동물자원지 : 46(1) 61~68, 2004 J. Anim. Sci. & Technol. (Kor.) 46(1) 61~68, 2004

The Effects of Condensed Molasses Solubles(CMS) / Molasses Mixtures on Ruminal Microbial Protein Synthesis

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Condensed Molasses Solubles(CMS) / 당밀 혼합물이 반추위 미생물 단백질 합성량에 미치는 영향

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적 요

본 연구는 monosodium glutamate의 생산 후 발생되는 부산물인 condensed molasses solubles(CMS)가 반추가축의 질소 공급원으로서 반추위 미생물 단백질 합성에 미치는 효과를 조사하기 위하여 수행하였다. 반추위 canulae가 부착된 4마리의 비착유소를 4 × 4 라틴 방각법에 적용하여 실험을 수행하였으며, 4개의 처리구는 다음과 같다. (1) 기초사료(앞착된 보리 3kg/일과 보리짚의 자유채식), (2) 기초사료에 당밀 200g/일 그리고 물 300g/일 첨가, (3) 기초사료에 당밀 200g/일, CMS 100g/일 그리고 물 200g/일 첨가 (4) 기초사료에 당밀 200g/일, CMS 200g/일, 그리고 물 100g/일 첨가. CMS의 첨가수준에 따른 반추위내 발효양상은 처리구간에 유의적인 차이가 없었으나, CMS를 200g/일을 첨가한 처리구가 다른 처리구들에 비해 반추위내 미생물 단백질 합성량의 표시로서 이용된 allantoin/creatinine의 비율을 증가시키는 경향이 나타났다($P < 0.10$).

(**주요어** : Allantoin, Condensed molasses solubles, Rumen, By-products)

I. INTRODUCTION

The rate of ammonia utilization is strongly dependent on the amount of available energy source in the diet and the capacity of the microorganism to utilize that energy in an efficient way(Johnson, 1976). In turn, the fast rate of ammonia production from NPN source needs to be synchronized with a fast rate of carbohydrate breakdown, like that from readily fermentable carbohydrate sources in rumen, for maximum utilization of ammonia.

Although the most widely used source of NPN is urea, by-products from fermentation of molasses have been used as a source of NPN for ruminants. The condensed molasses solubles (CMS) used in the present experiment is a by-product from the fermentation of molasses to produce monosodium glutamate and contained high levels of ammonia nitrogen. There were several reports which have shown the effects of condensed molasses solubles(CMS) on nitrogen retention in the rumen(Karalazos and Swan, 1977; Chen et al., 1981; Potter et al., 1985). However, the composition of CMS, especially its nitrogen content, was very variable in each

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experiment and also there were no reports using the specific by-product from monosodium glutamate production. In addition, due to increased feed prices recently, the demand for cheaper feed sources is increasing. So it will be advantageous for farmers or feed companies to use CMS. But prior to using CMS, uncertainties about variations in its chemical composition and variable levels of dietary inclusion must be considered.

In view of these observations, it was decided to investigate the effects of CMS/molasses mixture on microbial protein synthesis of non-lactating dairy cows given barley straw by using the ratio of the urinary output of purine derivatives to creatinine concentrations as an index of microbial protein supply to the animal.

II. MATERIALS AND METHODS

1. Animals and their management

Four non-lactating Holstein cows fitted with permanent rumen cannulas were used. The animals were housed individually in metabolism stalls with water freely accessible. Average body weight of the animals was approximately 560kg(range 502 to 658 kg). Food was given at 7:00 and 15:00 each day. The intake of barley straw was determined daily and feeding was adjusted to ensure a feed refusal of approximately 15% of that offered.

2. Experimental treatments and design

The treatments were applied in a sequence according to a 4×4 Latin square design with four treatments and four 14-day periods including a 14-day preliminary period. The experimental treatments were (1) basal diet consisting of barley straw *ad libitum* and 3 kg/d of rolled barley, (2) basal diet plus 200 g/d molasses and 300 g/d water, (3) basal diet plus 200 g/d molasses, 100 g/d CMS and 200 g/d water, (4) basal diet plus 200 g/d molasses, 200 g/d CMS and 100 g/d water. The chemical compositions of the dietary ingredients are shown in Table 1 and 2. Samples of rumen liquor

Table 1. The chemical composition of barley, barley straw, condensed molasses solubles (CMS) and molasses

	Barley	Barley straw	CMS	Molasses
	% DM	% DM	% DM	% as fed
Dry matter	91.0	86.6	59.6	ND*
CP	12.4	3.1	57.5	4.1
Ammonia-N	ND	ND	8.05	ND
Sugar	ND	ND	ND	47.2
NDF	ND	86.0	ND	ND
ADF	ND	57.5	ND	ND

*Not determined.

Table 2. The amino acid composition of (%, DM) of condensed molasses solubles(CMS)

Amino acids	
Aspartic acid	1.73
Glutamic acid	7.76
Serine	0.28
Histidine	0.29
Glycine	0.40
Threonine	0.33
Arginine	0.42
Alanine	2.01
Tyrosine	0.43
Methionine	0.20
Valine	2.72
Phenylalanine	0.45
Isoleucine	0.39
Leucine	0.62
Lysine	0.46

were taken at 09:30, 11:00, 12:00, 13:00 and 15:00 on the last day of each period. The pH of the ruminal fluids was taken immediately after they were withdrawn. The samples were quickly strained through muslin cloth, centrifuged to remove solids at 1,000 g for 5 minutes and the supernatant fluid was stored at -20℃ until analysed. The complete output urine was collected via a bladder catheter into 500 ml of 4 M H_2SO_4 during the last 3 days of each period.

3. Chemical analysis

A known weight of sample was oven dried at 100℃ to constant weight and the dry matter(DM) expressed as a percentage of fresh weight. The nitrogen content of feed samples was measured by a Kjeldahl procedure using a Kjeltec Auto 1030 analyser(Foss UK Ltd, Didcot, Oxon, UK). Analyses of neutral-detergent fiber(NDF) and acid-detergent fiber(ADF) were done by the methods of Van Soest and Wine(1967) and Van Soest(1963), respectively. Total soluble sugars were determined by a method of Somogyi (1945). The amino acid composition of CMS was determined by the modified method of Umagat et al.(1982) using HPLC with ortho-phthaldialdehyde/2-mercaptoethanol precolumn derivatization. Ruminal ammonia N was analysed by a colorimetric method(Chaney and Marbach, 1962). Total and individual volatile fatty acids in rumen fluid was analysed by the method of Cottyn and Boucque(1968). The concentration of creatinine in urine was determined using color reagents in a commercial kit(Sigma, Dorset, UK). Allantoin was determined by the method of Borchers(1977) and to convert xanthine, hypoxanthine and uric acid to allantoin, the enzymic procedure(Fujihara et al., 1987) was used. Urea-N in urine was analysed by a colorimetric method(Watt and Chrisp, 1954). Urea was extracted from the urine with approx. 0.02 M HCl to inhibit the activity of any urease naturally present and color was removed with charcoal and nitrogenous substances were precipitated with zinc ferrocyanide.

4. Statistical analysis

For statistical analysis, mean values for feed intake were taken for the last 7 days of each experimental period. The results were subjected to analysis of variance and simple regression analysis to examine the treatment effects using Genstat 5(Lawes Agricultural Trust, 1990). All differences between treatments were inspected by the least significant difference(LSD) method at the 10 % level.

Ⅲ. RESULTS AND DISCUSSION

The effects of supplementation of molasses and the CMS/molasses mixture on ruminal fermentation are given in Table 3 to 6. Although there was a significant difference in daily mean ruminal pH between treatments($P < 0.05$), it remained at surprisingly high levels in all treatments and showed little variation during the day between treatments(Table 3). This is probably because the cows consumed diets having a high ratio of a low quality roughage to concentrate and this led to a high saliva secretion, which maintained ruminal pH at high levels. According to Nolan and Leng(1972), the amount of saliva produced can be greatly influenced by the physical structure of the diet, i.e. it increases with increasing proportion of roughage in diet. Again, the animals ate their food steadily throughout the day, leading to a fairly constant concentration of total VFA in the rumen(Table 4). Although Karalazos and

Table 3. The daily pattern of variation in ruminal pH

	Basal[1]	Molasses[2]	Molasses/CMS mixture[3]		s.e.d.[4]	P value[5]
			1	2		
Time (h) after feeding						
Prefeeding(0)	7.28	7.43	7.40	7.39	0.121	0.653
0.5	7.06	7.29	7.31	7.18	0.130	0.310
2	7.14	7.22	7.37	7.22	0.075	0.112
3	7.12	7.25	7.36	7.23	0.076	0.118
4	7.29	7.29	7.36	7.33	0.108	0.952
Mean	7.18[a]	7.29[b]	7.36[c]	7.27[b]	0.026	0.005

[1] The basal diet contained 3 kg/d barley and barley straw *ad libitum*.
[2] Basal diet plus 200 g/d molasses and 300 g/d water.
[3] The mixture 1 consisted of the basal diet supplemented with 200 g/d molasses, 100 g/d CMS and 200 g/d water ; The mixture 2 consisted of the basal diet supplemented with 200 g/d molasses, 200 g/d CMS and 100 g/d water.
[4] Standard error of differences.
[5] Statistical significance of treatment effects by *F*-test.

Table 4. The daily pattern of variation in ruminal concentration(mmol/l) of total VFAs

	Basal[1]	Molasses[2]	Molasses/CMS mixture[3]		s.e.d.[4]	P value[5]
			1	2		
Time (h) after feeding						
Prefeeding(0)	69	70	74	67	6.9	0.804
0.5	70	76	77	80	7.8	0.658
2	76	77	80	79	8.1	0.961
3	75	79	78	75	5.9	0.868
4	72	77	77	76	4.8	0.707
Mean	73	76	77	75	4.1	0.752

[1] The basal diet contained 3 kg/d barley and barley straw *ad libitum*.
[2] Basal diet plus 200 g/d molasses and 300 g/d water.
[3] The mixture 1 consisted of the basal diet supplemented with 200 g/d molasses, 100 g/d CMS and 200 g/d water ; The mixture 2 consisted of the basal diet supplemented with 200 g/d molasses, 200 g/d CMS and 100 g/d water.
[4] Standard error of differences.
[5] Statistical significance of treatment effects by *F*-test.

Swan(1977) reported that the total concentration of VFA in the rumen was not affected by CMS treatments, their results showed that the concentration of butyric acid was increased at the expense of propionic acid, which are different from those in the present study where molasses/CMS treatments did not affect the concentration of individual VFA(Table 5).

There was no difference in the concentration of ammonia-N in the rumen between treatments(Table 6), although the intake of nitrogen in molasses/CMS mixture treatments was high-

Table 5. The molar proportion(mmol/mol) of individual VFA in the rumen(daily mean values)

	Basal[1]	Molasses[2]	Molasses/CMS mixture[3]		s.e.d.[4]	P value[5]
			1	2		
Acetic acid	639	621	634	634	19.3	0.811
Propionic acid	166	192	177	196	18.4	0.420
Isobutyric acid	14	8	14	5	4.5	0.269
Butyric acid	149	152	143	141	11.7	0.759
Isovaleric acid	18	15	18	14	1.6	0.105
Valeric acid	15	12	14	11	1.4	0.129

[1] The basal diet contained 3 kg/d barley and barley straw *ad libitum*.
[2] Basal diet plus 200 g/d molasses and 300 g/d water.
[3] The mixture 1 consisted of the basal diet supplemented with 200 g/d molasses, 100 g/d CMS and 200 g/d water ; The mixture 2 consisted of the basal diet supplemented with 200 g/d molasses, 200 g/d CMS and 100 g/d water.
4 Standard error of differences.
5 Statistical significance of treatment effects by *F*-test.

Table 6. The daily pattern of variation in ruminal concentration(mg/l) of ammonia-N

	Basal[1]	Molasses[2]	Molasses/CMS mixture[3]		s.e.d.[4]	P value[5]
			1	2		
Time (h) after feeding						
Prefeeding(0)	77	77	59	79	10.3	0.303
0.5	95	85	100	108	13.6	0.487
2	91	74	87	86	12.0	0.547
3	85	73	83	95	11.1	0.389
4	95	72	69	78	9.2	0.124
Mean	88	76	79	89	6.9	0.271

[1] The basal diet contained 3 kg/d barley and barley straw *ad libitum*.
[2] Basal diet plus 200 g/d molasses and 300 g/d water.
[3] The mixture 1 consisted of the basal diet supplemented with 200 g/d molasses, 100 g/d CMS and 200 g/d water ; The mixture 2 consisted of the basal diet supplemented with 200 g/d molasses, 200 g/d CMS and 100 g/d water.
4 Standard error of differences.
5 Statistical significance of treatment effects by *F*-test.

er than that of control and molasses treatment (Table 7). Presumably high rates of absorption of ammonia and high rates of urea recycling might have contributed to the constant concentration of ammonia in the rumen. Some support for this interpretation comes from the output of total nitrogen in urine. Although there were no significant differences between treatments, the total nitrogen in urine was higher in molasses/CMS mixture treatments than in control and molasses treatment(Table 7). Owens and Richard(1988) reported that, at high pH (above 7), the rate of ammonia absorption was high because the un-ionized ammonia more readily penetrates the lipid surface of the mucosa and vice versa. Again, Smith(1975) concluded that, at ruminal pH values less than 6.5, there was little evidence of appreciable

Table 7. Intake and utilization of nitrogen

	Basal[1]	Molasses[2]	Molasses/CMS mixture[3]		s.e.d.[4]	P value[5]
			1	2		
Intake(DM basis)						
Barley straw (kg/d)	4.6	4.2	4.3	4.6	0.26	0.382
Nitrogen (g/d)	85.7	74.1	93.8	105.0		
Nitrogen excretion in urine						
Total-N (g/d)	37.9	36.7	45.1	47.4	7.30	0.447
Urea-N (g/d)	16.7	15.4	18.5	19.1	3.89	0.777
Ammonia-N (g/d)	13.8	12.0	17.9	20.1	3.55	0.211
Allantoin/creatinine ratio	1.43[a]	1.51[a]	1.45[a]	1.68[b]	0.079	0.085

[1] The basal diet contained 3 kg/d barley and barley straw *ad libitum*.
[2] Basal diet plus 200 g/d molasses and 300 g/d water.
[3] The mixture 1 consisted of the basal diet supplemented with 200 g/d molasses, 100 g/d CMS and 200 g/d water ; The mixture 2 consisted of the basal diet supplemented with 200 g/d molasses, 200 g/d CMS and 100 g/d water.
[4] Standard error of differences.
[5] Statistical significance of treatment effects by *F*-test.

absorption of ammonia from the rumen. Urea can be recycled and used as a source of nitrogen for the rumen microorganisms. Plasma urea enters the rumen by two routes-with saliva and by simple diffusion through the rumen wall (Owens and Richard, 1988). The extent to which urea is returned via saliva appears to be directly related to the blood urea concentration and to the amount of saliva produced.

In ruminants, urinary excretion of purine derivatives(allantoin, uric acid, xanthine and hypoxanthine) reflects the absorption of microbial purines and can be used as an index of microbial protein supply(Chen et al., 1995). In the present experiment, when the allantoin/creatinine ratio was used as an index of microbial protein production(Table 7), there was a suggestion of some benefit with the higher level of inclusion of CMS($P < 0.10$). The results from several studies in which not only different kinds of CMS, but also different kinds of basal diets were used are very variable. Karalazos and Swan(1977) reported that the apparent digestibility of dry matter, organic matter and gross energy in sheep were not affected when 10 % or 20 % of barley was replaced by CMS. They also concluded that microbial protein synthesis was not affected by treatments. But there were significant differences between treatments in rumen ammonia concentration(55 mg/l and 290 mg/l for the control and the CMS treatment, respectively). According to this result, nitrogen from CMS might be poorly utilized by rumen microbes. But Chen et al.(1981) reported that urinary nitrogen excretion with CCMS(citrus condensed molasses solubles) was lower than with control when dry corn and soybean meal were replaced by 10 % and 20 % of CCMS, suggesting that nitrogen from CCMS was well utilized. In this study, CCMS had no effect on organic matter digestibility in lams, but crude fiber and ether extract digestibility increased.

No clear picture emerges from the results of this experiment. The much higher than expected intakes of straw resulted in a much higher

roughage to concentrate ratio than planned and the associated effects on ruminal pH did not provide the most favorable conditions for efficient microbial capture of ammonia in the rumen. It is also worth remembering that the differences between treatments in the nitrogen content of the diets were quite small. It may be that the effects of these small differences on ruminal concentrations of ammonia (and hence on microbial protein synthesis) were masked by the effects of urea recycling to the rumen.

Ⅳ. ABSTRACT

An experiment was conducted to evaluate condensed molasses solubles(CMS, a by-product from monosodium glutamate production) as a source of nitrogen for ruminant with particular reference to its effects on microbial protein synthesis. Four non-lactating dairy cows fitted with rumen cannulas were used in a 4 × 4 Latin square with 14-day periods. The four treatments were (1) basal diet consisting of barley straw *ad libitum* and 3 kg/d of rolled barley, (2) basal diet plus 200 g/d molasses and 300 g/d water, (3) basal diet plus 200 g/d molasses, 100 g/d CMS and 200 g/d water, (4) basal diet plus 200 g/d molasses, 200 g/d CMS and 100 g/d water. Ruminal pH remained at high levels and showed little variation during the day between treatments. The concentration of total and individual VFA in the rumen was similar between treatments. There was no difference in the concentration of ammonia in the rumen between treatments, although the intake of nitrogen in molasses/CMS mixture treatments was higher than that of control and molasses treatment. But there was a suggestion of an increased synthesis of microbial protein with the higher level of inclusion of CMS when the allantoin/creatinine ratio was used as an index of microbial protein production($P < 0.10$).

(**Key words** : Allantoin, By-products, Condensed molasses solubles, Nitrogen, Rumen)

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(접수일자 : 2003. 10. 23. / 채택일자 : 2003. 12. 24.)