THE EFFECT OF MOLASSES SUPPLEMENTATION ON RUMEN FERMENTATION, MICROBIAL PROTEIN SYNTHESIS AND NITROGEN RETENTION IN SHEEP KEPT UNDER HIGH AMBIENT TEMPERATURE AND FED UREA-TREATED BARLEY STRAW

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SUMMARY

This experiment was designed to examine the possibility that soluble carbohydrate was deficient in barley straw given to the sheep. A different level of molasses in the dry matter (DM) of urea-treated barley straw was fed to lambs at 40±3 ^{0}C and at 40-50% relative humidity for a 7 week period. Sixteen Merino sheep were fed a basal diet of urea treated chaffed barley straw with one of 4 level of molasses supplements: (1) 0% (control), (2) 6%, (3) 12%, and (4) 18%. All diets were supplemented with minerals and vitamins. Feed was offered at 09.00 and 16.00 h daily and drinking water was available *ad libitum*. The parameters recorded were rumen ammonia nitrogen (N) concentration, rumen pH, rumen volatile fatty acid (VFA) concentration, microbial protein synthesis (MPS) and nitrogen content in the feed, faeces and urine.

There was no significant effect of molasses on rumen ammonia concentration (P>0.05). The means of ammonia N concentration were 125±6.1 and 192±5.8 mg NH_3-N/L before and 2 h after feeding. Rumen pH was also not affected by treatment (P>0.05) and averaged 6.7±0.04.

Total VFA concentration and butyric acids as a percentage of total VFA in the rumen fluid increased progressively with increasing the levels of molasses in the diet. The lowest levels, 53.1±2.1 mmol/L and 6.1±0.21% were recorded in the controls and the highest levels (70.9±2.89 mmol/L and 10.9±0.2%, respectively) in the diet with 12 and18% of molasses. In contrast, as the molasses level increased in the diet, the proportion of acetic acid declined from 75.0±0.6 to 71.1±1.1%. The proportion of propionic acid (16.1±0.29%) was not affected by level of molasses (P>0.05).

An increase in MPS from the rumen was recorded as the molasses level increased (P<0.05), from 3.4±0.35 g N/d in controls to 4.6±0.07 g N/d at 18% molasses. All sheep had positive N retention, but the level of molasses did not affect N intake, total N excretion (faecal and urinary) as well as N retention (P>0.05; means of 8.6±0.21, 3.3±0.09, 3.7±0.08, 1.6±0.2 g/d, respectively).

Key Words : Molasses, rumen, microbial protein synthesis, sheep, barley straw

PENGARUH SULEMENTASI MOLASSES TERHADAP FERMENTASI RUMEN, SINTESIS PROTEIN MIKROBIA, DAN RETENSI NITROGEN PADA SAPI YANG DIBERI PAKAN JERAMI BARLEY DENGAN PERLAKUAN UREA DAN DIPELIHARA PADA KONDISI LINGKUNGAN TEMPERATUR TINGGI

RINGKASAN

Penelitian ini dilaksanakan untuk menguji kemungkinan adanya kekurangan kandungan zat karbohidrat yang mudah larut pada jerami barley yang diberikan kepada domba. Level tetes yang berbeda dalam berat kering pada jerami barley yang telah ditambahi urea, diberikan kepada domba yang dikandangkan pada suhu 40±3 ^{0}C dengan kelembaban relatif sebesar 40-50% selama 7 minggu. Parameter yang diamati meliputi konsentrasi amonia N pada rumen, pH rumen, konsentrasi VFA pada rumen, produksi protein mikroba, dan kandungan nitrogen pada pakan, feses, dan urin.

Pengaruh dari level tetes terhadap konsentrasi ammonia N pada rumen adalah tidak nyata, dengan rataan sebesar 125±6,1 dan 192±5,8 mg NH_3-N/L sebelum (0 jam) dan 2 jam setelah pemberian pakan. pH cairan rumen juga tidak dipengaruhi secara nyata oleh perlakuan (P>0,05) dengan rataan 6,7±0,04.

Konsentrasi total VFA dan prosentase asam butirat dalam cairan rumen meningkat seiring dengan meningkatnya level dari tetes di dalam pakan (P<0.05). Konsentrasi yang paling rendah adalah 53,1±2,1 mmol/L dan 6,1±0,21% untuk ternak tanpa mendapatkan tetes dan konsentrasi tertinggi adalah 70,9±2,89 mmol/L dan 10,9±0,2% untuk pakan dengan tambahan 18% tetes. Sebaliknya, proporsi asam asetat menurun dari 75,0±0,6 menjadi 71,1±1,1% seiring dengan meningkatnya level tetes dalam pakan. Proporsi asam propionat (16,1±0,29%) tidak dipengaruhi secara nyata (P>0,05) oleh level pemberian tetes.

Sintesis protein mikroba dalam rumen secara nyata (P<0,05) meningkat seiring dengan meningkatnya level tetes, dari 3,4±0,35 g N/hari pada ternak kontrol menjadi 4,6±0,07 g N/hari pada ternak dengan penambahan tetes sebesar 18%. Semua domba perlakuan mengalami retensi N yang positif, tetapi level tetes dalam pakan tidak berpengaruh secara nyata (P<0,05) terhadap konsumsi N, total ekskresi N (melalui feses dan urin) atau retensi N (P>0,05; dengan rataan masing-masing sebesar 8,6±0,21, 3,3±0,09, 3,7±0,08, dan 1,6±0,2 g/hari.

Kata Kunci : Tetes, rumen, sintesis protein mikroba, domba, dan jerami barley

INTRODUCTION

The metabolisable energy intake of ruminants given roughages depends largely upon their intake and digestibility. Microorganisms in the rumen are primarily fermenters of plant materials. To digest plant carbohydrates from straw,

these organisms require a balanced supply of sources of available energy, nitrogen and minerals to maximise their metabolism and growth (Hegarty *et al*., 1996). Under those conditions their overall digestive ability and the supply of nutrients (primarily VFAs and microbial protein) to the host is also optimised.

Leng (1990) suggested that when the energy substrate is mainly in the form of fibrous materials, supplementation of ruminants with urea and minerals is unlikely to support reasonable levels of production. Therefore, to achieve improved digestibility on low quality roughage it may require the addition of some readily available energy sources, as well as N sources. The aim of this experiment was to establish the level of molasses in the diet to achieve this effect on barley chaff sprayed with 2% urea in relation with rumen fermentation, nitrogen retention and microbial protein synthesis.

MATERIALS AND METHODS

Location and time

This experiment was carried out at the animal house and at laboratory of the Department of Animal Science of the University of New England, Armidale Australia during the period August to October 1997.

Animals, experimental design and diets

Sixteen Merino lambs were penned individually in floor pens (0.75 x 2.10 m) in a climate chamber set at 20±3 (SE) ^{0}C for the first week. From the second week, mean room temperature was increased by 3 ^{0}C each day until 40±3 ^{0}C was reached. Relative humidity was set at 40-50% and lighting was provided from

06.00 to 18.00 h daily. During the first two weeks, the lambs were allowed to adapt to experimental feeding (barley chaff *ad libitum* in individual feeders), and were then divided randomly into four treatment groups (n=4) based on straw consumption over the previous seven days.

The diet was chaffed barley straw, separate portions of which were fed at 09.00 and 16.00 h daily. Molasses was mixed with urea and water (in ratio 1:0.5:4 W/W/V) then sprayed onto the barley straw in a large mixer. The amount of feed offered each day was set at 20% more than that consumed by the same sheep on the previous day. The treatments imposed were: (1) 0% molasses (control), (2) 6% molasses (3) 12% molasses and (4) 18% molasses. The basal diet was supplemented with 2% urea, sulfurs as Na_2SO_4 (2 g S/kg basal diet) and Pfizer 422 mineral and vitamin supplement (1 g/kg basal diet). Fresh drinking water was freely available at all times and its intake was recorded at 09.00 h daily.

Data was collected from weeks 3 to 7. From week 5 to week 7, the lambs were moved into metabolism crates. Total collection of faeces to measure total and straw dry matter digestibility (DMD) were made over the last 7 consecutive days, after the lambs had been allowed for one week to adapt to the conditions of the metabolism cages.

Measurements and chemical analysis

Samples of ruminal fluid were taken through a stomach tube using a 140-ml syringe connected to a plastic tube before feeding (0 h) and at 2 hours after the 09.00 feeding on day 28. Samples (15 ml) of rumen fluid for chemical analysis were drawn immediately into tubes containing 0.2 ml of 98% H_2SO_4, while

ruminal pH was measured on fresh fluid immediately after ruminal sampling. Each sample was centrifuged at approximately 3000 rpm for 10 minutes and the resultant supernatant was stored at –20 ^{0}C prior to an analysis for VFA and ammonia N. Nitrogen retention, for individual sheep, was calculated as the difference between N intake and N excretion in faeces and urine.

Total urine was also collected and sampled daily during the last 7 days of experimental period. Thirty ml of urine sub-samples from each sheep was taken daily for allantoin analysis and the pH was reduced below 3 by adding 3 to 5 drops of concentrated sulphuric acid. Fresh urine sub-samples were also taken for N analysis.

Ammonia N concentrations in the rumen fluid of each sheep at each sampling time were determined on centrifuged samples by placing 0.1 ml of supernatant into vials containing sodium salicylate and nitro-prusside, and measuring colour development with the addition of an alkaline dichloro-isocyanurate solution (Havilah *et al*., 1977). Total VFAs and their proportions were determined on the concentrated distillates by gas liquid chromatography (Erwin *et al*., 1961). The excretion of total purine derivatives (PD) in urine (to predict MPS in the rumen) was determined by using the method described by Chen and Gomes (1992). Nitrogen in the feed samples was determined by the Micro Kjeldahl method.

Statistical Analysis

Data obtained were analysed by analysis of variance for repeated measures on the Statview 4.0 program on a Macintosh P.C.

RESULTS AND DISCUSSION

Effect of levels of molasses supplementation on rumen metabolism

There was no significant effect of molasses level in the diet on the rumen ammonia N concentration, either before feeding (0 h) or 2 h after feeding ($P>0.05$; Table 1), although at both times, the trend was a consistent decline as the molasses intake increased. The overall means of rumen ammonia N concentration were 125±6.1 and 192±5.8 mg NH_3-N/L for samples taken before feeding and 2 h after feeding, respectively. Similarly, rumen pH did not differ between treatments ($P>0.05$), with group means ranging from 6.6 – 6.7 (Table 1), and the overall mean being 6.7±0.04.

Although rumen ammonia concentration was higher at 2 h after feeding than before feeding, ammonia N concentration in the rumen fluid was not affected by diet ($P>0.05$; Table 1). Despite the fact that the rumen ammonia N concentration before feeding was lower than at 2 h after feeding (125 vs 192 mg NH_3-N/L) both means (at 0 and 2 h after feeding) were higher than the value of 50 mg/L considered by Satter and Slyter (1974) to be adequate to maintain maximum MPS. More recently, Perdok and Leng (1990) reported that concentrations ranging from 100 to 200 mg/L are needed to promote maximal microbial growth. The values in the current experiment were within that range. From the available data, it is thus concluded that the 2% rate of urea supplementation adopted was sufficient to elevate rumen ammonia levels into the optimal range, and that rumen ammonia is unlikely to have had a differential effect, between treatment, on the current results. It is interesting to note, however that increasing the molasses level in the diet from 0 to 18% led to a progressive decline in rumen ammonia levels

before and after feeding (132 to 115 and 204 to 179 mg NH_3-N/L, respectively). This result is inconsistent with a progressive increase in microbial growth and protein synthesis as the molasses level was increased the utilising of the available rumen NH_3-N.

The mean total VFA concentration and the corresponding molar proportions are presented in Table 1. Total VFA concentration and the concentrations of acetic and butyric acids were significantly affected by treatment, but those of propionic, isobutyric and valeric acids were not ($P>0.05$). The concentrations of VFA and of butyric acids increased progressively with increasing the level of molasses, with the lowest levels of being 53.1±2.1 mmol/L and 6.0±0.21% in controls and the highest levels of 70.9±2.89 mmol/L, and 10.91±0.2% respectively, in the 18% molasses group. In contrast, as the molasses levels increased in the diet, the concentration of acetic acid decreased progressively from 75.0±0.6 to 71.1±1.1%. The overall group mean values for the molar percentages of propionic, isobutyric, valeric and isovaleric acid were 17.0±0.29, 0.8±0.08, 0.2±0.02 and 0.7±0.06, respectively.

Total VFA concentration was higher ($P<0.05$) at high rather than low levels of molasses (Table 1), probably as a result of higher total dry matter intake (TDMI) (Marsetyo, 2004) and the consequent increase in the energy available for rumen fermentation. A progressive increase in VFA concentration with increasing TDMI has been reported by both Staples *et al*. (1984) and Ludden and Kerley (1997). The lowest and the highest total VFA concentrations of 53.1 and 70.9 mmol/L, respectively (Table 1) are values which correspond to the lower end of the range considered ‘normal’ suggested by France and Siddons (1993) of 70 to

130 mmol/L. The current VFA concentration were progressively and positively related to TDMI (R^2= 0.90; P<0.05) as has previously been reported by Staples *et al*. (1984) and Ludden and Kerley (1997). The low levels of rumen VFA observed were consistent related with the low values of TDDMI and with the losses in liveweight experienced by all groups of sheep (Marsetyo, 2004).

Table 1. Rumen ammonia concentration (NH_3-N, mg N/L), pH and total VFA (mmol/L) and molar proportion of VFA (%) of Merino sheep fed urea-treated barley straw supplemented with different molasses levels at 40 ^{0}C ambient temperature

Rumen parameters	Molasses level (%)				P value
	0	**6**	**12**	**18**	
NH_3-N (0 h)	132±11.2	130±12.1	123±17.2	1115±13.6	0.80
NH_3-N (2 h)	204±7.6	195±7.8	187±15.4	179±14.1	0.49
PH (2 h)	6.7±0.03	6.7±0.04	6.6±0.03	6.6±0.04	0.13
Total VFA	53.1±2.1	58.9±3.0	64.0±2.8	70.9±2.89	0.01
Acetic	75.0±0.6	73.6±0.6	72.4±1.0	71.1±1.1	0.04
Propionic	17.2±0.8	17.1±0.4	17.1±0.7	16.6±0.4	0.91
Butyric	6.0±0.2	7.3±0.5	9.1±0.4	10.9±0.2	0.01
Isobutyric	0.9±0.1	0.9±0.2	0.6±0.2	0.6±0.2	0.68
Valeric	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.97
Isovaleric	0.7±0.0	0.9±0.1	0.7±0.0	0.5±0.0	0.12

Molasses level had no effect on the proportions of propionic, isobutyric, valeric and isovaleric acids but it reduced the proportion of acetate and increased butyrate. As a consequence, increasing the level of molasses in the diet tended to decrease the acetate:butyrate ratio. Such an effect has been reported previously. Marty and Preston (1970) noted that supplementation with molasses lead to establishment of a stable VFA pattern with a characteristically high molar content of butyric acid. Eadie *et al*. (1970) and Marty and Preston (1970) both reported that high protozoal densities appear to be associated with the low rumen acetate:butyrate ratios which are frequently observed on molasses based diets.

Acetate and butyrate are readily converted to acetyl-CoA in the liver and may contribute indirectly to gluconeogenesis by reducing the requirement for complete oxidation of proprionate (Hungate, 1966), although only proprionate is capable of directly contributing to glucose supply. The increase in butyrate proportions in diets containing molasses is probably due to the stimulation of large numbers of small protozoa which produce butyric and acetic acids as end products of their fermentation (Abou Akkada and Howard, 1960). However, as the basal diet in this experiment was barley straw which is high in fibre, the proportion of acetate was still high (it ranged from 71 to 75%), whereas the mean for propionate was only 17% and butyrate ranged from 6 to 10.9%. These values vary somewhat from the ratio of 70:20:10% considered 'normal' in the review of France and Siddons (1993).

Effect of levels of molasses supplementation on microbial protein synthesis and nitrogen retention

MPS increased progressively with increasing molasses levels (Table 2). In addition, all sheep had a positive N retention. However, neither the level of molasses supplementation had any significant effect on N intake, total N excretion (faecal plus urinary N excretion) or N retention ($P>0.05$) (Table 2), the mean values of which were 8.6±0.21, 3.3±0.09, 3.7±0.08 and 1.6±0.2 g/d, respectively.

Table 2. Microbial protein synthesis (g N/d/animal) and nitrogen retention (g/d) of Merino sheep fed urea-treated barley straw supplemented with different levels of molasses at 400C ambient temperature (mean ± SE, N=4).

Parameters	Molasses level (%)				P value
	0	6	12	18	
MPS	3.4±0.35	3.9±0.32	4.2±0.17	4.6±0.07	0.01
N intake	8.8±0.56	8.3±0.34	8.4±0.44	8.7±0.47	0.21
N in faeces	3.4±0.56	3.2±0.22	3.3±0.16	3.2±0.20	0.89

N in urine	3.9±0.16	3.6±0.02	3.5±0.13	3.8±0.26	0.07
N retention	1.5±0.60	1.5±0.19	1.6±0.44	1.7±0.55	0.86

With supplementation used in this study, MPS showed a progressive increase with increasing molasses intake, probably as a consequence of increased TDDMI (Balcells *et al*., 1993) readily fermentable energy (Lindsay and Laing, 1996), sulphur (Lindsay and Laing, 1996) and also to an improved fractional outflow rate from the rumen (Gomes *et al*., 1994). There was no sign of a decrease in the MPS in this study, even at the highest level (18%) of molasses supplementation. Therefore, on a urea-treated barley straw diet, a level of at least 18% of molasses is recommended in order to achieve maximum MPS.

N intake, N excretion and N retention in the current experiment did not differ among treatments ($P>0.05$); all sheep were in positive N balance. The relatively similar levels of N retention between groups of sheep is possibly due to similar input of dietary urea among the treatments. This suggestion is in agreement with previous findings (Martin *et al*., 1981; Ortigues *et al*., 1988) which indicate that N retention is more influenced by urea supplementation than by the addition of molasses or other readily available carbohydrate. On the other hand, Sultan and Loerch (1992) found that lambs fed high energy diets (9.8 MJ ME/kg) retained 23% more N than those fed low energy diets (7.6 MJ ME/kg), probably due to an increased capture of urea N by bacteria in the rumen.

CONCLUSIONS

The main conclusion that can be drawn from the current experiment is that under moderate heat stress, sheep fed-urea treated barley straw with 18% molasses experienced significant increases in total VFA concentrations and microbial protein synthesis. However with respect to rumen ammonia concentration, there was no significant effect of molasses supplementation on rumen pH and nitrogen retention,.

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REFERENCES

Abou Akkada, A.R. and Howard, B.H., 1960. The biochemistry of rumen protozoa. 3. The carbohydrate metabolism of Entodinium. *Biochem. J.*, **76**:445-451.

Balcells, J., Guada, J.A., Castrillo, C. and Gasa, J., 1993. Rumen digestion and urinary excretion of purine derivatives in response to urea supplementation of sodium-treated straw fed to sheep. *Br. J. Nut.*, **69**:721-732.

Chen, X.B. and Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives. *An Overview of The Technical Details.* Occasional Publication. *Rowwett Res. Inst.*, Aberdeen, Scotland. pp. 1-22.

Eadie, J.M., Hyldgaard-Jensen, J., Mann, S.O., Reid, R.S. and Whitelaw, F.G., 1970. Observations on the microbiology and biochemistry of the rumen in cattle given different qualities of a pelleted barley ration. *Br. J. Nut* , **24**:157-177.

Erwin, E.S., Marco, G.J. and Emery, E.M., 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J. Dairy Sci*., **44**:1768-1771.

France, J. and Siddons, R.C., 1993. Volatile fatty acid production. In: Quantitative Aspects of Ruminant Digestion and Metabolism. Forbes, J.M. and France, J. (eds.). CAB International, Wallingford, England. pp. 107-121.

Gomes, M.J., Hovel, F.D. Deb., Chen, X.B., Nengomasha, E.M. and Fakiremariam, D., 1994. The effect of starch supplementation of straw on microbial protein supply in sheep. *Anim. Feed Sci. Tech*., **49**:277-286.

Havilah, E.J., Wallis, D.M., Morris, R. and Woolnough, J.A., 1977. A micro-colorimetric method for determination of ammonia in Kjeldahl digest with a manual spectrophotometer. *Lab. Prac*., **26**:545-556.

Hegarty, R., Godwin, I. and Nolan, J.V., 1996. Animal Metabolism, Digestion and Nutrition. *Dep. Anim. Sci. UNE*. Armidale, Australia.

Hungate, R.E., 1966. The Rumen and Its Microbes. *Acad. Press, N.Y.*

Leng, R.A., 1990. Factors affecting the utilisation of poor quality forages by ruminants particularly under tropical conditions. *Nut. Res. Rev*., **3**:277-303.

Lindsay, J.A. and Laing, A.R., 1996. The place for molasses in drought feeding strategies. In: *A Users Guide to Drought Feeding Alternatives.* Rowe, J. and Cossins, N (eds.). Dep. Anim. Sci. UNE, Armidale, Australia. pp.55-59.

Ludden, P.A. and Kerley, M.S., 1997. Amino acid and energy interrelationships in growing beef steers: I. The effect of level of feed intake on ruminal characteristics and intestinal amino acids flows. *J. Anim. Sci*., **75**:2550-2560.

Marsetyo, 2004. Effect of levels of molasses supplementation on intake, digestion and liveweight change of sheep fed urea-treated barley straw at high ambient temperature. Agrisains, **5**: 30-36.

Martin, L.C., Ammerman, C.B., Henry, P.R., and Loggin, P.E., 1981. Effect of level and form of supplemental energy and nitrogen on utilisation of low quality roughage by sheep. *J. Anim. Sci.*, **53**:479-487.

Marty, R.J. and Preston, T.R., 1970. Molar proportions of the short chain volatile fatty acids (VFA) produced in the rumen of cattle given high-molasses diets. *Cuban J. Agric. Sci*., **4**:183-186.

Perdok, H.B. and Leng, R.A., 1990. Effect of supplementation with protein meal on the growth of cattle given a basal diet of untreated or ammoniated rice straw. *Asian-Aust. J. Anim. Sci*., **3**:269-279.

Ortigues, I., Fontenot, J.P. and Ferry, J.G., 1988. Digesta flows in sheep fed poor quality hay supplemented with urea and carbohydrates. . *J. Anim. Sci.*, **66**:975-985.

Satter, L.D. and Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Br. J. Nut*., **32**:199-208.

Staples, C.R., Fernando, R.L., Fahey, G.C. Jr., Berger, L.L. and Jaster, E.H., 1984. Effect of intake of a mixed diet by dairy steers on digestion events. *J. Dairy Sci.*, **67**:995-1006.

Sultan J.I. and Loerch, S.C., 1992. Effect of protein and energy supplementation of wheat straw-based diets on site of nutrient digestion and nitrogen metabolism of lambs. *J. Anim. Sci*., **70**:2228-2234.