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Effects of different proportions of dietary structural and nonstructural carbohydrates on ruminal fermentation and microbial growth efficiency in sheep*

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ABSTRACT

Four sheep were used in a 4×4 Latin square design to study the effect of different dietary proportions of structural carbohydrate (SC) and nonstructural carbohydrate (NSC) (SC:NSC ratio: 0.55, 1.12, 2.25 and 5.24) on rumen fermentation and microbial growth efficiency. As the dietary SC: NSC ratio increased, ruminal pH and ammonia concentration increased ($P<0.001$), but total rumen VFA concentration decreased ($P<0.001$). When dietary SC:NSC ratio increased from 0.55 to 1.12, the molar percentage of acetate increased ($P<0.001$) and the molar proportion of propionate decreased ($P<0.001$). However, when the ratio increased from 1.12 to 5.24, there were no changed molar percentages of acetate and of propionate. Both daily microbial nitrogen production and microbial efficiency decreased with increasing dietary SC:NSC ratio.

KEY WORDS: sheep, SC : NSC, rumen fermentation, microbial growth efficiency

INTRODUCTION

The dietary fibre composition, specially the content and proportion of structural carbohydrates (SC, mainly cell wall carbohydrates) and nonstructural carbohydrates (NSC, including sugars, starch, organic acids and soluble fibre), which could alter the

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fermentable energy available for microbial protein (MCP) production, has raised interest in ruminal protein research (Meng et al., 1999; Bodine et al., 2001). Correspondingly, the Cornell Net Carbohydrate and Protein System (CNCPS) submodel divides the rumen population into two groups, NSC fermenters and SC fermenters in terms of their difference in the requirement of nitrogen for their growth. Many studies regarding the dietary NSC to SC ratio have been done in dairy and beef cattle, but there is no information available on sheep. Therefore, the objective of this study was to determine the effects of different proportions of dietary SC and NSC (ratio of SC:NSC) on ruminal fermentation characteristics and microbial growth efficiency in sheep.

MATERIAL AND METHODS

Four diets with different SC:NSC ratio (0.55, 1.12, 2.25 and 5.24) were fed to four sheep (1/2 Mongolian and 1/2 Dorset Down; averaged body weight 36 kg), cannulated in the rumen and duodenum in a 4×4 Latin square design (Table 1; DM basis). The animals were housed separately and fed twice (8.00 and 16.00) daily. The diets were formulated based on NRC (1985) with identical contents of CP, Ca and P, but different contents of fibre and total nonstructural carbohydrates.

Table 1. Ingredient and chemical composition of four complete diets

Item	Dietary ratio of SC : NSC			
	0.55	1.12	2.25	5.24
Ingredient, %				
maize meal	57.59	36.15	14.68	0
soyabean meal	12.20	8.00	4.00	0
blood corpuscle protein meal	1.70	2.50	3.00	3.50
stone meal	1.40	0.98	0.40	0
bone meal	0.56	0.77	1.09	1.30
mineral and vitamin premix[1]	0.10	0.10	0.10	0.10
salt	0.40	0.40	0.40	0.40
vegetable oil	1.00	1.00	1.00	1.00
maize stalks	0	8.00	20.00	0
rice straw	20.00	12.00	0	12.65
soya hulls	0	25.00	50.00	75.00
lucerne meal	5.00	5.00	5.00	5.00
Chemical composition, %				
OM	94.28	93.25	91.65	89.23
CP	13.98	13.70	14.01	13.98
NDF	39.79	46.13	54.38	57.46
ADF	12.81	22.83	35.06	41.99
Ca	0.79	0.79	0.74	0.75
P	0.35	0.34	0.34	0.33

per kg contained, IU: vit. A 3000, vit. D_3 1200, vit. E 10; mg: Cu 8, Fe 50, Zn 30, Mn 40, Co 0.1, Se 0.2, I 0.5

The rumen digestibility of DM, OM, NDF and ADF was carried out using Cr-labelled soyabean hulls as an external marker of solid phase, and PEG-4000 as a marker of liquid phase. Each experimental period contained 15 days for adaptation and 5 days for sampling. During the sampling period, 100 ml rumen digesta were collected daily through rumen and duodenum cannulae. Ruminal pH was measured immediately using a pH meter equipped with a glass electrode. A 10-ml aliquot of ruminal fluid was acidified (1 ml 20% H_2SO_4/50ml ruminal fluid) and stored at -20°C. NH_3-N concentration was analysed according to Broderick and Kang (1980), and volatile fatty acid (VFA) was determined according to Li and Meng (2006) after centrifuged at 10,000 *g* for 15 min. The total 5-day rumen digesta samples were pooled, homogenized, and strained through two layers of cheesecloth and then centrifuged at 500 *g* for 10 min to remove feed particles and protozoa. The supernatant fluid was centrifuged again at 20,000 *g* for 20 min. After discarding the liquid phase, the bacterial pellet was resuspended and recentrifuged again after washing. The final pellet was dispersed in distilled water and then lyophilized for further analysis, such as dry matter (DM) and other rumen fermentation parameters.

The samples of feed, isolated microorganism, and digesta of rumen and duodenum were analysed for dry matter (DM), organic matter (OM), Cr, PEG and nitrogen (AOAC, 1990). The content of Cr in the rumen NDF and ADF were determined according to Van Soest et al. (1991). Content of purines in bacteria was measured by the procedure of Zinn and Owens (1986). Microbial N of digesta was calculated based on the RNA-to-N ratio of isolated microorganism in combination with the RNA content of the digesta. Microbial protein synthesis efficiency was expressed as grams of microbial N per kg of OM truly digested.

Data were analysed using the GLM procedure of SAS (1999). Significance was declared as $P<0.05$.

RESULTS AND DISCUSSION

Increased dietary SC : NSC ratio resulted in a linear decrease in the digestilities of DM and OM ($P<0.001$; Table 2). This result is in consistent with the reports of Stokes et al. (1991) and Hristov and Ropp (2003). The result might be due to the lower digestibility of SC compared to NSC. With increased SC : NSC ratios, there was a quadratic increase in digestibilities of NDF and ADF ($P<0.001$). The highest digestiblity occurred when dietary SC : NSC ratio was 2.25. It seemed likely that higher dietary SC : NSC ratio would improve the growth of fibrolytic microorganisms against the organisms fermenting NSC; however, this advantage may decrease when dietary ratio of SC to NSC reached a certain maximum.

Table 2. Effect of dietary SC: NSC ratio on true digestibilities of DM, OM, NDF and ADF[1]

Item	Dietary SC : NSC ratio				SEM	P	
	0.55	1.12	2.25	5.24		L	Q
DMD	68.81[a]	65.17[b]	60.89[c]	55.19[d]	1.119	0.001	0.376
OMD	72.32[a]	67.06[b]	63.03[c]	57.96[d]	0.873	0.001	0.915
NDFD	37.09[d]	50.73[c]	60.06[a]	55.31[b]	0.739	0.001	0.001
ADFD	33.59[d]	46.97[c]	56.12[a]	53.11[b]	1.156	0.001	0.001

[1] L - linear effect of dietary SC : NSC ratio; Q - quadratic effect of SC : NSC ratio
[a, b, c] means in the same row with different superscripts differ significantly (P<0.05)
DMD - true digestibility of DM; OMD - true digestibility of OM; NDFD - true digestibility of NDF; ADFD - true digestibility of ADF

As dietary SC : NSC ratio increased, the pH of rumen contents increased (Table 3). This result might be attributed to the decreased organic acids in the rumen resulting from the decreased growth of rumen microorganisms fermenting NSC. Additionally, rumen pH could affect ruminal fibre digestion. It was known that the optimal pH for fibre digestion was between 6.7 and 7.1, and fibre digestion would be weakened if ruminal pH declined below 6.2 (Caton and Dhuyvetter, 1997). The concentration of NH_3-N which was used for microbial protein synthesis increased as SC:NSC increased, so the free ammonia concentration increased with more dietary SC and less NSC.

Table 3. Effect of dietary SC: NSC ratio on ruminal pH, ammonia and VFA concentration[1]

Item	Dietary SC:NSC ratio				SEM	P	
	0.55	1.12	2.25	5.24		L	Q
pH	6.19[d]	6.38[c]	6.50[b]	6.64[a]	0.024	0.001	0.297
NH_3-N, mg/dl	2.40[d]	5.69[c]	8.88[b]	10.9[a]	0.373	0.001	0.123
Total VFA, mmol/l	72.5[a]	65.3[b]	59.3[c]	50.7[d]	1.744	0.001	0.704
Individual VFA, molar %							
acetate	47.4[b]	72.1[a]	72.8[a]	75.0[a]	0.971	0.001	0.001
propionate	33.9[a]	19.2[b]	17.3[b]	18.2[b]	2.221	0.001	0.001
butyrate	11.8[a]	9.8[ab]	8.9[ab]	7.7[b]	0.874	0.006	0.671

[1] L - linear effect of dietary SC : NSC ratio; Q - quadratic effect of SC:NSC ratio
[a,b,c] means in the same row with different superscripts differ significantly (P<0.05)

Increasing dietary SC : NSC ratio caused a decreased total VFA production (P<0.001). Febel et al. (2000) also reported that there was less total VFA production when dietary NSC level decreased from 38 to 23% in the cannulated sheep. This result is also in accordance with the result of DM and OM digestibilities. When SC : NSC increased from 0.55 to 1.12, acetate molar percentage was significantly

increased, while propionate molar percentage was decreased (P<0.001); however, further increasing dietary SC:NSC ratios did not result in any change in molar percentages of acetate and propionate. Changes in individual VFA molar percentages reflected a shift or alteration in microbial species and microbial metabolism toward their dietary carbohydrate fermentation.

Increased dietary SC:NSC ratio resulted in a significant decrease in daily microbial nitrogen production (DMNP) and microbial growth efficiency (MOEFF) (P<0.001; Table 4). The result of decreased DMNP was in agreement with other study (Stokes and Hoover, 1991). In contrast, no significant differences were observed on DMNP and MOEFF as dietary NSC decreased from 48 to 40% in lactating cows (Hristov and Ropp, 2003). This discrepancy may be due to the different compositions and dietary ratio of NSC and SC. Microbial growth in the rumen requires energy derived from ruminal fermentation and ammonia. In the present study, NH_3-N concentrations were all higher than the suggested threshold values (5 mg/dl) for maximum microbial growth. Therefore, energy is likely a key factor limiting maximum microbial growth. It has been suggested that although dietary fat and protein can contribute to the total energy, the main source of ATP and the determinant content for microbial protein synthesis is the carbohydrate fermented in the rumen (Karsli, 2003; Oba and Allen, 2003). Rumen microbes that ferment SC grow slowly and utilize ammonia as the N source for their protein synthesis. Rumen microbes that ferment NSC grow more rapidly than those SC fermenters, and utilize either ammonia or amino acids as the N source. The growth rates of both groups are directly proportional to the rate of carbohydrate digestion, as long as a suitable N source is available (NRC, 2001). When dietary SC:NSC ratio was high, the energy came from rumen fermentation could not meet the maximum microbial growth. As dietary SC: NSC ratio decreased, the increased requirements of NSC and energy were used for microbial growth in order to increase DMNP and MOEFF.

Table 4. Effect of dietary SC and NSC ratio on rumen microbial production and microbial efficiency[1]

Proximal duodenum digesta N	Dietary SC:NSC ratio				SEM	P	
	0.55	1.12	2.25	5.24		L	Q
Total N,g/d	32.41[a]	29.57[b]	27.54[c]	25.21[d]	0.375	0.001	0.516
DMNP [d], g/d	26.72[a]	23.45[b]	20.74[c]	17.15[d]	0.408	0.001	0.707
NANMN [e], g/d	5.69[d]	6.12[c]	6.80[b]	8.41[a]	0.143	0.001	0.003
MOEFF[f]	30.66[a]	29.24[b]	28.13[c]	25.25[d]	0.351	0.001	0.065

[1] L - linear effect of dietary SC:NSC ratio; Q - quadratic effect of SC:NSC ratio

[a, b, c] means in the same row with different superscripts differ significantly (P<0.05)

[d] daily microbial nitrogen production; [e] non-ammonia non-microbial N; [f] microbial efficiency expressed as grams of microbial N/kg of organic matter truly digested

CONCLUSIONS

Dietary structural:nonstructural carbohydrates (SC:NSC) ratio significantly influenced the rumen digestibilities of DM, OM, NDF and ADF, and ruminal fermentation parameters. As the dietary SC:NSC ratio increased, ruminal pH and ammonia concentration increased, but total rumen VFA concentration decreased. Both daily microbial nitrogen production and microbial efficiency decreased with increasing dietary SC:NSC ratio.

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