MICROBIAL PROTEIN PRODUCTION IN THE RUMEN OF STEERS FED LOW QUALITY FORAGE SUPPLEMENTED WITH VARIOUS LEVELS OF BARLEY GRAIN OR BARLEY GRAIN PLUS PROTEIN

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ABSTRACT

An experiment was conducted to examine the effects of increasing intake of barley grain or barley grain plus protein as supplement on microbial crude protein (MCP) production, efficiency of microbial crude protein production (eMCP) in the rumen of Brahman steers consuming low quality forage. Ten steers were used and allocated to two supplement groups namely barley grain (B) and barley plus protein (BP). The experimental design was two incomplete 5x5 Latin Squares, each with three runs. Each run consisted of a 21 d adaptation and 7 d collection period. Steers received Green panic hay *ad libitum* with one of five levels of B or BP (0; 0.5; 1.0; 1.5 and 2.0% of body weight per day (W/d)). MCP production in the rumen increased linearly ($P<0.05$) with increasing intake of B or BP supplements. The MCP in the rumen increased linearly ($P<0.05$) and quadratically ($P<0.05$) by increased B and BP intakes, respectively. Generally, steers supplemened with BP had greater ($P<0.01$) MCP production and eMCP in the rumen than B supplemented steers. The concentration of rumen NH_3-N (samples taken at 3 h after feeding) increased linearly with increased B or BP intakes. For samples taken at 24 h post feeding, the concentration of rumen NH_3-N was not changed ($P>0.05$) with increased B intake but increased linearly ($P<0.01$) with increased BP intake. Ruminal pH at 3 h after feeding decreased linearly ($P<0.05$) due to increased B intake, but was not altered ($P>0.05$) by increased BP intake. For samples taken at 24 h post feeding in contrast, was not influenced ($P>0.05$) by intake of B or BP. It was concluded that although both supplements increased MCP production and eMCP in the rumen of steers receiving low quality forage, BP supplementation had much more beneficial effect than B supplementation.

Keywords: barley, protein, rumen, steer, forage

INTRODUCTION

Faster growth rate of cattle is the main goal in feedlot industries. However, this goal cannot be achieved by providing low quality forage alone. Supplementation with cereal grain as energy sources is one strategy to boost the liveweight gain of cattle. Barley grain has long been used to feed cattle in many feedlot industries in developed countries. This because the starch of barley grain is more extensively fermented in the rumen and allows less starch to escape and be digested in the small intestine compared to other grains, such as corn or sorghum (Orskov, 1986; Owens *et al*., 1986).

However, the use of the cereal grains, such as barley grain often decreases forage intake and digestibility (Hoover, 1986). The main reason for these decreases is due to the lack of rumen degradable protein (RDP) and the depression of rumen pH which results in the depression in ruminal microbial growth and activities.

The addition protein on feed high in energy content offers an alternative to stimulate the growth and activity of microbes in the rumen. Preston and Leng (1987) indicated that there are two possible effects, which might arise from this strategy. Primarily this can satisfy the requirements of rumen microbes for nitrogen and energy. The

specific amino acids or carbon chains resulting from the supplement lead to a more optimal fermentation of the substrate to produce energy (volatile fatty acids) and microbial protein. Secondly, this strategy may help to satisfy the protein and energy requirements of the host animal by enhancing microbial protein synthesis and the supply of undegradable protein and energy (Kempton *et al*. 1977; Preston and Leng, 1987). Both of those effects will directly or indirectly change the balance of substrates available in the rumen, or nutrients absorbed in the small intestine. The balance between protein and energy in the rumen has a major role in stimulating microbial protein synthesis, with improved utilisation of fibre in the rumen. This experiment examined the microbial protein production and ruminal parameters of varying levels of the two supplemental groups (barley vs barley plus protein) of steers fed a low quality basal diet.

MATERIALS AND METHODS

Treatment and Feeding

The experiment was undertaken at the University of Queensland Mt. Cotton Research Farm. Australia, from April to June 2002. Ten Brahman crossbred steers {202 kg ± 5.69 (SE) mean initial live weight} kg and 12 months old) were used. The steers were randomly allocated on a stratified unfasted weight basis, to two treatment groups, barley and barley plus protein supplements. Prior to the commencement of the experiments, steers were given an extra 14 days to accustom them to the pens, feeding procedure and metabolic crates. All steers were treated with ivomec (1 mL per 10 kg of liveweight) for internal and external parasites at the beginning of this pre-adaptation period.

The experimental design was two 5 x 5 incomplete Latin Square. In each Latin Square, there were two types of supplements with 5 feeding levels of supplement intake equivalent to 0.0, 0.5, 1.0, 1.5 and 2.0 % of body weight. There were three runs and each run consisted of one steer, treatment or replication thus there were three replicates for each treatment. The basal diets given to steers were mature Green panic hay which represent low quality forage. The dietary supplements offered were cracked barley (B) and a mixture of cracked barley plus protein sources (BP). The BP composition was 50:25:25 for barley, copra meal and cottonseed meal, respectively. The supplement offered was on a dry matter basis. After the steers were allocated to the treatment, they remained in similar supplement types during the experimental period, but changed to the levels based on the random allocation.

Each run lasted 28 days, consisting of a 21 d adaptation period followed by 7 d of sample collections. Steers were housed in individual pens during the adaptation period and moved into metabolism crates during the collection period. The liveweight of the steers were recorded at the commencement of the preliminary period and then every seven days including the beginning and completion of the collection period. The supplement allocation was determined by the liveweight at the beginning of the preliminary period and then adjusted just prior to the collection period.

Hay was chopped into lengths of 2-10 cm. The hay was offered individually *ad libitum* once a day at 0830h with offered amounts adjusted to give 10-15% refusal. The dietary supplements were given separately to the hay in a plastic container placed in the feed bin and offered individually twice daily at 0800h and 1200h. The supplement given was gradually increased over six days during the adaptation period until the full ration was achieved. The levels of supplement (0.0; 0.5; 1.0; 1.5 and 2.0% W/d) tested were similar to the levels of supplement used in the study of Bolam (1998) using sorghum grain. These levels represent a control (no supplement), low, medium and high levels of supplement to draw the regression equation. The chemical composition of the basal diet and supplements is presented in Table 1. Steers had *ad libitum* access to fresh water continuously during the experimental period. No mineral blocks were given to the steers.

Measurements

Urine sampling for predicting microbial protein production

The measurement of urine production of individual steers per day was done by total collection into trays located under metabolism crates. The urine was added with 10% H_2SO_4 (approximately 150-200 mL) into individual trays at the start of each daily collection to maintain the pH below 3. For each steer, urine collected over a 24 h period

Table 1. Chemical Composition of Basal and Supplemental Feed Offered to Steers during Experiment

	OM	CP	NDF	EE
		(%)		
Green panic hay	90.1	5.7	67.2	1.7
Barley grain	95.4	12.5	22.8	2.1
Barley grain plus protein	93.0	23.4	28.6	3.6

OM = organic matter, CP= crude protein, NDF = neutral detergent fibre, EE = ether exctract

was mixed and a 5% aliquot was taken and bulked over the collection period into a plastic container stored in a refrigerator. At the end of each collection period, a 5 mL sub-sample was taken from the bulked samples from each steer, diluted to 50 mL with ammonium phosphate stock buffer and frozen awaiting purine derivative (PD) analysis. High Performance Liquid Chromatography (HPLC) procedures (Bolam, 1998) was used to measure the concentration of PD in sub-samples of the urine.

The exogenous purine supply (X, mmol/day) attributable to the microbial population of the rumen was estimated as the total purine excretion (Y, mmol/d) less the endogenous contribution to this total, divided by a recovery factor. Verbic *et al*. (1990) suggested an endogenous purine contribution of 0.385 mmol/kg $W^{0.75}$ and a recovery coefficient of 0.85 for absorbed purines. The calculation thus becomes:

$$Y = 0.85\,X + 0.385\,W^{0.75}.$$

However, in the study of Bowen (2003) found that *Bos indicus* cattle has a lower value of endogenous purine excretions (0.190 mmol/kg $W^{0.75}$). As the current experiment used Brahman steers (*Bos indicus*), hence the calculation of absorption of PD daily (Y; mmol/d) used the value of Bowen (2003), which became:

$$Y = 0.85\,X + 0.190\,W^{0.75}$$

The value of X (endogenous purine supply) was then used in determining estimated microbial nitrogen production (EMNP, g/d) through the following equation :

$$EMNP = (70X) / (0.83 \times 0.116 \times 1000)$$

where 0.83 is the assumed digestibility of the microbial protein and 0.116 represents the ratio of purine nitrogen to total microbial nitrogen (Chen *et al*., 1992). A factor of 6.25 is applied to convert the EMNP to a microbial crude protein (MCP) production (g/d).

pH and rumen NH_3-N concentration in the rumen fluid

Rumen fluid samples were taken at 3 h after supplement feeding on day 7 of the collection period and 24 h on the next day. The samples were collected by inserting a plastic tube down the oesophagus and into the rumen and withdrawing a sample using a vacuum pump. The pH of the rumen fluid was immediately measured on fresh samples. Immediately after the measurement of rumen pH, a sub-sample (20 mL) of rumen fluid for chemical analysis was drawn into 2 tubes (10 mL capacity), each containing 0.2 mL of concentrated H_2SO_4, and stored at -20^0C prior to determination of NH_3-N concentration.

Chemical analysis

The procedures of AOAC (1980) were employed to analyse the dry matter and organic matter of samples of feed, refusals. Samples were ground (1 mm screen) before analysed. The nitrogen content of the samples were determined using an automatic total nitrogen analyzer (LECO FP-428). Neutral detergent fibre was determined by procedures of Goering and Van Soest (1970) using a fibre extraction unit (ANKOM 220). The ether extract content of the hay and supplements was analysed using a solvent extraction unit (Soxtec HT6, Tecator, Sweden). The concentration of NH_3-N in the rumen fluid was determined by a distillation method using a Buchi 321 distillation unit (Buchi Scientific Apparatus Flawil, Switzerland), and an automatic titrator. The reagents were 2% boric acid (H_3BO_3) solution, a saturated sodium tetraborate solution (>260 g/L), and 0.01M HCL (normality 0.0095). Twenty-five mL of the boric acid solution.

Statistical analysis

The effect of supplementation were tested and described by fitting general linear models with pen, run, supplement type and supplement level as fixed effect by using the Genstat 6th edition program (Lawes Agricultural Trust, 2002). Polynomials were used to describe the responses to level of supplement. The differences between control treatment from B and BP groups were analysed by using Genstat 6th edition. As there were no differences between the control treatments in both treatment groups tested, a single intercept was used which represented six control steers from the two treatment groups.

RESULTS AND DISCUSSION

Microbial Protein Production

The values of MCP production and eMCP in response to increasing levels of B and BP supplementation in steers receiving a low quality forage are presented in Table 2. The mean values of MCP production of control steers fed low quality forage was 165 g MCP/d. For both supplement types, MCP production increased linearly ($P< 0.05$) with increasing intake of supplement. The slopes of regression lines drawn showed that BP had greater ($P<0.01$) MCP production than B supplemented. eMCP increased linearly with increasing levels of B intake (Table 2). However, eMCP increased quadratically ($P<0.01$) with increasing intake of BP. BP had a greater ($P<0.01$) eMCP than B supplemented steers (Table 2).

The mean value of the eMCP of control steers fed a low quality basal diet in this study was 93 g MCP/kg digestible organic matter intake (DOMI), which only reached 71.5% of the lower range of values recommended by SCA (130 g MCP/kg DOMI). However, this value is consistent with previous values observed for steers fed low quality tropical forages in many other studies (Prior *et al*., 1998; and Bowen 2003). One possible explanation for the low eMCP is the inadequacy of RDP and soluble carbohydrate. The crude protein (CP) content of Green panic hay used in this study was 5.7% (Table 1). From the intake data (Table 2), and assuming that hay CP degradability in the rumen is 75% for Green panic hay (McLennan *et al*., 1997), it was calculated that RDP availability would be only 88 g RDP/kg DOMI and so MCP production matches RDP supply in agreement with principles in all feeding standards. This calculation clearly suggests that RDP supply was insufficient to meet the recommended level (130 to 170 g RDP/kg DOMI) by the current feeding systems (SCA, 1990; NRC, 2000). Therefore, in any feeding strategy to improve the eMCP, additional RDP sources are likely to be more effective under this feeding con-

Table 2. Effect of Feeding Barley (B) and Barley plus Protein (BP) on the Microbial Protein Production (MCP), the Efficiency of MCP Production (eMCP), Hay Dry Matter (DM) Intake, Rumen NH_3-N Concentration and Rmen pH of Steers Received Low Quality Forage

	Supplement intake (%W/d)					Probability	
	0.0	0.5	1.0	1.5	2.0	Linear	Quad
MCP production (g/d)							
B	165±14.1	279±25.2	364±25.6	470±25.7	523±24.4	<0.01	0.23
BP		369±25.3	462±24.2	635±24.3	734±25.2	<0.01	0.10
eMCP (g CP/kg DOMI)							
B	93±3.0	105±5.1	115±5.3	125±5.3	139±5.1	0.04	0.78
BP		137±5.3	153±5.1	174±5.1	189±5.3	<0.01	<0.01
Hay DM intake (%W/d)							
B	1.7±0.1	1.6±0.1	1.3±0.1	1.1±0.1	0.7±0.1	0.67	<0.01
BP		1.7±0.1	1.4±0.1	1.1±0.1	0.8±0.1	0.05	<0.01
Rumen NH_3-N –3 h (mg/L)							
B	45±2.5	67±4.3	81±4.5	96±4.5	107±4.3	<0.01	0.14
BP		78±4.5	117±4.3	144±4.3	181±4.5	<0.01	0.76
Rumen NH_3 N –24 h (mg/L)							
B	37±1.9	45±3.3	51±3.4	63±3.4	76±3.3	0.20	0.25
BP		62±3.4	77±3.3	91±3.3	121±3.4	<0.01	0.39
Rumen pH-3 h							
B	7.0±0.1	6.7±0.1	6.6±0.1	6.4±0.1	6.2±0.1	0.03	1.0
BP		7.0±0.1	6.9±0.1	6.9±0.1	6.8±0.1	0.96	0.52
Rumen pH-24 h							
B	6.9±0.0	6.9±0.1	6.8±0.1	6.8±0.1	6.7±0.1	0.53	0.68
BP		6.9±0.1	6.±0.1	6.8±0.1	6.9±0.1	0.39	0.58

dition.

Steers supplemented with B grain from 0 to 2.0% W/d, linearly increase the eMCP from 93 to 139 g MCP/kg DOMI (P< 0.05) (Table 2). It was noted that the eMCP reached the value recommended by the feeding standards at the highest level of B intake of steers consuming the low quality forage. The increase in eMCP with increased B grain supplementation was probably the result of increased NH_3-N concentration and starch availability in the rumen. Rapidly available energy from B allows the microbes in the rumen to increase their growth rate and there was a higher rumen NH_3-N concentration. This indicates that the most important factor influencing eMCP was RDP supply.

BP supplementation increased eMCP much more than B supplementation. The value of eMCP increased quadratically and linearly, respectively (P<0.01), and reached the values adopted by the feeding standards from the first level of supplementation. The eMCP is influenced by a number of factors including the supply of nutrients such as nitrogen (N), sulphur, synchronisation between level of N and energy supply in the rumen, level of intake and rumen dilution rate (Hoover and Stokes, 1991). The rate of carbohydrate digestion in diets and the synchronisation of this rate with that of N release from protein sources may partly explain the greater eMCP from BP than from B intakes. A further factor is that the level of RDP and hence supply of peptides and branch chain fatty acids would increase under BP and these are essential elements for the growth of cellulolytic bacteria (Allison and Bryant, 1963; Russell and Hespell, 1981).

NH_3-N concentration and pH in the rumen fluid

The effect of feeding B and BP supplements on rumen NH_3-N concentration in steers fed low quality hay are shown in Table 2 for samples taken at 3 and 24 h after feeding. The concentration of rumen NH_3-N was low at 3 h after feeding (45 ± 2.5 mg/L) for unsupplemented steers and increased linearly (P<0.01) with increased B intake. For samples taken at 24 h after feeding, the mean NH_3-N concentration of control was 37 ± 1.9 mg/L and was not changed (P>0.05) with increased B intake. With each increment of BP, rumen NH_3-N concentration increased linearly (P<0.01) in samples taken at either 3 h or 24 h post feeding and were higher than B samples.

The mean concentrations of NH_3-N in the rumen fluid of control, measured at 3 and 24 h after feeding (45 and 37 mg/L, respectively), were slightly below the minimum value suggested by Satter and Slyter (1974) for optimum rumen function. This low level of rumen NH_3-N concentration is not surprising as the CP content of the Green panic hay fed to the steer was quite low (5.7%).

The rumen NH_3-N concentration increased linearly (P<0.05) as both B and BP intakes increased for samples taken at 3 h after morning feeding. This is most likely related to the CP content of the supplements which were degraded in the rumen and contributed to the rumen ammonia pool. For samples taken 24 h after feeding, increased B supplementation did not significantly (P>0.05) affect the rumen NH_3-N concentration. Increased BP intake on the other hand significantly (P<0.01) increased NH_3-N concentration even at 24 h after feeding. In general, increasing the level of supplemental BP resulted in a greater increase (P<0.01) in ruminal NH_3-N concentration. The greater increase in ruminal NH_3-N concentration was probably primarily due to the provision of RDP from both copra meal and cottonseed meal which had a greater CP content and rumen degradability. Some studies (Mathis *et al*., 2000) demonstrated this increased ruminal NH_3-N with CP supplementation of low quality forage. The ruminal fluid NH_3-N concentrations of supplemented steers were all above the recommended levels for maximum microbial growth (50 mg/L) suggested by Satter and Slyter (1974). Hence fibre digestion should not have been depressed by low NH_3-N levels.

No significant effect (P>0.05) was noted with increased B supplements on ruminal pH of steers receiving low quality forage for samples taken at 24 h after feeding. However, ruminal fluid pH at 3 h after feeding was linearly decreased with the increase of B intake. A similar trend was observed in previous studies (Mould and Orskov, 1983; Mould *et al*., 1983) with grain supplementation. One common problem associated with supplementing forages with high grain is the reduction in rumen pH. Low rumen pH is generally associated with a reduction both in cellulose digestion and intake of forage (Orskov and Fraser, 1975; Van Soest, 1994). With increased BP supplementation on the other hand, ruminal pH was not significantly affected (P>0.05). The range of ruminal pH of BP

supplemented steer across level and sampling times in this study was 6.8 to 7.0, indicating that rumen pH should not have affected ruminal fibre digestion in the rumen.

CONCLUSION

It is concluded that although both B and BP supplementation increased MCP production and efficiency of MCP production in the rumen of steers fed low quality forage, the steers supplemented with BP had greater increases than B supplemented steers.

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