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Ruminal Peptide and Ammonia Nitrogen Concentrations in Holstein Steers Fed Diets Differing in Concentrate to Alfalfa Hay Ratios

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Abstract: The objective of this study was to evaluate ruminal peptide and ammonia nitrogen concentrations and Crude Protein (CP) degradation in Holstein steers fed diets differing in concentrate to alfalfa hay ratios. Four rumen fistulated Holstein steers (216±27 kg, body weight) with were used. Animals fed 7 kg of Dry Matter (DM) of diets differing in concentrate (155 g CP kg^{-1} of DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCo3, 0.5% mineral and vitamin premix, 0.2% salt) to alfalfa hay (155 g CP kg^{-1} of DM) ratios as 60:40 (C_{60}:L_{40}), 70:30 (C_{70}:L_{30}), 80:20 (C_{80}:L_{20}) and 90:10 (C_{90}:L_{10}) in a 4×4 Latin square design (28 days of each period). Steers fed the experimental diets as total mixed ration twice daily at 0800 and 2000 h. Ruminal fluid was taken, by suction, via rumen fistula on days 24 to 28 of each period. Then, pH and NH3-N, soluble protein-N and peptide-N concentrations were determined. In addition, in situ CP and DM degradation kinetics of various feeds (alfalfa hay, barley grain, soybean meal and fish meal) were determined. Ruminal pH and ammonia-N concentration were significantly affected by the treatments and sampling time ($p< 0.05$). Ruminal pH and ammonia-N concentration decreased from 6.48 (C_{60}:L_{40}) and 19.86 (C_{70}:L_{30}) to 5.86 (C_{90}:L_{10}) and 14.81 (C_{90}:L_{10}), respectively, when level of concentrate was increased from 60 to 90% ($p<0.05$). Results indicated that the ruminal peptide-N and soluble protein-N concentrations were not significantly influenced by the diets and sampling time. In steers fed a high concentrate:alfalfa hay ratios (C_{80}:L_{20}), ruminal peptide-N concentration was lower than those fed C_{60}:L_{40} and C_{70}:L_{30}. In general, ruminal soluble protein-N was high in samples taken before feeding, intermediate at 6 h and low at 4 h after feeding. The results showed that the DM and CP degradation parameters of the feeds evaluated in the present experiment are influenced by the diet composition. It was concluded that the extent of ruminal CP degradation was affected by rumen pH.

Key words: Peptide, ammonia, protein degradation, steer

INTRODUCTION

In dry and semiarid regions, because of low pasture availability, ruminant diets are based on concentrates (Danesh Mesgaran and Stern, 2005). High-grain diets might promote particular rumen characteristics such as latent acidosis, reduced rumination and saliva secretion and lower ratios of acetate to propionate in the volatile fatty acids produced by rumen microorganisms (Beauchemin and Buchanan-Smith, 1990). In this system, the important goal of dairy cow nutrition is to feed as much concentrate as possible, in order to maximize production, without causing ruminal acidosis (Marie Krause and Garrett, 2006). Ruminal adaptation to diets high in fermentable carbohydrates apparently has two key aspects, microbial adaptation and lengthening of the ruminal papillae (Dirksen *et al*., 1985). In addition, data of *in situ* ruminal degradability of some dairy feeds have usually been obtained in animals fed high forage diets, with forage to concentrate ratio higher than commonly used in intensive dairy production systems (Rotger *et al*., 2006). It has been reported that in high concentrate diets, protein degradation is usually reduced (Ganev *et al*., 1979; Lindberg, 1981; Molero *et al*., 2004). This reduction has been attributed to lower ruminal pH, which causes changes in protein solubility (Loerch *et al*., 1983) and reduces fibrolytic activity of rumen micro flora (Hoover, 1986; Mould and Ørskov, 1983). In addition, there is limited information concerning the requirements for Rumen Degradable Nitrogen (RDN) when high-grain diets are given to steers and most of that information has been obtained using urea as the source of RDN (MartõÂn-Orue *et al*., 2000). However, few experiments have been conducted to determine whether pre-formed

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degradable true protein (e.g., casein) is required to generate optimum levels of rumen fermentation and microbial growth (MartõÂn-Orue *et al*., 2000). In addition, individual Amino Acid (AA) in feedstuffs disappeared at different rates in the rumen and in the intestinal tract. Consequently, the proportion of feedstuffs protein and AA entering the intestine must be considered (Taghizadeh *et al*., 2005). Considering the positive effect of peptides, amino acid and/or other growth factors on starch-fermenting bacteria (Argyle and Baldwin, 1989), a source of degradable true protein may be needed to optimize rumen fermentation of high-grain diets (Russell *et al*., 1992). Peptides are intermediates in the conversion of ingested protein to ammonia in the rumen and their accumulation depends upon the nature of diet (Mesgaran and Parker, 1995). Transient accumulation of peptides occurs after feeding and then their concentrations declines. In addition, it has been suggested that the production of peptides in the rumen was not altered by the protein supplements when diets provided similar effective rumen degradable protein (Mesgaran and Parker, 1995). The objective of this experiment was to evaluate the ruminal nitrogen metabolism in Holstein steers when fed diets differing in concentrate to Alfalfa hay ratios as 60:40 (C_{60}:L_{40}), 70:30 (C_{70}:L_{30}), 80:20 (C_{80}:L_{20}) and 90:10 (C_{90}:L_{10}).

MATERIALS AND METHODS

Animals and diets: Four Holstein steers (216±27 kg, body weight) with rumen fistulae were used. Animals fed 7 kg of DM of diets differing in concentrate (155 g CP kg^{-1} of DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCo3, 0.5% mineral and vitamin premix, 0.2% salt) to alfalfa hay (155 g CP kg-1 of DM) ratios as 60:40 (C_{60}:L_{40}), 70:30 (C_{70}:L_{30}), 80:20 (C_{80}:L_{20}) and 90:10 (C_{90}:L_{10}) in a 4×4 Latin square design (28 days of each period). Steers were housed in individual pens (6 m^2) and fed the experimental diets as total mixed ration twice daily at 0800 and 2000 h. Clean drinking water was available in plastic buckets.

Sampling and analysis: Ruminal fluid was taken, by suction, via rumen fistula on days 24 to 28 of each period. The pH of the ruminal fluid samples was measured immediately with a portable pH meter (Metrohm 744) before the morning feeding (0.0 h) to 8 h post feeding (interval 15 min) on all ruminal collection days of each experimental period. At day 24 of the each experimental period, ruminal fluid samples of before the morning feeding and 4 and 6 h post feeding were prepared for peptide-N analysis using sulphate-tungstate method described by Chen. The percholoric and tungstate acid-precipitates nitrogen (reflecting the soluble protein and peptide nitrogen, respectively) were assayed by a standard macro-Kjeldahl procedure (Kjeltec 1030 Analyzer tecator). At day 28 of the each period of experiment, ruminal fluid samples of the morning feeding and 0.5, 1, 2, 3, 4 and 6 h post feeding were prepared for NH3-N analysis. In this procedure, 10 mL of ruminal fluid from each collection point was acidified with 10 mL of 0.2 N HCl. Samples were analyzed for NH3-N using distillation method (Kjeltec 1030 Analyzer tecator).

***In situ* dry matter and crude protein degradability:** Disappearance of Dry Matter (DM) and Crude Protein (CP) from various feeds (alfalfa hay, barley grain, soybean meal and fish meal) were determined using the in situ procedure as described by Ørskov and McDonald (1979). The plant feeds originated from the Iranian varieties and fish meal from a variety located in Caspian Sea. Samples were dried using a forced-air oven at 96°C for 48 h. All feed samples were ground to pass through a 2-mm screen. The samples were incubated in the rumen of the steers in each period using polyester nylon bags (12×17 cm, 48 μm pore size). Approximately 5 g DM of each sample was placed in each bag (8 bags per each feed) and incubated in the rumen of each steer for 0.0, 2, 4, 8, 16, 24, 48, 72 and 96 h. After removal from the rumen, bags were washed using cold water. Finally, bags were dried in a forced-air oven (70°C, 24 h) and weighed to determine DM disappearance. Nitrogen concentration of ruminal pre-incubated and incubated sample was determined using the Kjeldahl method (Kjeltec 1030 Analyzer tecator).

Calculations and statistical analysis: Data of pH and NH3-N, soluble protein-N and peptide-N concentrations were analyzed as repeat measures of mixed model of SAS [$Y_{ijkL} = \mu+A_i+B_j+C_k+D_l+AD_{il}+E_{ijkL}$; where Y_{ijkL} is the dependent variable; μ is the overall mean; A_i is the treatment effect (i= 1,2,3,4); B_j is the period effect (j= 1,2,3,4); C_k is the steer effect (k= 1,2,3,4); D_l is the time effect (l=1,…,23); AD_{il} is the time × treatment effect and E_{ijkL} is the residual]. Means were compared using Duncan procedure at ($p<0.05$).

Data of CP and DM degradation were adjusted to a negative exponential model [$P = a+b\ (1-e^{-ct})$, where a= rapidly degradable fraction, b = slowly degradable fraction, c = fractional degradation rate constant (h^{-1}) and t = incubation time].

Table 1: Ruminal pH and ammonia-nitrogen concentration (mg dL^{-1}) in Holstein steers fed diets differing in concentrate:alfalfa hay ratios

Item	Concentrate:alfalfa hay ratio†	Time*(h) 0	0.5	1	2	3	4	6	Treatment effect** SEM[4]	*P*	Time effect** SEM	*P*
pH												
	C_{60}:L_{40}	6.91	6.64	6.48	6.49	6.43	6.30	6.43	0.03	0.01	0.07	0.01
	C_{70}:L_{30}	6.76	6.51	6.60	6.45	6.30	6.20	6.51				
	C_{80}:L_{20}	6.52	6.44	6.34	6.08	5.83	5.94	6.21				
	C_{90}:L_{10}	6.50	6.25	6.17	5.85	5.60	5.50	5.63				
NH_3-N												
	C_{60}:L_{40}	12.48	17.13	22.99	23.03	21.27	18.87	13.74	1.00	0.02	1.32	0.01
	C_{70}:L_{30}	19.48	20.31	21.27	22.21	21.36	19.98	14.41				
	C_{80}:L_{20}	15.75	15.82	18.78	18.61	17.18	13.87	10.52				
	C_{90}:L_{10}	12.97	13.09	17.73	19.10	19.45	15.42	5.94				

†: C_{60}:L_{40}= 60% concentrate+40% alfalfa hay, C_{70}:L_{30}= 70% concentrate+30% alfalfa hay, C_{80}:L_{20}= 80% concentrate+20% alfalfa hay and C_{90}:L_{10}= 90% concentrate+10% alfalfa hay, *: When the difference between means is greater than two times the SEM, it is considered as significant ($p<0.05$). Values were reported as the mean of four sampling periods, **: SEM= Standard Error of Mean; *P*: Probability

Table 2: Soluble protein and peptide nitrogen concentrations (mg dL^{-1}) in the rumen fluid of Holstein steers fed diets differing in concentrate:alfalfa hay ratios

Item	Time† (h)	Concentrate:alfalfa hay ratio* C_{60}:L_{40}	C_{70}:L_{30}	C_{80}:L_{20}	C_{90}:L_{10}	Treatment effect** SEM	P	Time effect** SEM	P
Soluble protein-N	0	2.41	7.46	1.56	2.16	1.06	0.15	0.92	0.24
	4	0.04	2.51	2.27	0.25				
	6	0.15	1.37	4.99	0.31				
peptide-N	0	5.67	4.92	1.49	0.18	1.30	0.15	1.12	0.98
	4	2.07	0.13	0.07	8.97				
	6	6.95	3.23	0.24	1.66				

†: Time after the morning feeding, *: C_{60}:L_{40}= 60% concentrate+40% alfalfa hay, C_{70}:L_{30}= 70% concentrate+30% alfalfa hay, C_{80}:L_{20}= 80% concentrate+20% alfalfa hay and C_{90}:L_{10}= 90% concentrate+10% alfalfa hay. Values were reported as the mean of four sampling periods, **: SEM= Standard Error of Mean; P: Probability

Table 3: *In situ* dry matter and crude protein degradation parameters (mean±SE) of various feeds determined in steers fed diets differing in concentrate:alfalfa hay

Feeds	Parameter†	Concentrate:alfalfa hay*£ C_{60}:L_{40}	C_{70}:L_{30}	C_{80}:L_{20}	C_{90}:L_{10}
Dry matter					
Alfalfa hay	a	0.309±0.007	0.326±0.008	0.326±0.008	0.326±0.008
	b	0.391±0.010	0.306±0.010	0.306±0.010	0.314±0.011
	c	0.042±0.003	0.053±0.005	0.053±0.005	0.044±0.004
Barley grain	a	0.239±0.036	0.267±0.022	0.267±0.037	0.228±0.047
	b	0.564±0.040	0.550±0.024	0.541±0.040	0.563±0.051
	c	0.212±0.036	0.330±0.033	0.248±0.044	0.227±0.049
Soybean meal	a	0.220±0.014	0.221±0.012	0.214±0.013	0.224±0.012
	b	0.566±0.031	0.586±0.028	0.529±0.026	0.557±0.049
	c	0.035±0.005	0.034±0.004	0.039±0.005	0.024±0.004
Fish meal	a	0.162±0.010	0.144±0.007	0.141±0.011	0.138±0.006
	b	0.344±0.017	0.385±0.014	0.383±0.023	0.363±0.011
	c	0.042±0.006	0.037±0.003	0.037±0.006	0.042±0.020
Crude protein					
Alfalfa hay	a	0.398±0.013	0.381±0.011	0.398±0.010	0.398±0.008
	b	0.436±0.015	0.491±0.014	0.408±0.012	0.446±0.010
	c	0.070±0.007	0.058±0.004	0.083±0.006	0.057±0.003
Barley grain	a	0.209±0.038	0.252±0.031	0.297±0.031	0.214±0.050
	b	0.612±0.043	0.570±0.034	0.553±0.036	0.605±0.056
	c	0.141±0.026	0.227±0.033	0.117±0.020	0.173±0.040
Soybean meal	a	0.055±0.023	0.058±0.015	0.055±0.017	0.074±0.045
	b	0.620±0.149	0.894±0.159	0.721±0.132	0.557±0.085
	c	0.019±0.009	0.015±0.004	0.018±0.006	0.041±0.017
Fish meal	a	0.189±0.014	0.189±0.011	0.176±0.011	0.169±0.011
	b	0.514±0.054	0.472±0.028	0.436±0.021	0.433±0.021
	c	0.025±0.006	0.032±0.005	0.039±0.005	0.039±0.005

*: C_{60}:L_{40}= 60% concentrate+40% alfalfa hay, C_{70}:L_{30}= 70% concentrate+30% alfalfa hay, C_{80}:L_{20}= 80% concentrate+20% alfalfa hay and C_{90}:L_{10}= 90% concentrate+10% alfalfa hay, †: a= rapidly degradable fraction, b= slowly degradable fraction, c= fractional degradation rate constant (h^{-1}), £: in each raw, when mean±SE is grater than the others, it is considered as significant ($p<0.05$)

RESULTS AND DISCUSSION

Rumen pH and NH3-N, soluble protein and peptide nitrogen concentrations at different sampling time are shown in Table 1. Ruminal pH and ammonia-N concentration were significantly affected by the treatments and sampling time ($p<0.05$). The results of the present experiment suggested that diets containing higher conce-ntrate had a significant effect on ruminal pH and ammonia-N concentration. Ruminal pH and ammonia-N concen-tration decreased from 6.48 (C_{60}:L_{40}) and 19.86 (C_{70}:L_{30}) to 5.86 (C_{90}:L_{10}) and 14.81 (C_{90}:L_{10}) as shown in Table 1, respectively, when level of concentrate was increased from 60 to 90% ($p<0.05$). Shriver *et al.* (1986) reported a decrease in ammonia N concentration at low pH. The results of the present study demonstrated that the increasing of concentrate in ruminant diets caused to decrease the ruminal pH and ammonia-N concentration. The increasing of concentrate level may reduce proteolysis in the rumen (Klevesahl *et al.*, 2003). Furthermore, provided ruminal energy facilitates microbial yield and the demand for ruminally available N (Owens and Goetsch, 1988). Therefore, the delayed appearance of substantial amounts of ruminal ammonia in steers given high concentrate level reflects increased demand for readily available N by amylolytic organisms (Klevesahl *et al.*, 2003). The optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0 according to Kopecny and Wallace (1982). Cardozo *et al.* (2000, 2002) conducted two dual flow continuous culture fermentation studies comparing high forage *vs.* high concentrate rations at pH ranging from 4.9 to 7.0 and demonstrated that protein degradation was reduced as pH decreased with both types of rations. Amylolytic bacteria tend to be more proteolytic than cellulolytic bacteria (Siddons and Paradine, 1981; Wallace *et al.*, 1997). In addition, Lana *et al.* (1998) reported that a decrease in ruminal pH from 6.5 to 5.7 reduced ruminal ammonia concentration only when bacteria were obtained from cattle fed a 100% forage ration, whereas, bacteria from cattle fed 90% concentrate had lower ammonia N concentration regardless of pH. These results indicated that protein degradation is affected by pH and type of ration, which may dictate the predominant type of microbial population present in the rumen.

The soluble protein-N and peptide-N concentrations at each sampling time are shown in Table 2. Results indicated that the ruminal peptide-N and soluble protein-N concentrations were not significantly influenced by the diets and sampling time. Results of the present study showed there was not constant pattern in ruminal soluble protein and peptide-N concentrations regarding the treatment or time effect. However, when animals fed a high concentrate:alfalfa hay ratios (C_{80}:L_{20}), ruminal peptide-N concentration was lower than those fed C_{60}:L_{40} and C_{70}:L_{30}. In general, ruminal soluble protein-N was high in samples taken before feeding, intermediate at 6 h and low at 4 h after feeding. In the present experiment, there was a fluctuation in soluble protein-N and peptide-N concentrations. It seems when diets with similar CP fed to steers, the protein and peptide nitrogen concentrations follow a non-significant pattern and not influence by time after feeding.

The results of the present study showed that the DM and CP degradation parameters of the feeds evaluated in the present experiment are influenced by the diet composition (Table 3). It was consider that, the extent of ruminal CP degradation was affected by rumen pH. The effect being more pronounced on the fractional degradation rate (c). This parameter was stimulated by C_{80}:L_{20}, but, decreased when the animals fed C_{60}:L_{40}. The reason(s) for these differential effects was not clear. Among the feeds evaluated in the present study, soybean meal and fish meal were the most feedstuffs which were influenced by the differing in ruminal ecosystem. A reduction in CP degradation at low pH has been previously reported (Erfle *et al.*, 1982). The proteolytic enzymes produced by rumen microbes are generally active across a wide range of pH (Wallace and Cotta, 1989). The amylolytic bacteria tend to be more proteolytic than cellulolytic bacteria (Wallace and Cotta, 1989). In the conditions of the present experiment, as in many acidic conditions, it is likely that the reduced protein degradation may be related to the reduction in the digestibility of fiber associated with the protein within feeds. The undigested fiber within feed will reduce the access of bacteria and enzymes to the protein and therefore reduce protein degradation. This hypothesis was suggested by Wallace and Cotta (1989) and Devant *et al.* (2000). Devant *et al.* (2001) incubated soybean meal and heat-processed soybean meal in the rumen of dairy cattle fed a 60:40 forage-to-concentrate ration or in the rumen of beef cattle fed a 10:90 forage-to-concentrate ration using the *in situ* technique. Results demonstrated that protein degradation was lower with the beef-type ration, despite the fact that pH was 6.0 in both types of animals, illustrating that the reduction of protein degradation is not only due to a pH effect, but is also related to

type of substrate being fermented or the predominant microbial population induced by a particular ration. The combined effect of pH and substrate on ruminal protein degradation may be explained by the resulting predominant microbial population. It is obvious that protein degradation occurs by the action of proteolytic enzymes, but there is evidence that supports the importance of other enzymatic activities on the degradation of protein. Assoumani *et al.* (1992) demonstrated that starch interferes with protein degradation. They noted that the addition of amylase increased total ruminal protein degradation of cereal grains between 6 and 20% units. Positive effects of amylases on protein degradation were also reported by other workers (Aufre`re and Cartailler, 1988; Toma´nkova´ and Kopecny, 1995). Debroas and Blanchart (1993) found that NDF-bound protein was degraded by proteolytic bacteria only after microbial depolymerization of cellulose began. Many plant proteins are trapped in a fiber matrix that needs to be degraded before proteases can gain access to proteins for degradation. Therefore, it appears that protein degradation in the rumen requires the presence of several proteolytic and non-proteolytic enzymes and the combination of several microbial and enzymatic activities are required for maximum protein degradation (Bach *et al*., 2005). This fact is clearly illustrated in a study by Endres and Stern (1993), who observed a reduction in CP and NDF digestion when pH decreased from 6.3 to 5.9. Proteolytic bacteria counts were not affected by pH, but cellulolytic bacteria counts were reduced by about 50% (Bach *et al*., 2005). It is likely that with a high-concentrate ration, even if pH is high, starch-degrading bacteria predominate and fiber digestion is limited by the reduced number of cellulolytic bacteria, reducing the degradation of protein (Mould and Ørskov, 1983). Therefore, the effect of pH and (or) the substrate being fermented may affect the predominant microbial population and modify protein degradation caused by interactions among nutrients.

CONCLUSION

The results of the present study demonstrated that the increasing of concentrate in diets caused to decrease the ruminal pH and ammonia-N concentration. Furthermore, the increasing of concentrate may reduce ruminal DM and CP degradation. However, It was not affected the ruminal soluble protein and peptide nitrogen concentrations.

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