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Effects of peptide source on microbial protein synthesis and nitrogen digestion in steers fed high concentrate diets

(Kesan sumber peptida terhadap sintesis protein mikrob dan pencernaan nitrogen pada lembu yang diberi diet konsentrat yang tinggi)

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Key words: peptide-N, microbial synthesis, duodenal N, N digestibility, ammonia

Abstract

Six steers fitted with rumen and duodenal cannulas were used in a replicated 3 x 3 latin square to determine the effects of peptide source on the efficiency of microbial protein synthesis and N digestibility. Steers were fed corn-based diets supplemented with three different sources of nitrogen. The three treatment diets consisted of 1) corn + casein (CC), 2) corn + soybean meal (CSBM), and 3) corn + urea (CU).

Mean ruminal NH_3-N concentrations for CC, CSBM and CU diets were 7.13, 5.93 and 9.27 mg/dl, respectively. Steers supplemented with SBM had higher ruminal peptide-N concentration (p <0.02) and amino acid-N concentration (p <0.03) than steers supplemented with casein. Mean ruminal concentrations of peptide-N for CC, CSBM and CU diets were 56.67, 66.82 and 1.66 mg/liter respectively. Efficiency of microbial protein synthesis was not altered by ruminal peptide-N concentrations and averaged 13.89 g of microbial N/kg of organic matter (OM) digested for all treatments.

Supplementation with SBM and casein tended to increase microbial N and NANMN flow but the difference between them was not significant (p <0.08). Mean microbial N entering duodenum averaged 65.0, 67.2 and 59.0 g/d for treatments CC, CSBM and CU respectively. Steers receiving SBM had 7.1% more microbial N flow to duodenum than steers receiving urea. Faecal N excretion was greater for steers receiving urea or casein than for steers receiving SBM (p <0.02, p <0.04) but total tract N digestibilities were similar among treatments. Efficiency of microbial protein synthesis was not increased by feeding diets that increased the concentration of peptides in the rumen.

Introduction

Ammonia, a central intermediate in the degradation and assimilation of dietary N in the rumen, is required by many species of bacteria (Nolan 1975). However, most species of rumen bacteria will grow with ammonia as their sole source of N provided that certain volatile fatty acids (VFA) are present as amino acids (AA) precursors (Bryant 1973).

Indeed ruminants can survive for a prolonged period with urea as N; the sole source in the diet (Virtanen 1966). When grown in vitro in mixed cultures, microbial

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growth is often greater when additional dietary N is provided in the form of peptides or amino acids (Cotta and Russell 1982; Merry et al. 1990; McAllan 1991). With in vitro studies, it is difficult to distinguish whether the benefit from an increased supply of preformed amino acids in the diet is due to effects on the rumen microorganisms or to increased escape of some dietary protein to the abomasum (McAllan 1991).

Because peptides and amino acids stimulate the growth of rumen microorganisms grown in vitro, and they are often considered essential for optimal growth rates of many bacteria species growing on rapidly fermented substrates; in a rich medium, bacteria may prefer preformed amino acids as a source of N (or as source of carbon, too) for using amino acid N for synthesis of more than 90% of their amino acids. Cellulolytic species are an exception in this respect, but still about half of their cell N in vitro is derived from N obtained from preformed amino acids. However, the extent to which bacteria use ammonia vs. peptides and amino acids for protein synthesis also depends on the concentrations of each; consequently, preformed amino acids and peptides probably are used to a lesser extent in vivo than these in vitro experiments would suggest.

Cruz Soto et al. (1994) presented evidence suggesting that bacterial stimulation by peptides and amino acids does not always occur. However, peptides and amino acids might be expected to benefit even cellulolytic rumen bacteria, when such bacteria are grown on cellobiose, instead of cellulose. Subsequent experiments (Chikunya et al. 1996), in which peptides were supplied with diets containing either rapidly or slowly degraded fibre, suggested that the benefit of peptides would be evident only when the energy source supported a very rapid growth rate. Results from these studies indicate that the in vivo situation is much more complicated than the simplistic assumptions about the effect of peptides and amino acids on microbial growth efficiency in that form part of the Cornell Net Carbohydrate and Protein Model (NRC 1996).

The objective of this experiment was to determine the effect of peptide source on microbial protein synthesis in the rumen and N digestibility of steers fed corn-based diets.

Materials and methods

Six crossbred steers with an average weight of 402 kg were used to measure the influence of different N sources on efficiency of microbial protein synthesis in the rumen. The steers were randomly allotted to individual 3 x 5 meter stalls and had free access to water. The steers were assigned to three dietary treatments in a replicated 3 x 3 latin square experiment. Treatment diets consisted of supplemented ground corn diet containing 18% cottonseed hulls; N was added from either a) casein (C), b) soybean meal (SBM) or, c) urea (U). Ingredients and chemical composition of each diet are shown in *Table 1*. Diets were fed twice daily at 0800 h and 1600 h in equal portions. Feed dry matter was provided at a rate of 1.8% of body weight daily. Chromium sesquioxide ($Cr_2O_3 1.5H_2O$) was used as a nonabsorbable marker for measurement of digesta flow at the rate of 0.2% for each diet.

Before the start of the experiment, all steers were fed diet U for a period of three weeks for diet adaptation. Each experimental period lasted 21 d, with 16 d for adjustment and 5 d for sampling. On day 17 through 19, approximately 250 ml of duodenal digesta and 200 g of wet faeces were collected at 2 and 8 h after feeding. On day 20, approximately 1,000 ml of strained rumen fluid was collected at 2 h and 8 h after feeding and frozen for later isolation of bacteria. On day 21 approximately 250 ml of rumen fluid was withdrawn at 1, 2, 4 and 8 h after feeding. The samples were frozen and used later for ammonia and peptides analyses. All rumen fluid collected was

Table 1. Composition of experimental diets (% of dry matter)

	Casein	Soybean meal	Urea
Ground corn	71.2	66.4	74.4
Cottonseed hulls	18.0	18.0	18.0
Molasses	3.59	3.59	3.59
Supplements			
Urea	–	–	1.4
SBM	–	9.4	–
Casein	4.6	–	–
Dicalcium phosphate	1.4	1.4	1.4
Limestone, 38%	0.7	0.7	0.7
Trace mineralized salt	0.3	0.3	0.3
Vitamin A	0.01	0.01	0.0
Chromic oxide	0.2	0.2	0.2
Crude protein (%)	11.7	11.5	12.1
Starch (%)	50.3	52.3	52.4

strained through four layers of cheesecloth and the pH was measured immediately. Before freezing, each rumen sample was acidified with 1 ml of 20% (v/v) sulfuric acid per 50 ml strained fluid to stop microbial activity.

Feed samples were obtained prior to each sampling day and composited within each diet and period. All samples were ground through a Wiley Mill fitted with a 2 mm screen and stored for analysis. Feed, duodenal and faecal samples were analysed for dry matter (DM), organic matter (OM), ash (AOAC 1984), starch (Herrera-Saldana and Huber 1989) and chromium (Cr) (Fenton and Fenton 1979). The N content of feed, duodenal digesta, bacterial composites, and faeces was analysed by macro-Kjeldahl analysis (AOAC 1984). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) in feed, duodenal and faecal samples were analysed using procedures of Goering and Van Soest (1970). Rumen NH_3-N was analysed colorimetrically using a spectrophotometer (UV-VIS Spectrophotometer, Gilford-Respond Series 1987) following the procedures of Broderick and Kang (1980).

Bacteria were isolated from the ruminal fluid using the procedures of Weakley and Owens (1983). Dried duodenal and bacterial samples were analysed for nucleic acid-N by the procedure of Zinn and Owens (1986). To improve recovery of RNA pellets after precipitation with silver chloride, the RNA pellets were washed with 100 ml of solution consisting of 5 ml of 12.5% $HClO_4$ in 0.0285 M $NH_4H_2PO_4$ + 5 ml of 0.4 M $AgNO_3$ + 90 ml of 0.2 M $NH_4H_2PO_4$. Rumen samples for peptide-N analysis were prepared using the procedures of Chen et al. (1987). Prehydrolysed and hydrolysed rumen fluid samples were analysed colorimetrically at 570 nm using a spectrophotometer (UV-VIS Spectrophotometer, Gilford-Respond Series 1987). The concentrations of ninhydrin reactive material in hydrolysed and unhydrolysed were measured and leucine was used as a standard (Moore 1968). The concentration of peptide-associated α-amino N was calculated as the difference between the α-amino N content of each hydrolysed and its corresponding unhydrolysed samples. The concentrations of α-amino N in the hydrolysed and unhydrolysed were corrected for NH_3-N in samples and ninhydrin (by subtracting NH_3-N concentrations in the samples and ninhydrin from α-amino N concentrations in the hydrolysed and unhydrolysed samples).

Flows of DM at the duodenum were calculated by dividing daily Cr intake (grammes) by Cr concentration (g/kg) in

duodenal digesta. Nutrient flows were calculated by multiplying DM flow by the concentration of the given nutrient in duodenal DM. Bacterial N flow (g/d) at the duodenum was calculated by multiplying daily N flow at the duodenum by the proportion of bacterial N in the duodenal N. This proportion was calculated by dividing the bacterial N:purine ratio of duodenal digesta by the ratio found for ruminal bacteria isolated from each steer in each period (Weakley and Owens 1983).

Daily amounts of non-microbial DM and N flowing past the duodenal cannula were calculated by subtracting the microbial contributions from the total. Bacterial DM was determined by oven drying the freeze-dried bacteria samples (ground) at 60 °C for 24 h. As a percentage of DM, these samples ranged from 7.9–9.1% N. Daily duodenal organic matter (OM) flow, corrected for microbial contributions, were calculated from the corrected duodenal DM flow x duodenal OM percentage.

Variables measured were analysed as replicated 3 x 3 Latin square with animal (6), period (2) and dietary treatment (2) as factors (SAS Inst. 1988). Differences between treatments were determined using a multiple comparison test (PDIFF options of SAS Inst. 1988).

Results and discussion

Source of N supplementation of the diet casein, SBM or urea had no significant effect on rumen pH. The mean pH values for C, SBM and U diets were 6.26, 6.27 and 6.24 respectively *(Table 2).* Rumen pH declined from 1 h to 3 h after feeding but then increased until 8 h after feeding with all diets *(Figure 1).* Ruminal NH_3-N concentrations were significantly lower (p <0.02) for SBM and C than for U but no significant difference (p <0.12) was detected between SBM and C. Mean ruminal NH_3-N concentrations for C, SBM and U were 7.13, 5.93 and 9.27 mg/dl, respectively *(Table 2).*

Concentrations of NH_3-N at 8 h were below the 5 mg/dl concentrations that were recommended by Satter and Slyter (1974) for optimum microbial synthesis in vitro. Kang-Merznarich and Broderick (1981) suggested that ruminal NH_3-N concentrations between 3.3 and 8.5 mg/dl were required for maximal microbial growth when diets contained 74% corn grain. Supplementing SBM as a protein source resulted in the lowest NH_3-N concentrations; but despite the lower NH_3-N concentration, SBM fed animals had the highest microbial N flows to the duodenum, and a higher microbial efficiency when compared with urea and casein. According to Cotta and Russell (1982), Chen et al. (1987), Williams and Cockburn (1991), when ruminal

Table 2. Ruminal pH, concentrations of nitrogen-containing compounds and microbial protein synthesis in rumen of steers fed a ground corn-based diet supplemented with isonitrogenous amounts of urea, casein or soybean meal

	Diets				Contrast	
	Casein	Soybean meal	Urea	SEM	Urea vs. others	Soybean meal vs. casein
pH	6.26	6.27	6.24	0.03	0.41	0.64
NH_3-N, mg/dl	7.13a	5.93a	9.27b	0.33	0.02	0.12
Amino acid-N, mg/liter[1]	4.13a	5.06b	2.40c	0.11	0.004	0.03
Peptide-N, mg/liter[2]	56.67a	75.31b	1.66c	2.64	0.003	0.04
Microbial efficiency[3]	13.73	14.27	13.67	0.25	0.39	0.27

Means in a same row with different letters differ significantly (p <0.05)
[1]Prehydrolysed fluid
[2]Hydrolysed fluid
[3]g microbial N per kg OM fermented

ammonia concentrations are low, ruminal microbes may preferentially utilise soluble amino acids and peptides as N source, so these relatively low ruminal ammonia concentrations should enhance any potential benefits from non-ammonia sources of N.

Mean concentrations of peptide-N and amino acid-N concentrations were significantly higher (p <0.004, p <0.003) in ruminal samples from steers supplemented with SBM than in samples from steers receiving either casein or urea *(Table 2).* Concentrations of peptide-N in the rumen averaged 40 and 34 times greater (p <0.04) for steers fed SBM or casein than for steers fed urea. However, temporal patterns differed with N source. For steers supplemented with SBM, the ruminal peptide-N concentration peaked at 2 h after feeding, but for steers fed casein, peptide-N concentrations peaked at or before 1 h after feeding *(Figure 2).* Amino acid-N concentrations peaked at 1 hour after feeding for both SBM and casein treatments. Concentrations of both peptide-N and amino acid-N declined rapidly after peaking. This trend is similar to that reported by other workers (Chen et al. 1987; William and Cockburn 1991; Kim et al. 1998). However, according to Williams and Cockburn (1991) accumulation of peptide in rumen contents does not appear to be related to either the rate or the extent of degradation of the protein supplement.

Although supplementing diets with SBM or casein was sufficient to markedly increase ruminal peptide-N and amino acid-N concentrations, it failed to increase (p > 0.39) efficiency of microbial growth (MOEFF). The correlation coefficient between mean peptide-N in ruminal fluid and MOEFF was not significant (r = 0.39, p = 0.71). In vitro data from Russell et al. (1983), Argyle and Baldwin (1989) and Grisswold et al. (1996) demonstrated that preformed amino acids stimulated microbial growth. In vivo data from McAllan and Smith (1983) and Rooke et al. (1987) also

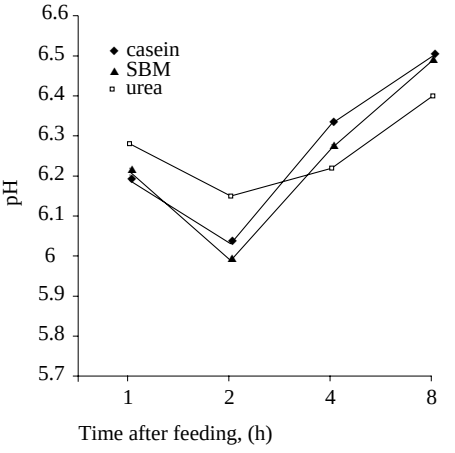

Figure 1. Changes of pH in the rumen of steers fed corn-based diet supplemented with isonitrogenous amounts of casein, SBM and urea

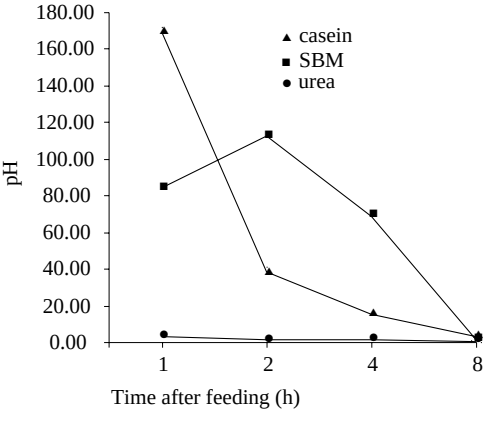

Figure 2. Changes in ruminal peptide-N concentrations in steers fed corn-based diet supplemented with isonitrogenous amount of casein, SBM and urea

suggested that bacterial protein synthesis was stimulated by peptides and amino acids.

In contrast, Cruz Soto et al. (1994) infused urea, amino acids and peptides into the rumen of sheep fed hay diet and reported that microbial protein flow to duodenum was unaffected by concentrations of either peptides or amino acids in the rumen. They concluded that fermentation by rumen bacteria is not limited by availability of peptides or amino acids when ammonia is available in sufficient quantities and bacterial growth rate is limited by a slowly

degradable energy source. Even though this statement might suggest that rumen microbial growth should respond only when a concentrate energy source is provided, results from this experiment found no benefit in MOEFF even when concentrate was provided in the diet. With concentrate diets, the microbial population of the rumen increases so that the supply of readily available soluble energy in the rumen still remains limited.

Intake of N, duodenal N flow, ruminal N digestion, non-ammonia non-microbial nitrogen (NANMN) and apparent total tract N digestibility were not significantly different among diets *(Table 3).* Supplementation with SBM and casein tended to increase microbial N and NANMN flow, but the difference between them was not significant (p <0.08). Mean microbial N entering duodenum averaged 65.0, 67.2 and 59.0 g/d for treatments CC, CSBM and CU respectively. Steers receiving SBM had 7.1% more microbial N flow to duodenum than steers receiving urea. Faecal N excretion was greater for steers receiving urea or casein than for steers receiving SBM (p <0.02, p <0.04) but total tract N digestibility was similar among treatments, which suggested that the faecal N in the faeces was not being derived from the diets.

Conclusion

Concentrate diets supplemented with different nitrogen (N) sources (casein, soybean meal, urea) produced markedly different concentrations of peptides in ruminal samples. Diets producing higher ruminal peptide and amino acid concentrations tended to increase duodenal flow of bacterial N slightly. But efficiency of microbial protein synthesis in the rumen, calculated as grammes of bacterial N per kilogramme of organic matter fermented in the rumen, was not increased when ruminal peptide and amino acid concentrations were elevated. These results suggest that ruminal peptide concentrations do not alter efficiency of microbial growth in vivo as was proposed by NRC (1996) based on in vitro experiments.

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Table 3. Nitrogen (N) digestion in steers fed a ground corn-based diet supplemented with isonitrogenous amounts of urea, casein or soybean meal (SBM)

	Diets				Contrast	
	Casein	SBM	Urea	SEM	Urea vs. others	SBM vs. casein
Nitrogen intake, g/d	136.3	136.2	136.1	–	–	–
Entering duodenum, g/d	128.7	133.4	124.6	1.3	0.06	0.013
Microbial N, g/d	65.0	67.2	59.0	1.8	0.08	0.47
NANMN, g/d[1]	56.7	60.0	57.6	2.7	0.86	0.49
Ruminal digestion, %						
Unadjusted	5.6a	2.1b	8.4c	0.4	0.01	0.01
Adjusted[2]	58.4	55.9	57.7	1.4	0.79	0.33
Faecal N, g/d	33.1a	32.0b	33.7a	0.1	0.02	0.04
ATT digestion, %[3]	75.7	78.8	75.2	1.5	0.37	0.27

Means in a same row with different letters differ significantly (p <0.05)

[1]Non-ammonia non-microbial nitrogen

[2]Adjusted for microbial and ammonia nitrogen

[3]Apparent Total Tract Digestibility

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Abstrak

Enam ekor lembu jantan kembiri yang dipasang kanula pada rumen dan duodenum telah digunakan dalam kajian menggunakan replikat 3 x 3 reka bentuk latin segi empat untuk menentukan kesan sumber peptida terhadap kecekapan sintesis protein mikrob dan pencernaan N. Lembu telah diberi diet berasaskan jagung dengan penambahan tiga jenis sumber peptida. Tiga diet perlakuan terdiri daripada 1) jagung + kasein (CC), 2) jagung + mil kacang soya (CSBM), dan 3) jagung + urea (CU).

Purata nilai konsentrasi NH_3-N dalam rumen bagi perlakuan CC, CSBM dan CU ialah 7.13, 5.93 dan 9.27 mg/dl. Lembu yang diberi penambahan SBM masing-masing telah mencatatkan konsentrasi peptida-N (p <0.02) dan asid amino-N (p <0.03) yang lebih tinggi daripada lembu yang diberi penambahan kasein. Purata nilai konsentrasi peptida-N bagi perlakuan CC, CSBM dan CU ialah 56.67, 66.82 dan 1.66 mg/liter. Kecekapan sintesis protein mikrob didapati tidak berubah dengan perubahan konsentrasi peptida-N di dalam rumen. Nilai purata bagi semua perlakuan ialah 13.89 g mikrob N bagi setiap kilogram bahan organik (OM) yang dicerna.

Penambahan bahan SBM dan casein telah mengakibatkan peningkatan N mikrob dan pengaliran N bukan-ammonia dan bukan-mikrob tetapi perbezaan antara perlakuan tidak ketara (p <0.08). Purata N mikrob yang memasuki duodenum ialah 65.0, 67.2 dan 59.0 g/hari masing-masing bagi perlakuan CC, CSBM dan CU. Lembu yang menerima SBM telah mencatatkan 7.1% lebih banyak N mikrob memasuki duodenum daripada lembu yang menerima urea. Nilai perkumuhan N lebih tinggi bagi lembu yang menerima urea atau kasein daripada lembu yang menerima SBM (p <0.02, p <0.04) tetapi pencernaan N bagi keseluruhan saluran penghadaman adalah sama bagi kesemua perlakuan. Kecekapan sintesis protein mikrob tidak meningkat dengan peningkatan konsentrasi peptida di dalam rumen.

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