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ORIGINAL ARTICLE

Associative effects of supplementing rice straw-based diet with cornstarch on intake, digestion, rumen microbes and growth performance of Huzhou lambs

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ABSTRACT

Thirty-six male Hu lambs consuming a rice straw-based diet were used in a 60-day trial to study the associative effects of cornstarch supplementation on intake, digestion, ruminal microbial population and growth performance. All animals were fed rice straw *ad libitum* together with 160 g/day of rapeseed meal and supplemented with cornstarch at levels of 0 (control), 60, 120 or 180 g/day, respectively. Increment of supplementary cornstarch showed little influence on rice straw intake. Optimal growth performance and highest apparent digestibility of organic matter was achieved in the 120 g/day cornstarch group ($P < 0.05$), while the digestibilities of neutral detergent fiber and crude protein were significantly decreased by 180 g/day cornstarch ($P < 0.05$). Similar results were observed for carboxymethyl cellulose activity and relative populations of cellulolytic bacteria (*Ruminococcus albus, Ruminococcus flavefaciens and Fibrobacter succinogene*). Blood urea nitrogen was reduced by supplementary cornstarch, indicating enhanced protein utilization efficiency. Carcass traits were all significantly improved by supplementary cornstarch. These results suggested that proper amounts of starch supplementation (within 0.5% BW) has little adverse effect on forage utilization, but could effectively improve growth performance. High levels of cornstarch, however, would decrease cellulase activity and populations of cellulolytic bacteria, and hence the digestibility of forage.

Key words: *associative effects, cornstarch, growing lambs, rice straw, ruminal microbes.*

INTRODUCTION

Rice straw is one of the largest by-products of Chinese agriculture, and is a potentially useful resource for ruminant production in China. However, it is low in nutritive value and poor in digestibility. When animals are fed on rice straw, a supplementary strategy is necessary for optimal performance.

Protein is typically considered as the primary limiting nutrient for ruminants consuming low-quality forage (Bodine & Purvis 2003). Supplements with a high content of protein can increase intake (Church & Santos 1981; Guthrie & Wagner 1988; Stokes *et al.* 1988) and digestibility (Stokes *et al.* 1988; DelCurto *et al.* 1990a) of low-quality forages. However, increasing forage intake with protein supplements may not result in adequate increases in energy intake to achieve a desired rate of gain (Bowman & Sanson 1996). Summaries of previous researches mostly indicated that forage intake and digestibility will be decreased by supplementary corn, barley, or other cereal grains as a result of negative associative effects (Kartchner 1980; Chase & Hibberd 1987; Lusby & Wagner 1987; Carey *et al.* 1993). However, there is also evidence for improved forage intake (Henning *et al.* 1980) and digestibility (Pordomingo *et al.* 1991;

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Lardy *et al.* 2004) when low levels of grains are supplemented, probably resulting from promoted microbial growth in the rumen and consequent positive associative effects on forage utilization. The data cited above have included confounding effects such as differing amounts of protein or mineral content in cereal grains, suggesting that the specific impact of starch on low-quality forage utilization remains unclear.

For deep insight into the effects of supplementary starch on forage utilization, researchers traditionally focused on ruminal pH, volatile fatty acid production and other fermentation parameters (Fieser & Vanzant 2004). Recently, molecular-based techniques such as rRNA hybridization, competitive PCR and real-time PCR have been successfully used by microbiologists to study rumen microbial community structure or population of special species of bacteria. Therefore, the objective of this study was to investigate the effects of pure cornstarch on rice straw utilization and growth performance of lambs consuming rice straw-based diet, and attempted to obtain new information on the microbial profiles.

MATERIAL AND METHODS

Animal and diets

This experiment was approved by the Institutional Animal Care and Use Committee at Zhejiang University and conducted in accordance with the National Institutes of Health guidelines for the care and use of experimental animals.

Thirty-six male Hu lambs (3.5 months), with an initial live weight of 20 ± 1.5 kg, were divided into four equal groups of nine each according to body weight. Lambs in each group were kept in 3 pens (three each). All animals were given free access to rice straw and water, together with 160 g/day of rapeseed meal and 15 g of mineral mixture, as a basal diet, and supplemented with cornstarch at levels of 0 (control), 60, 120 or 180 g/day, respectively. The crude protein ingested from rapeseed meal and rice straw was presumed to provide a crude protein allowance for 100 g of average daily gain (MOA 2004). Supplements were given twice daily at 08.00 and 16.00 hours. The rice straw used was from the late season rice, cultivated in Zhejiang province. Following the harvest, rice straw was air-dried and manually chopped to 5–10 cm lengths. The cornstarch was a commercial product (Cerestar Jiliang Maize Industry Co., Ltd., Beijing, China). Contents (g/100 g DM) of organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) were 85.5, 4.8 and 68.6 for rice straw, and 90.5, 40.8 and 20.9 for rapeseed meal, respectively.

Sampling procedures and measurements

The feeding trial lasted for 60 days, with the first 15 days for adaptation. Rice straw offered and refused was recorded daily, and straw orts was recorded every 7 days after routine weekly pen cleaning, and were used to calculate daily intake. Each lamb was weighed for two consecutive days at the beginning and the end of the trial. A digestion trial was carried out on the 20th day of the measurement period, and consisted of a 3-day adjustment to faecal bags, followed by a 6-day total faecal collection. At the completion of the digestion trial, subsamples of feed offered and refused and dried faecal samples were ground in a mill to pass a 1 mm screen and stored at room temperature in sealed containers until they were analyzed. Procedures outlined by AOAC (1990) were used to determine chemical composition of feedstuffs.

On the morning of the last day of the feeding trial, blood samples were taken before morning feeding by jugular venipuncture from six lambs of each group randomly, and centrifuged at 3000 *g* for 15 min for separation of serum. Biochemical reagent kits (Sigma, St. Louis, MO, USA) were used to determine serum total proteins, albumin, globulin, urea nitrogen, triacylglycerol and glucose.

Four lambs from each group were slaughtered in the morning of the next day after the feeding trial. Hot carcass weight was taken within 10 min of slaughter, including kidneys and 'kidney fat'. Dressing percentage was the ratio of hot carcass weight to the unshorn weight just before slaughter, multiplied by 100. Ribeye area was measured by tracing an outline of the cross-section of the *longissimus* muscles from both the right and left sides of the carcass between the 12th and 13th thoracic vertebrae on a piece of acetate. The area was then measured in square centimeters by a polar planimeter (Model WDY-500; Harbin Optical Gaging Products Co. Ltd., Harbin, Heilongjiang, China) and the two sides were averaged. The muscle and bone of each right side of the carcasses were separated for calculation of 'bone to muscle ratio'. All the necessary cutting of the carcasses was made by experienced technicians.

Rumen fluid was collected immediately after the lambs were slaughtered, and then filtered for separation of solid from liquid. Enzyme extraction from the solid-bound microbes was performed according to the methods by Manyuchi *et al.* (1992). Carboxymethyl cellulase (CMCase) activity was determined by measuring reducing sugar formation from sodium carboxymethyl cellulose (Manyuchi *et al.* 1992). Total DNA from the each rumen liquid sample was extracted as described by Chen *et al.* (2007). The primer pairs of total bacteria, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*, as described by Denman and McSweeney (2006), and Koike and Kobayashi (2001) were listed in Table 1. Species-specific real-time quantitative PCR was performed in triplicates using the ABI 7500 real time PCR system (Applied Biosystems) with fluorescence detection of SYBR green dye. Amplification consisted of an initial denature at 95°C for 15 s followed by 40 cycles of 95°C for 5 s and 60°C for 34 s. Specificity of amplified products was confirmed by melting temperatures and dissociation curves after each amplification. Amplification efficiencies for each primer pair were investigated by examining dilution series of total rumen microbial DNA template on the same plate in triplicate.

Calculation and statistical analyses

The results obtained were analyzed by one-way analysis of variance (SAS 1996). The difference of means for the

Table 1 Primers for qPCR assay

Target species	Forward / reverse	Primer sequence	Amplicon (base pairs)
Total bacteria†	F	CGGCAACGAGCGCAACCC	130
	R	CCATTGTAGCACGTGTGTAGCC	
R.. albus‡	F	CCCTAAAAGCAGTCTTAGTTGG	176
	R	CCTCCTTGCGGTTAGAACA	
R.flavefacien†	F	CGAACGGAGATAATTTGAGTTTACTTAGG	132
	R	CGGTCTCTGTATGTTATGAGGTATTACC	
F. succinogenes†	F	GTTCGGAATTACTGGGCGTAAA	121
	R	CGCCTGCCCCTGAACTATC	

†Cited from Denman and McSweeney (2006). ‡Cited from Koike and Kobayashi (2001).

Table 2 Effects of supplementary cornstarch on intake, digestion and growth performance in lambs fed a rice straw-based diet

	Supplemental cornstarch (g/day)				SEM
	0	60	120	180	
Dry matter intake (g/day)					
Rice Straw	386.2	404.8	401.4	398.1	9.82
Total	560.1[d]	632.6[c]	683.2[b]	733.9[a]	9.82
Apparent digestibility (g/kg)					
Organic matter	579[b]	593[b]	628[a]	591[b]	10.5
Neutral detergent fiber	547[a]	525[a]	519[a]	458[b]	11.8
Crude protein	655[a]	615[b]	609[b]	533[c]	11.6
Growth performance					
Initial weight (kg)	20.7	21.0	21.2	21.0	0.43
Average daily gain (g/day)	37.2[c]	61.2[b]	91.6[a]	84.4[a]	3.58
Supplementary efficiency†	–	0.40[a]	0.45[a]	0.26[b]	0.03

[a,b,c,d]Means within row with different superscripts differ ($P < 0.05$). †Ratio of the difference in weight gain of cornstarch-supplemented lambs relative to the non-supplemented lambs and the amount of cornstarch consumed. SEM, standard error of means.

treatments was tested by using Tukey Kramer multiple comparison. Quantification for *R. albus*, *R. flavefacien* and *F. succinogenes* were expressed as a proportion of total rumen bacterial 16S rDNA, according to the equation: relative quantification = $2^{-(ct\ target-ct\ total\ bacteria)}$, where Ct represents threshold cycle.

RESULTS

Intake, digestion and growth performance

Effects of supplementary starch on feed intake, digestion and growth performance are shown in Table 2. Supplementation of cornstarch showed little influence on rice straw intake, while intake of total DM was increased linearly ($P < 0.05$) with the increment of cornstarch. Apparent digestibility of OM in lambs supplemented with 120 g/day of cornstarch was significantly higher than that of other groups ($P < 0.05$), while the apparent digestibility of NDF with 180 g/day of cornstarch was significantly lower than that of other groups ($P < 0.05$). Supplementation with 60 and 120 g/day of cornstarch decreased apparent digestibility of CP ($P < 0.05$) and a further decrease was observed by 180 g/day ($P < 0.05$). Average daily weight gain (ADG) of lambs was increased by supplementary cornstarch, with the optimal performance level of 120 g/day. Supplement efficiency, calculated as the ratio of the difference in weight gain of cornstarch-supplemented lambs relative to the non-supplemented lambs and the amount of cornstarch consumed, was the highest for 120 g/day cornstarch supplementation, and lowest for 180 g/day cornstarch supplementation ($P < 0.05$).

Carboxymethyl cellulase activity and rumen microbes

Relative population of the ruminal cellulolytic bacteria and CMCase activity are shown in Table 3. *R. albus* increased at 60 g/day of cornstarch, and then decreased with additional cornstarch supplementa-

Table 3 Effects of supplementary cornstarch on carboxymethyl cellulose activity and population of the ruminal cellulolytic bacteria in rumen of lambs fed a rice straw-based diet

	Supplemental cornstarch (g/day)				SEM
	0	60	120	180	
Carboxymethyl cellulose activity (IU)	11.8[a]	10.4[a]	10.5[a]	7.0[b]	1.03
Cellulolytic bacteria (% of total rDNA)					
R.albus	0.09[b]	0.14[a]	0.07[c]	0.05[c]	0.008
R.flavefaciens	0.30[a]	0.19[b]	0.18[b]	0.05[c]	0.012
F.succinogenes	1.18[a]	0.89[b]	0.62[bc]	0.55[c]	0.015

[a,b,c]Means within row with different superscripts differ ($P < 0.05$). SEM, standard error of means. IU, Enzyme activity: releasing 1 μmol of reducing sugar per minute per gram of dry matter.

Table 4 Effects of supplementary cornstarch on serum biochemical indexes in lambs fed a rice straw-based diet

	Supplemental cornstarch (g)				SEM
	0	60	120	180	
Total protein (g/L)	65.4	64.8	63.4	62.9	1.79
Albumin (g/L)	30.0	29.2	29.2	28.6	0.55
Globulin (g/L)	36.0	36.3	34.9	34.8	1.25
Urea nitrogen (mmol/L)	9.35[a]	8.09[b]	7.09[c]	6.69[c]	0.309
Triacylglycerol (mmol/L)	0.22	0.24	0.26	0.26	0.024
Glucose (mmol/L)	2.82[b]	3.04[ab]	3.29[a]	3.37[a]	0.140

[a,b,c]Means within row with different superscripts differ ($P < 0.05$). SEM, standard error of means.

tion. Populations of both *R. flavefaciens* and *F. succinogenes* were decreased by increment of cornstarch, and the lowest values were detected in the 180 g/day of cornstarch group. Cornstarch supplementation at levels of 60 and 120 g/day showed little adverse effect on CMCase activity, while 180 g/day of cornstarch reduced it significantly ($P < 0.05$).

Serum biochemical parameter

Serum biochemical parameters for lambs consuming straw-based diet supplemented with different levels of cornstarch are shown in Table 4. Concentrations of total protein, albumin and globulin were not decreased significantly by cornstarch supplementation. Serum urea N concentration was reduced linearly by supplementary cornstarch, while serum glucose was increased by high levels of cornstarch ($P < 0.05$). Few differences in triacylglycerol were found between the treatments.

Carcass traits

Effects of cornstarch on carcass traits of Hu lambs are shown in Table 5. Slaughter weight, carcass weight, dressing percent and ribeye area were all significantly increased by supplementation of cornstarch, but the differences between three supplementary levels were not significant. Weight of kidney fat was increased with increment of energy, with the highest in 180 g/day of cornstarch group ($P < 0.05$), and lowest in the control group ($P < 0.05$). Compared to the control group, weight of bone was significantly higher in 60 and 180 g/day of cornstarch, while all the supplemented lambs produced significantly more lean meat ($P < 0.05$). Therefore, the 'bone to muscle ratio' of lambs supplemented with 120 g/day of cornstarch was the lowest among four treatments, significantly lower than that of the control group ($P < 0.05$).

DISCUSSION

Forage intake and digestibility

It is typical that forage intake was decreased by energy supplementation with cereal grains (Sanson & Clanton 1989; Martin & Hibberd 1990; Hess *et al.* 1996). However, the protein level in the majority of these works was low. DelCurto *et al.* (1990b) reported that increased supplementary energy reduced forage intake when supplement CP was low, while there was no reduction in forage intake when supplementary energy was accompanied with higher levels of CP. This is consistent with our finding, in which forage intake was not greatly affected by increasing cornstarch,

Table 5 Effects of supplementary cornstarch on carcass traits of lambs fed a rice straw-based diet

	Supplemental cornstarch (g/day)				SEM
	0	60	120	180	
Slaughter weight (kg)	21.9[b]	23.9[a]	25.4[a]	24.5[a]	0.82
Carcass weight (kg)	7.9[b]	9.6[a]	10.4[a]	9.9[a]	0.39
Dressing percent (%)	36.1[b]	40.1[a]	40.9[a]	40.4[a]	0.16
Bone (kg)	1.90[b]	2.13[a]	2.04[ab]	2.16[a]	0.065
Muscle (kg)	4.86[b]	6.50[a]	6.84[a]	6.62[a]	0.211
Bone: muscle ratio	0.39[a]	0.33[ab]	0.30[b]	0.33[ab]	0.024
Kidney fat (kg)	0.04[c]	0.05[bc]	0.06[ab]	0.08[a]	0.004
Ribeye area (cm^2)	5.89[b]	6.97[ab]	8.32[a]	7.99[a]	0.337

[a,b,c]Means within row with different superscripts differ ($P < 0.05$). SEM, standard error of means.

while total DM intake was increased linearly with starch supplementation. It was also possible that less bulk of cornstarch resulted in constant rice straw intake in the present study, as the amount of cornstarch accounted for less than 25% of total intake even at the highest supplementation level.

Results of the effect of energy supplementation as cereal grains on forage digestibility in ruminants have been varied. Most researchers reported negative effects on forage digestibility (Chase & Hibberd 1987; Sanson 1993), while there were also reports suggested no effect (Sanson & Clanton 1989) or even a positive effect (Pordomingo *et al.* 1991; Lardy *et al.* 2004). When considering response of forage digestibility to energy supplementation, it is important to differentiate the effects between the digestibilities of forage and supplement. In the current study, apparent digestibility of OM was significantly increased by 120 g/day of supplementary cornstarch, which should be primarily attributed to the high digestibility of supplementary cornstarch, rather than an evidence for improved forage utilization.

Rice straw was the largest source of NDF in the diets, apparent digestibility of diet NDF might also represent the digestibility of forage. Supplementation with 60 and 120 g/day (about 0.25% and 0.5% of BW, respectively) of cornstarch showed little effect on NDF digestibility, indicating no associative effect. However an additive effect was found between basal diet and cornstarch. Statistical significance was found when 180 g/day (about 0.75% of BW) of cornstarch was supplemented. Similarly, a model proposed by Bowman and Sanson (1996) to predict forage utilization indicated that supplemental starch would not depress the intake of low-quality forage by beef cattle until feeding levels exceeded 0.5% BW. These suggested that proper level of starch supplementation (within 0.5% of BW) is acceptable when considering forage intake and digestibility.

Carboxymethyl cellulase activity and rumen microbes

Researchers traditionally focused on ruminal pH, volatile fatty acids and other fermentation parameters (Fieser & Vanzant 2004), when they analyzed the associative effects of supplementation on forage utilization. In our study, the rumen fermentation characteristics of rumen sample were measured after the slaughtering trial. However, little difference between different treatments in ruminal fermentation parameters, such as pH, ammonia nitrogen, microbial protein, acetate and propionate was detectable, only total VFA content and butyrate percentage were linearly increased with starch supplementation (data not shown). These might mainly due to the long interval between feeding and sampling (about 16 to 18 h), or supplementing twice daily had diluted the influence of starch on rumen fermentation. However, the impact of starch supplementation on rumen fermentation was far from severe.

Although the influence of supplementary energy on rumen fermentation may be diluted with time, the structure of the rumen microbial ecosystem and related fibrolytic enzyme secretion should be relatively stable. *R. albus*, *R. flavefaciens* and *F. succinogenes* are the three species of cellulolytic bacteria of general concern when considering fiber digestion (Krause *et al.* 1999). *In vivo* studies on the effects of dietary composition on the populations of these bacteria have been limited. The data in Table 3 shows that both *R. flavefaciens* and *F. succinogenes* deceased with increment of cornstarch, and all reached their lowest values at the level of 180 g/day. *R. albus* increased with 60 g/day of cornstarch supplementation, and then went downward with more cornstarch. This indicates that high levels of starch in diets would generally reduce the proportion of major cellulolytic bacteria in rumen. CMCase, a major fibrolytic enzyme that highly correlated with fibre degradation (Silva *et al.* 1987) was only moderately influenced by 60 and 120 g/day of cornstarch supplementation, but significantly decreased ($P < 0.05$) by 180 g/day of cornstarch. This was consistent with the result of NDF degradation, and might be a reasonable explanation for the reduced NDF digestibility observed in the digestion trial.

Growth performance, carcass traits and blood indexes

Data available on energy supplementation for growing animals mostly show positive effects on growth performance (Gerrits *et al.* 1996; Schoonmaker *et al.* 2003). In the present study, the control lambs were offered an energy-limiting diet. It is clearly demonstrated that the feeding of rice straw and rapeseed meal alone was inadequate for growth of lambs (37.2 g/day live weight gain). Supplementary cornstarch increased ADG drastically, with 65, 146 and 126% higher in 60, 120 and 180 g/day of cornstarch treatments than control, respectively. These results suggested that energy supply for growing animals is critical, while too much readily fermentable energy will not be beneficial, and the proper energy supply is also related to dietary protein level. The supplement efficiencies (Table 2) were similar to those suggested in a review paper by McCollum and Horn (1990) for either protein- or energy-supplemented grazing livestock. Supplement efficiency of less than 0.33 might be a result of substitution or inefficient utilization of the supplemental nutrients (negative associative effect) (Bodine & Purvis 2003), as observed in 180 g/day of starch treatment, whereas a greater response could be a result of energy supplementation alone (positive associative effect), as the observation in 60 and 120 g/day of starch treatments. These indicated that starch supplementation may negatively affect forage utilization, but the response in animal performance can be different according to the level of supplementation. The most direct benefit that supplementary starch brought to the animal was the elevated amount of available glucose, as shown in blood glucose (Table 4). The increased amount of glucose would possibly result in more deposition of fat. However, in the present study, body conditions of lambs were far from fat, considering the fairly low 'kidney fat weight' in all treatments (Table 5).

NRC (1996) adopted an equation about efficiency of protein utilization for growing cattle based on the assumption that metabolizable protein is utilized with the same efficiency. However, dietary energy would strongly affect the efficiency of protein utilization (Gerrits *et al.* 1996; Clint 2006; Schroeder *et al.* 2006), which challenges the assumption of a constant efficiency of protein use. In the current study, the changes in protein digestion and utilization were notable. Apparent digestibility of CP was significantly decreased by supplementary cornstarch. However, major indexes for protein status, such as serum total protein, albumin and globulin were little influenced by supplementary cornstarch. Blood urea N, the most useful indicator for protein utilization in ruminants (Kohn *et al.* 2005), was significantly reduced by supplementary cornstarch, indicating higher efficiency of protein utilization. This was consistent with the results of 'bone to muscle ratio' and 'ribeye area', which both indicated that more protein was deposited in body tissue. Similar results of reduced protein digestion in digestive tracts while enhanced protein synthesis in body tissue by supplementary starch were also observed by Dror *et al.* (1969) and Fujita *et al.* (2006). Moreover, the positive effect of energy on protein synthesis overwhelmed its negative effect on digestion in this study, resulting in more protein deposition in body tissue of lambs in the starch supplemented groups. The influence of supplementary energy on utilization of protein has been mostly neglected when exploring supplementary strategies for ruminants consuming low-quality forage. Further investigations are needed to give into the impact of energy on protein utilization at the metabolism level in body tissue.

Implication

When lambs were fed on rice straw and limited rapeseed meal, forage intake was not affected by cornstarch supplementation, and apparent digestibility of NDF was only slightly decreased when cornstarch supplementation was not high (within 0.5% BW). This was associated with the change in fibrolytic enzyme activity and relative populations of cellulolytic bacteria in rumen. Though cornstarch supplementation showed no positive effect on forage utilization, it would effectively improve growth performance and efficiency of body protein deposition. For better understanding the effect of energy supplementation on growth performance, more efforts should be taken into investigating the metabolic process of protein deposition.

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