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Effects of Increasing Level of Dietary Rice Straw on Chewing Activity, Ruminal Fermentation and Fibrolytic Enzyme Activity in Growing Goats

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ABSTRACT: Effects of increasing dietary rice straw on chewing activity, ruminal fermentation, and fibrolytic enzyme activity in growing goats were investigated in a 4×4 Latin Square experiment. The goats were offered four diets with an increasing proportion of rice straw (i.e. 0.05, 0.10, 0.15 and 0.20, respectively, on dry matter basis). Increasing level of rice straw increased ($P_{linear\ effect}<0.05$) the time spent on eating, ruminating, and chewing. The ruminal pH and acetate: propionate ratio were increased ($P_{linear\ effect}<0.05$), while the NH_3-N concentration was decreased ($P_{linear\ effect}<0.01$). Increasing level of rice straw in the diet increased ($P_{linear\ effect}\leq0.01$) molar proportion of acetate and isovalerate, and decreased ($P_{linear\ effect}<0.01$) molar proportion of propionate. The CMCase, xylanase and cellobiase activities in the rumen were decreased ($P_{linear\ effect}<0.05$) with increasing level of dietary rice straw, whereas the avicelase activity was increased ($P_{linear\ effect}<0.01$). In summary, increased level of rice straw elevated the dietary neutral detergent fibre (NDF) content in the diet and had a great impact on chewing activity and ruminal fermentation. (**Key Words :** Rice Straw, Dietary NDF, Ruminal Fermentation, Fibrolytic Enzyme, Chewing Activity)

INTRODUCTION

Rice is the most widely grown cereal crop and provides an important traditional feed source for ruminant animals in China. Increasing the level of rice straw in the diet leads to an increased dietary neutral detergent fibre (NDF) content. Studies have shown that increasing dietary rice straw negatively affects nutrient digestibility in ruminants (Fimbres et al., 2002; Zhao et al., 2007). However, increasing dietary NDF content may have a positive effect on the maintenance of normal rumen function, which is associated with adequate salivation, optimal pH for cellulolytic microorganisms and energy supply (Jaster and Murphy, 1983; Allen, 1997; Krause et al., 2002). Although some studies have been made in cattle and sheep, few have been done for goats.

Chewing activity, including eating and ruminating, has a direct relationship with the amount of saliva secretion, which is an important buffer for maintaining normal rumen functions (Allen, 1997). The rumen microorganisms, predominately bacteria, protozoa and phycomycete fungi, can secrete a wide range of fibrolytic enzymes (Imai, 1998; Chen et al., 2008). These enzymes, such as avicelase, CMCase, xylanase and cellobiase, account for the primary reactants on dietary carbohydrates and proteins (Santra et al., 2007). Higher dietary NDF content tends to increase salivation through eating and ruminating, and might benefit the growth of cellulolytic microbes (Lu et al., 2005). Therefore, it was important to examine the effect of increasing dietary rice straw and NDF level on fibrolytic enzyme activities, which have been rarely explored.

The effects of rice straw on site and extent of digestion and nitrogen (N) balance were reported in our previous study (Zhao et al., 2007). In the present study, the effect of increasing level of rice straw in the diet on chewing activities, ruminal fermentation and rumen fibrolytic enzyme activities were studied.

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MATERIALS AND METHODS

Animals and their management

A 4×4 Latin square experiment with 4 growing Liuyang black wether goats (a local breed, body weight of 19.3±2.1 kg) was conducted. The goats were surgically fitted with ruminal fistulae. About 30 d were allowed for recovery of the animal from surgery. The experimental procedure was approved by the Animal Care Committee, ISA.

Rice straw and maize stover were used as the main sources of forage in this study. RS5, RS10, RS15 and RS20 were the diets which contained 0.05, 0.10, 0.15 and 0.20 rice straw. The diets were formulated to meet 1.3 times maintenance requirements of metabolisable energy according to our previous study (Zhao et al., 2007; Wang et al., 2008). Ingredients and composition of diets are shown in Table 1. The amount of diet offered to each goat was restricted to 850 g/kg of its *ad libitum* intake to ensure that there was no refusal during the whole experimental period. The forage was fed once daily at 07:30 h and the concentrate twice daily in two equal amounts at 08:00 and 20:00 h. All goats had free access to fresh water, and were kept individually in stainless steel metabolism cages (41×127 cm) in a temperature-controlled (at 20°C) house with constant lighting.

Each experimental period lasted for 26 d, comprised of 12 d adaptation and 14 d sampling with at least 3 d between periods. The amount of feed offered was weighed daily for each goat. Samples of the whole diet (about 0.1 kg) were collected daily and composited into one sample per goat during d 13 to 22, and dried at 65°C for 48 h for the determination of nutrient intake and digestibility. Feces were collected daily during the last 5 sampling days using faecal collection bags, and 10% representative samples were composited within goat and across days for each period (Zhao et al., 2009). Samples were stored at -20°C until analyses.

Sampling

About 50 ml ruminal fluid was collected with a rumen filter probe tube via the ruminal cannula at 0.5, 3, 6, 9 and 12 h after the morning feeding on d 17 of each period.

Table 1. Dietary ingredients and chemical composition[1]

Item	Diet			
	RS5	RS10	RS15	RS20
Ingredient (%)				
Maize stover	20.0	20.0	20.0	20.0
Rice straw	5.00	10.0	15.0	20.0
Ground corn	41.0	35.4	29.7	24.0
Soybean meal	7.30	8.00	8.60	9.30
Wheat bran	19.2	19.2	19.2	19.2
Corn starch	0.10	0.10	0.10	0.10
Fish meal	4.60	4.60	4.60	4.60
Urea	0.17	0.17	0.17	0.17
Sodium chloride	0.50	0.50	0.50	0.50
Calcium carbonate	0.58	0.58	0.58	0.58
Premix[2]	1.50	1.50	1.50	1.50
Chemical composition[3]				
DM (%)	85.7	86	86.2	86.4
OM (%)	92.5	92.6	91.6	93.1
CP (%)	14.9	14.9	14.9	14.9
NDF (%)	31.5	34.2	36.7	39.7
NDF from forage (%)	16.6	19.9	23.2	26.6
ADF (%)	17.3	19.4	21.5	23.6
Ca (%)	0.63	0.64	0.65	0.67
P (%)	0.39	0.39	0.39	0.39
ME[4] (Mcal/kg DM)	2.44	2.37	2.29	2.21

[1] Values expressed on a dry matter basis.

[2] Premix contained per kilogram: 119 g $MgSO_4 \cdot H_2O$, 2.5 g $FeSO_4 \cdot 7H_2O$, 0.8 g $CuSO_4 \cdot 5H_2O$, 3 g $MnSO_4 \cdot H_2O$, 5 g $ZnSO_4 \cdot H_2O$, 10 mg Na_2SeO_3, 40 mg KI, 30 mg $CoCl_2 \cdot 6H_2O$, 95,000 IU vitamin A, 17,500 IU vitamin D, and 18,000 IU vitamin E.

[3] The chemical composition was analyzed except for metabolizable energy (ME) which was calculated from the data of Zhang and Zhang (1998); DM = Dry matter, OM = Organic matter, CP = Crude protein, NDF = Neutral detergent fibre, ADF = Acid detergent fibre, P = Phosphorus.

Samples were composited for analysis of fibrolytic enzyme activity. The fermentation was stopped by swirling the flasks in ice water. The volume of ruminal fluid was equally mixed with phosphate buffer solution (50 mM; pH = 6.0), and immediately squeezed through 4 layers of cheesecloth. The filtered fluid was centrifuged at 800×g for 15 min to obtain the supernatant fraction without small digesta particles, which was then treated by Ultrusonic Cell Disrupter and stored at -20°C for subsequent analysis of fibrolytic enzyme activities.

Ruminal pH and VFA were measured from d 18 to 19. Ruminal fluid samples (50 ml) were taken with a rumen filter probe tube via the ruminal cannula. Ruminal fluid samples (50 ml) were collected at 0.5, 2, 4, 6, 9, 12, 15, 18 and 21 h after the morning feeding on d 18 of each period. The pH was measured using a pH meter (REX, Shanghai instrument factory, pHS-3C). Ruminal samples were then immediately squeezed through 4 layers of cheesecloth. A 10 ml sample of filtered fluid was centrifuged at 20,000×*g* for 15 min at 4°C to obtain a clear supernatant which was then analyzed for ammonia using a phenol-hypochlorite assay (Zhao et al., 2009). For the determination of VFA, 10 ml of fluid was centrifuged at 500×*g* for 10 min at 4°C. The solution was then put into a plastic bottle containing 1 ml of 25% metaphosphoric acid and 1 ml of 0.6% 2-ethyl butyric acid (internal standard). The mixture was centrifuged at 20,000×g for 15 min at 4°C and the supernatant was stored at -20°C for-analysis.

Chewing activity

Chewing activity was measured according to the procedure used in the previous study (Zhao et al., 2009). Eating and ruminating activities were monitored visually every 5 min for 24 h from d 22 to 23. Each activity was assumed to persist for the entire 5 min interval. Eating was defined as at least 1 min of eating activity after at least 20 min without eating. A period of rumination was defined as at least 5 min of rumination occurring after at least 5 min without ruminating. Time spent eating or ruminating per gram of dry matter intake (DMI) and neutral detergent fibre intake (NDFI) were calculated. Eating and rumination activities were expressed as total minutes for the 24 h period or on the basis of DMI and NDFI. Chewing activities were calculated as a sum of eating and ruminating activities.

Chemical analysis

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the methods of Van Soest et al. (1991). The NDF was assayed with the addition of a heat stable amylase, but without sodium sulphite. Dry matter (DM), organic matter (OM) and nitrogen (N) were determined according to AOAC (2002).

Ammonia was determined by a phenol-hypochlorite assay (Chaney and Marbach, 1962). The VFA was separated on a packed column (model SP-1200, Supelco, Bellefonte, PA) with 2-ethyl butyric acid as the internal standard, and quantified by gas chromatography (Hewlett Packard 5890, HP, USA).

Enzyme activities were determined by measuring reducing sugar formation from 0.5% sodium carboxymethylcellulose (i.e. 0.5 g sodium carboxymethyl-cellulose in 100 ml 0.2 M phosphate buffer solution) for carboxymethyl cellulase (CMCase) activity, from 0.5% avicel microcrystalline cellulose (i.e. 0.5 g avicel microcrystalline cellulose in 100 ml 0.2 M phosphate buffer solution) for avicelase, from 0.5% xylan (i.e. 0.5 g xylan in 100 ml 0.2 M phosphate buffer solution) for xylanase, and from 0.5% salicin (i.e. 0.5 g salicin in 100 ml 0.2 M phosphate buffer solution) for cellobiase, respectively. The enzyme reactions were initiated by addition of 0.2 ml enzyme solution and 1 ml substrate, and then incubated at 50°C for 90 min. After adding 2 ml of 3,5-dinitrosalicylic acid (DNS reagent), the reactions were stopped by heating in a boiling water bath for 10 min. The color formed was read at 550 nm with a Shimadzu UV-2450 Spectrophotometers (Japan). Glucose was used as standard for CMCase, avicelase and cellobiase, and xylose for xylanase, respectively. One international unit (IU) was equivalent to the enzyme activity releasing 1 μmol of glucose or xylose per minute per ml of enzyme solution.

Statistical analysis

The data were analyzed as a 4×4 Latin square design using the GLM procedure of SAS software. The model used was: $Y_{ijkl} = \mu_i+G_i+P_j+T_k+e_{ijkl}$ where μ is the overall mean, G_i is the effect of goat (i = 1 to 4), P_j is effect of period (j = 1 to 4), T_k is fixed effect of treatment (k = 1 to 4), and e_{ijkl} is residual. Where the treatment effect was significant, differences among means were tested with Duncan's multiple range tests. Statistical significances were considered to exist if $p<0.05$. Orthogonal polynomial contrasts were used to examine the responses (linear and quadratic) to increased level of rice straw in the diets.

RESULTS

Chewing activity

Effects of increasing level of dietary rice straw on chewing activity in goats are shown in Table 2. Dietary rice straw level increased the time spent in eating, ruminating and chewing activities ($P_{linear\ effect}<0.01$). Increasing level of rice straw increased min/g DM ($P_{linear\ effect}<0.01$) and min/g NDFI ($P_{linear\ effect}<0.05$) for eating activities, min/g DM ($P_{linear\ effect} = 0.01$) for ruminating activity, and min/g DM ($P_{linear\ effect}<0.01$) and min/g NDFI ($P_{linear\ effect}<0.01$) for chewing activities.

Table 2. Effect of increasing level of rice straw on the eating, rumination and chewing activities of goats (n = 4) [1]

Item	Diets[2]				SEM	Effects	
	RS5	RS10	RS15	RS20		Linear	Quadratic
Eating							
Min/d	227[b]	250[b]	300[b]	362[a]	16.9	<0.01	0.29
Min/g DMI	0.45[b]	0.50[b]	0.59[b]	0.71[a]	0.03	<0.01	0.31
Min/g NDFI	1.43[b]	1.44[b]	1.60[ab]	1.81[a]	0.09	0.02	0.35
Rumination							
Min/d	223[b]	259[ab]	306[ab]	333[a]	21.8	<0.01	0.84
Min/g DMI	0.44[b]	0.51[ab]	0.60[a]	0.66[a]	0.04	0.01	0.80
Min/g NDFI	1.40	1.49	1.63	1.66	0.13	0.16	0.80
Chewing							
Min/d	450[c]	509[c]	606[b]	695[a]	20.1	<0.01	0.48
Min/g DMI	0.89[c]	1.00[c]	1.20[b]	1.37[a]	0.23	<0.01	0.46
Min/g NDFI	2.82[c]	2.94[bc]	3.24[ab]	3.46[a]	0.11	<0.01	0.64

[1] DMI, NDFI and SEM were dry matter per day, neutral detergent fibre intake per day and pooled standard error of means, respectively. Means with different letters within a row are significantly different ($p<0.05$).
[2] RS5, RS10, RS15 and RS20 contained 0.05, 0.10, 0.15 and 0.20 rice straw, respectively.

Ruminal fermentation

Effect of increasing level of rice straw on ruminal fermentation is shown in Table 3. Increasing dietary rice straw increased pH ($P_{linear\ effect}<0.05$), acetate: propionate ratio ($P_{linear\ effect}<0.01$), and decreased NH_3-N concentration ($P_{linear\ effect}<0.01$) in the rumen. Dietary rice straw greatly affected ($P_{quadratic\ effect}<0.01$) the total VFA concentration in rumen, with the lowest value observed in the RS10 group. Further investigating the individual VFA, increasing level of dietary rice straw also increased acetate ($P_{linear\ effect}<0.01$) and isovalerate ($P_{linear\ effect} = 0.01$), and decreased propionate ($P_{linear\ effect}<0.01$) in the rumen.

Fibrolytic enzyme activity

Increased level of dietary rice straw decreased CMCase ($P_{linear\ effect} = 0.02$), xylanase ($P_{linear\ effect}<0.01$) and cellobiase ($P_{linear\ effect}<0.01$) activities, but increased the avicelase ($P_{linear\ effect}<0.01$) activity (Table 4).

DISCUSSION

The goats spent 227-362, 223-333, and 450-695 min/d for eating, ruminating and chewing activities, and an elevated level of rice straw increased the time spent on these three activities. The results were consistent with reports on other small ruminants (Kawas et al., 1991; Fimbres et al., 2002), although the extent of increased chewing activity might be different. Research on lambs showed that eating, ruminating and chewing time ranged from 92 to 75, 143 to 413 and 235-558 min/d, respectively, when grass hay increased from 0 to 30% in the diet (Fimbres et al., 2002). Research on sheep showed that

Table 3. Effect of increasing level of rice straw on ruminal pH and fermentation metabolites of goats [1]

Item	Diets[2]				SEM	Effects	
	RS5	RS10	RS15	RS20		Linear	Quadratic
pH	6.21[b]	6.27[ab]	6.32[ab]	6.39[a]	0.04	0.01	0.95
NH_3-N (mg/100 ml)	25.3[a]	24.0[b]	23.1[bc]	22.4[c]	0.37	<0.01	0.43
Total VFA (mmol)	112[a]	100[b]	113[a]	113[a]	1.46	0.06	0.01
Acetate:propionate	1.46[b]	1.44[b]	1.65[b]	2.12[a]	0.07	<0.01	0.02
Individual VFA (% of total)							
Acetate	47.0[b]	47.0[b]	48.7[b]	53.0[a]	0.88	<0.01	0.09
Propionate	32.5[a]	34.3[a]	29.9[a]	26.4[b]	0.95	<0.01	0.02
Butyrate	15.2	13.9	16.2	14.8	0.97	0.04	0.40
Iso-butyrate	1.6	1.66	1.67	1.84	0.07	0.80	0.99
Iso-valerate	2.00[b]	1.80[c]	2.02[b]	2.39[a]	0.10	0.01	0.01
Valerate	1.64	1.34	1.50	1.53	0.09	0.59	0.06

[1] Means with different letters within a row are significantly different ($p<0.05$), SEM is pooled standard error of means.
[2] RS5, RS10, RS15 and RS20 contained 0.05, 0.10, 0.15 and 0.20 rice straw, respectively.

Table 4. Effect of increasing level of rice straw on activity of avicelase, CMCase, xylanase and cellobiase (n = 4)[1]

Item	Diets[2]				SEM	Effects	
	RS5	RS10	RS15	RS20		Linear	Quadratic
Avicelase	5.59[b]	6.38[a]	6.09[a]	6.33[a]	0.18	0.02	0.09
CMCase	3.96[a]	3.13[b]	3.77[a]	3.30[b]	0.11	<0.01	0.03
Xylanase	14.7[a]	13.8[b]	12.4[c]	13.4[b]	0.21	<0.01	<0.01
Cellobiase	3.83[a]	3.07[b]	3.09[b]	3.12[b]	0.08	<0.01	<0.01

[1] Means with different letters within a row are significantly different ($p<0.05$); SEM is pooled standard error of means.
[2] RS5, RS10, RS15 and RS20 contained 0.05, 0.10, 0.15 and 0.20 rice straw, respectively.

eating, ruminating and chewing time ranged from 324 to 372, 486 to 558 and 810-930 min/d, respectively, when high forage rations were fed (from 40 to 80% grass hay) (Kawas et al., 1991). Increasing forage level caused an increased dietary fibre in the diet, which showed a great impact on chewing activity (Armentano and Pereira, 1997; Yang et al., 2001). However, the extent of response of chewing activity to the dietary fibre might be different. Beauchemin (1991) reported that the total chewing time increased by 11% as NDF content of the diet increased from 31 to 37% in dairy cows. Oba and Allen (2000) observed that the total chewing time increased by 21% as NDF content of the diet increased from 29 to 38% in dairy cows. In our study, total chewing time by goats was more sensitive to changes in NDF content than in larger animals, and increased by 56% as NDF content increased from 31.5 to 39.7%.

Increasing level of dietary rice straw linearly increased the ruminal pH and acetate: propionate ratio, and decreased NH_3-N concentration. Increased acetate:propionate ratio in the rumen indicated a reduced energy efficiency. The results are in agreement with the generally accepted perception that dietary NDF maintains normal rumen function, which is associated with adequate salivation, optimal pH for cellulolytic microorganisms and energy supply (Beauchemin, 1991; Oba and Allen, 2000). Further investigating the molar proportion of individual VFA in the rumen, the increased acetate and iso-valerate and decreased propionate were consistent with many previous studies (Zinn et al., 1994; Lu et al., 2005). Lu et al. (2005) reported that a concentrate diet yielded a higher proportion of propionate, and a forage diet yielded more acetic, butyrate and iso-butyrate. Zinn et al. (1994) reported that increasing the forage level from 10 to 20% increased ruminal molar proportion of acetate, but did not affect molar proportions of butyrate. It was likely that the increased dietary rice straw elevated ruminal pH, and thus favored the growth of cellulolytic microbes (e.g. *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*) and thereby increased acetate production (Lu et al., 2005; Zebeli et al., 2008).

Increased level of dietary rice straw decreased NH_3-N concentration in the rumen. Decreased ruminal NH_3-N concentration might lead to a decreased efficiency of N use in ruminant animals, and was consistent with previous research on N balance, which showed that rice straw decreased N cycling back to the digestive tract in goats, as increased faecal and urea N, and decreased apparently absorbed and retained N (Zhao et al., 2007). Although increased avicelase enzyme was observed, the other three fibrolytic enzymes, CMCase, xylanase and cellobiase, were decreased. So, increasing the level of rice straw leaded to the low efficiency of use of nutritive substrates in the diet. The results were in agreement with our previous study, which showed that increased dietary rice straw decreased the potential digestibility of DM and fibre in goats (Zhao et al., 2007). Increased dietary fibre might be of benefit in maintaining rumen health, but sacrifices efficiency of nutritional use of the diet (Zhao et al., 2007).

CONCLUSION

Increasing level of dietary rice straw from 5-20% leaded to an increase of NDF content from 31.5 to 39.7% in the diet, which increased chewing activity and ruminal pH, and affected ruminal fermentation metabolites in goats. Increased acetate:propionate ratio in the rumen indicated reduced energy efficiency when dietary rice straw content was increased. In addition, analysis of fibrolytic enzymes indicated that increasing level of dietary rice straw decreased the ruminal CMCase, xylanase and cellobiase activities, and increased ruminal avicelase activity.

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