019). Ramie has the characteristics of rapid growth, strong branching ability, large leaf blade, high biological yield, and high nutritional value (especially high protein content). There is a new variety of ramie, "Zhongsizhu No. 1", for feeding, released by the Institute of Bast Fibre Crops, Chinese Academy of Agricultural Sciences with more cuts annually and 22% crude protein content in the stems and leaves, similar to that of lucerne hay (Kipriotis et al. 2015).

A previous study (Despal et al. 2017) showed that ramie leaves in the form of hay can be used to substitute feed concentrate and increase digested feed and nutrient intake in Jawaradu goats. De Toledo

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et al. (2008) studied the partial and total substitution of ramie for lucerne hay in feed for growing rabbits and found that replacing lucerne with ramie may improve growth performance. It is commonly believed that mastication reduces the particle size of ingested feed, and it is necessary for the consumption of feed. Furthermore, feeding forage of short particle size to ruminants decreases salivary buffer secretion, ultimately lowering rumen pH (Kononoff et al. 2003). Several studies have reported that chop lengths of lucerne silage or maize silage affect rumen fermentation to a certain degree (Bhandari et al. 2007; Nemati et al. 2015; Sharifi et al. 2016).

However, the effects of ramie silage particle size on goats remain unclear. Thus, the objective of this study was to investigate the effects of different chop lengths of ramie silage on ruminal fermentation, free amino acid content, and cellulase activity in goats.

MATERIAL AND METHODS

All protocols used in the study were approved by the Animal Care and Use Committee of the Institute of Bast Fibre Crops, Chinese Academy of Agricultural Sciences.

Ramie silage

Forage ramie (*Boehmeria nivea*, with a height of 1.2 m) was harvested and chopped into different lengths (1 cm, 2 cm, and 3 cm, respectively). The chopped forage ramie was packed (approximately 25 kg in each bag) with caster sugar at an inclusion of 0.5% of the wet weight of ramie. Ramie silage bags were stored in a feed storage room (four layers, approximately 1.5 m high) for 60 days at room temperature (27–34 °C) and then used in a total mixed ration (TMR) prepared using a complete mixed ration machine (Shandong Shengtai Machinery Manufacturing Co., Ltd., Qufu, China). The nutritive value of ramie silage is shown in Table 1.

Animals and experimental design

Twelve adult Liuyang black goats of an average age of 180 ± 10 days and body weight of 22.35 ± 2.16 kg were divided into three groups of four goats by body weight. The goats were kept in individual metabolism pens (1.5 × 2.0 m) with a plastic leakage dung plate. The grouped goats were randomly assigned to one of the three experimental diets that included ramie silage (RS) with short (1 cm, RS1), medium (2 cm, RS2), and long (3 cm, RS3) chop lengths. The diets were formulated with 60% RS, 40% concentrate, and mineral supplements (dry matter basis) to meet the nutrient requirement of goats according to the NRC (2007); the ingredients and chemical composition of the experimental diets are shown in Table 2. This study included a 14-day pre-feeding period and a 3-day data collection period. The goats were housed individually in 1.5 × 2.0 m pens, with feed offered twice in equal amounts at 08:00 and 20:00, with free access to fresh water throughout the experimental period.

Table 1. Analysis of routine nutrient composition of ramie silage (dry matter basis)[1]

Items (%)	Ramie silage chop length		
	1 cm	2 cm	3 cm
Crude protein	15.39 ± 1.48	17.12 ± 1.45	16.00 ± 1.04
Crude fat	3.53 ± 0.59	2.93 ± 0.14	2.87 ± 0.49
Crude fibre	29.84 ± 0.94	26.91 ± 1.21	27.93 ± 0.98
Neutral detergent fibre	54.18 ± 0.57	48.23 ± 0.67	50.76 ± 2.42
Acid detergent fibre	42.66 ± 0.95	38.71 ± 3.12	39.75 ± 0.90

[1]Data were obtained from Wang et al. (2018a)

Sampling and measurements

Before morning feeding on days 15 to 17, approximately 50 ml of rumen content was obtained from each animal via oral lavage using a polystyrene tube. The first 50 ml of rumen fluid was discarded to prevent saliva contamination. The pH of the rumen fluid was measured immediately using a pH meter (SevenCompact™ S210 pH meter; Mettler Toledo Instruments Ltd., Shanghai, China). The collected ruminal contents were immediately squeezed through four layers of cheesecloth, and subsamples were taken for volatile fatty acids (VFA), NH_3–N, amino acid profiles, and cellulase activity analysis. Briefly, the samples for VFA analysis were prepared and assayed by gas chromatography (GC7890A; Agilent Technologies, Santa Clara, CA, USA) as described by Wu et al. (2013). Free amino acids (AAs) in ruminal fluids were extracted using

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Table 2. Composition and nutrient levels of the experimental diets (air-dry basis)

Group	Ramie silage chop length		
	1 cm	2 cm	3 cm
Ingredient (%)			
Ramie silage of 1 cm	60.0	–	–
Ramie silage of 2 cm	–	60.0	–
Ramie silage of 3 cm	–	–	60.0
Corn meal	20.4	20.4	20.4
Wheat bran	12.72	12.72	12.72
Soybean meal	4.8	4.8	4.8
NaCl	0.48	0.48	0.48
Premix[1]	1.6	1.6	1.6
Total	100.0	100.0	100.0
Nutrient levels[2]			
Metabolic energy (ME, MJ/kg)	8.72	8.72	8.72
Crude protein (CP, %)	14.9	15.2	14.9
Starch (%)	15.49	15.48	15.48
Neutral detergent fibre (NDF, %)	37.5	36.7	37.3
Physical effectiveness NDF (peNDF > 1.18, %)	25.99	24.51	23.12
Acid detergent fibre (ADF, %)	26.6	26.4	26.8
Calcium (%)	2.87	2.86	2.88
Phosphorus (%)	0.31	0.32	0.31

[1]The premix contained per kg of diet: 120 g of $MgSO_4 \cdot H_2O$, 3 g of $FeSO_4 \cdot 7H_2O$, 1 g of $CuSO_4 \cdot 5H_2O$, 3 g of $MnSO_4 \cdot H_2O$, 5 g of $ZnSO_4 \cdot H_2O$, 10 mg of Na_2SeO_3, 40 mg of KI, 30 mg of $CoCl_2 \cdot 6H_2O$, 100 000 IU vitamin A, 18 000 IU vitamin D, and 20 000 IU vitamin E

[2]The contents of CP, starch, NDF, peNDF, ADF, Ca, and P were analysed, whereas the ME was recalculated

boiling water, separated using a sulfonic acid cation exchange column, and then heated at 135 °C and reacted with ninhydrin. The free amino acid content was determined using a Hitachi 8900 automatic amino acid analyser (Hitachi Ltd., Tokyo, Japan). The activities of pectase, carboxymethyl cellulase, filter paper enzyme, and xylanase were determined according to the instructions of the ELISA Kit (Jiangsu Baolai Biotechnology Ltd., Nanjing, China) using an automatic microplate reader (Model ELXS 808; Biotek Instruments Inc., Vermont, NE, USA).

Statistical analysis

Statistical analysis of the data was conducted using the one-way ANOVA procedure in SAS v9.2. For the analysis, experimental animals were included as a random effect, and treatment was included as a fixed effect. Tukey's test was used to determine differences between means. Least square means were expressed in tables, and significance was declared for P-values < 0.05 and trends at 0.05 < P < 0.10.

RESULTS AND DISCUSSION

The ruminal pH was numerically higher (P = 0.110) in RS3 than in RS1 (Table 3). Several studies have shown that an increase in forage particle length can stimulate chewing behaviour and saliva secretion, thus increasing ruminal pH, which is beneficial for the rumen function (Mertens 1997; Yang and Beauchemin 2007). A more recent study by Kmicikewycz et al. (2015) also reported a higher average rumen pH in cows fed long maize silage than in those fed short maize silage.

Increasing the ramie silage chop length decreased the ammonia concentration (P = 0.024), in contrast to the report by Thomson et al. (2017), who reported that cows fed diets containing 19 mm lucerne silage had higher ruminal NH_3–N content than those fed 14 mm silage. We speculate that the differences between the studies might be due to the higher protein content of ramie silage than in lucerne silage. Increasing the chop length of ramie silage leads to longer rumination time per unit of dry matter intake, thus reducing NH_3–N production.

Table 3. Effects of different chop lengths of ramie silage on the ruminal fermentation characteristics of goats

Items	Ramie silage chop length			SEM	P-value
	1 cm	2 cm	3 cm		
pH	6.8	6.9	7.0	0.04	0.110
Ammonia nitrogen (NH_3–N, mg/dl)	21.5[a]	18.7[ab]	17.2[b]	0.72	0.024
Molar %					
Acetic acid	69.4	68.5	68.4	0.65	0.818
Propionic acid	16.5	18.5	20.9	0.85	0.096
Butyric acid	12.7	11.8	9.54	0.77	0.233
Valeric acid	1.38	1.23	1.18	0.05	0.257
Ratio of acetate to propionate	4.22	3.72	3.37	0.16	0.083
Total volatile fatty acids (μmol/ml)	62.8	50.2	51.7	2.63	0.087

[a,b]Different upper-case letters denote significantly different expression levels in the same index (P < 0.05)

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Increasing the ramie silage chop length tended to increase the proportion of propionic acid (P = 0.096) and tended to decrease the acetate to propionate (A/P) ratio (P = 0.083), and the concentration of total VFA (P = 0.087). However, there was no effect on the molar proportions of acetic acid, butyric acid, and valeric acid. The effects of the chop length of silage on VFA production, especially the A/P ratio, are inconsistent among studies. Several studies have reported a lower A/P ratio and greater total VFA concentration in lactating dairy cows fed diets containing maize silage with a shorter chop length (Krause et al. 2002; Kmicikewycz and Heinrichs 2015), whereas Kononoff et al. (2003) reported a lower A/P ratio and total VFA concentration in lactating dairy cows fed a diet containing mostly long silage versus short silage. Furthermore, Beauchemin et al. (2003) reported that there was no significant effect on the A/P ratio or total VFA concentration when cows were fed short maize silage. This inconsistency in fermentation parameters among studies might be ascribed to different types of forage, sampling time, and experimental animals.

The amino acid profiles, including essential amino acids (EAA) and nonessential amino acids (NEAA), are shown in Table 4. The chop length of RS affected (P < 0.05) the concentration of most EAA except phenylalanine and histidine and affected (P < 0.05) the concentration of NEAA, such as serine, glutamic acid, and glycine, in the ruminal fluid of goats, with the concentration of these amino acids linearly decreasing with increasing RS chop lengths. Taken together, total EAA, total NEAA, and total AA concentration linearly (P < 0.01) decreased as the RS chop length increased. This might be so because a shorter particle size is beneficial for the attachment of rumen microorganisms as it increases the surface area for degradation and release of AAs. The supply of individual AAs to the rumen is important to ruminants for rumen microbial protein synthesis and a deficiency in EAA can limit animal production (Johansen et al. 2018). The AA profile of feedstuff differs with feed variety and their respective profiles after rumen degradation. The change in the AA concentration in the rumen might be due to different ruminal AA degradability or feedstuff sources. Wang et al. (2018b) found that the concentration of total NEAA was higher than that of total EAA in the rumen fluid, which indicates that AAs in feed are not equally degraded by ruminal microbes. This is consistent with the results of the present study.

Table 4. Effects of different chop lengths of ramie silage on the amino acid profiles of the ruminal fluid of goats (μg/ml)

Parameter	Ramie silage chop length			SEM	P-value
	1 cm	2 cm	3 cm		
Essential amino acid (EAA)					
Threonine	73.7^{a}	44.0^{b}	28.9^{c}	6.76	0.000
Valine	68.6^{a}	41.9^{b}	32.0^{c}	5.67	0.000
Methionine	34.6^{a}	20.8^{b}	13.2^{c}	3.29	0.001
Isoleucine	42.7^{a}	40.6^{a}	24.7^{b}	3.43	0.030
Leucine	58.3^{a}	47.0^{ab}	30.3^{b}	5.16	0.054
Phenylalanine	41.7	31.4	21.9	4.38	0.187
Lysine	136.7^{a}	85.9^{b}	59.6^{b}	12.35	0.004
Histidine	5.0	4.7	3.9	0.38	0.527
Arginine	1.32^{a}	0.87^{b}	0.72^{b}	0.10	0.002
Non-essential amino acid (NEAA)					
Aspartic acid	60.6	56.5	35.9	5.03	0.076
Cystine	4.67	3.96	2.83	0.48	0.330
Tyrosine	41.0	31.1	21.7	4.10	0.167
Proline	14.9	8.51	6.87	2.07	0.278
Serine	31.2^{a}	25.8^{ab}	17.0^{b}	2.55	0.042
Glutamic acid	232.1^{a}	153.0^{b}	122.7^{b}	17.14	0.001
Glycine	24.8^{ab}	32.7^{a}	17.3^{c}	2.64	0.025
Alanine	85.8	58.8	57.4	7.38	0.224
Total EAA	462.6^{a}	317.3^{b}	215.2^{c}	37.72	0.001
Total NEAA	495.1^{a}	370.3^{b}	281.8^{b}	33.55	0.003
Total AA	957.7^{a}	687.6^{b}	497.2^{c}	70.28	0.001

$^{a–c}$Different upper-case letters denote significantly different expression levels in the same index (P < 0.05)

The activity of pectase, carboxymethyl cellulase, and filter paper enzyme in the rumen fluid was not affected (P > 0.05) by the chop length of RS (Table 5). The activity of xylanase linearly (P = 0.043) decreased with increasing chop length

Table 5. Effects of different chop lengths of ramie silage on the activity of cellulase in the ruminal fluid of goats

Parameter	Ramie silage chop length			SEM	P-value
	1 cm	2 cm	3 cm		
Xylanase (IU/ml)	91.6^{a}	83.4^{ab}	78.0^{b}	2.36	0.043
CMCase (IU/ml)	114.0	104.8	96.8	3.43	0.115
Pectase (IU/ml)	58.2	52.3	51.4	2.37	0.483
FPase (IU/ml)	518.1	499.1	488.3	12.85	0.675

CMCase = carboxymethyl cellulase; FPase = filter paper enzyme

^{a,b}Different upper-case letters denote significantly different expression levels in the same index (P < 0.05)

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of RS. As rumen microorganisms play a key role in the degradation of forage in the rumen and adhere to feed particles and secrete enzymes to degrade the plant cell wall (Miron et al. 2001; Wang et al. 2018c), we speculate that the higher xylanase activity in goats fed RS with a shorter chop length is due to the larger surface area of the shorter ramie particles, which results in better adhesion and degradation of cellulose and hemicellulose components by ruminal cellulolytic bacteria compared with that of the long ramie particles. Our results agree with those of Zebeli et al. (2008), who found that decreasing the particle size of maize silage significantly increased the activity of non-starch polysaccharide-degrading enzymes, such as carboxymethyl cellulase and xylanase, in the rumen of dairy cows. Shaani et al. (2017) also observed that cows fed short-particle wheat hay TMR showed higher digestibility of cellulose and hemicellulose than those fed long-particle hay TMR.

CONCLUSIONS

The content of TVFA tended to decrease, and the total essential amino acid concentration and the activity of xylanase decreased with an increase in the particle length of ramie silage. Thus, a chop length of 1 cm exhibited more positive effects on goats under the current experimental conditions.

Conflict of interest

The authors declare no conflict of interest.

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