Altering Soluble and Potentially Rumen Degradable Protein for Prepubertal Holstein Heifers¹

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ABSTRACT

Four prepubertal Holstein heifers, average age 184 ± 2.1 d and 169.1 ± 15.8 kg of body weight, fitted with rumen cannulae were used to evaluate effects of altering the soluble CP and potentially rumen degradable protein fractions of diets containing 16% CP and 71.5% TDN. Parameters studied included rumen ammonia, volatile fatty acid production, N balance, total tract apparent digestibility, and urinary excretion of purine derivatives in a 4×4 Latin square design with a 2×2 factorial arrangement of treatments. Heifers were fed treatment rations containing 62.1 ± 0.8 g CP/Mcal ME at 2.0% BW with altered soluble CP fractions (AB_1) (33.6 or 40.6% of CP) and potentially rumen degradable protein fractions (B_2B_3) (20.9 or 28.2% of CP). Increased intake of AB_1 increased rumen ammonia and decreased total volatile fatty acid concentrations with molar proportions of isovalerate and isobutyrate decreased. Increased intake of B_2B_3 tended to increase volatile fatty acid concentrations, increased molar proportions of propionate, and decreased the acetate to propionate ratio. Nitrogen utilization was not affected by increased intake of AB_1 or B_2B_3 , although increased intake of AB_1 tended to increase urine urea excretion. Increasing solubility (40.6 versus 33.6% AB_1) of dietary CP when feeding a 16% CP and 71.5% TDN ration, decreased total volatile fatty acid concentrations and molar proportions of isovalerate and isobutyrate. Feeding 62.1 ± 0.8 g CP/Mcal ME with increased potentially rumen degradable protein (28.2 versus 20.9% B_2B_3) at 2.0% BW affected rumen fermentation but did not affect DM digestibility or N utilization.

(**Key words:** heifer metabolism, protein to energy ratio, soluble protein, potentially rumen degradable protein)

Abbreviation key: AB_1 = soluble protein, B_2B_3 = potentially rumen degradable protein, **CP:ME** = crude protein to metabolizable energy ratio, **HH** = high AB_1 -high B_2B_3 , **HL** = high AB_1 -low B_2B_3 , **LH** = low AB_1 -high B_2B_3 , **LL** = low AB_1 -low B_2B_3 , **ME** = metabolizable energy, **NSC:RDP** = non-structural carbohydrate to rumen degradable protein ratio, **PD** = purine derivatives, **PUN** = plasma urea nitrogen.

INTRODUCTION

The quantity and degradability of dietary protein affects rumen fermentation (Nocek and Russell, 1988), which ultimately affects net efficiency of absorbed nutrients. The NRC (2001) classifies dietary CP into two components, rumen degradable protein (RDP) and rumen undegradable protein (RUP), each of which possesses separate and distinct functions. RDP is the sum of protein fractions A, B_1 , B_2 , and B_3 (Sniffen et al., 1992) with degradation and passage rates as noted in the NRC (2001). Optimum utilization of dietary CP requires selection of complementary feed protein sources that provide the types and amounts of RDP that will meet, but not exceed, the N requirement of rumen microbes (NRC, 2001). Efficient utilization of dietary protein is of importance due to economic and environmental concerns.

Soluble protein (AB_1) is assumed to be immediately available for utilization by rumen microbes and chemically defined as protein fractions dissolved in a borate-phosphate buffer, typically fractions A and B_1 (Sniffen et al., 1992). Potentially RDP (B_2B_3) is not as immediately available for utilization as soluble protein but is fractionally degraded with time. Not all B_2B_3 may be rumen-degraded due to lignin-bound proteins, passage rates, and feeding management (Tamminga, 1979). Potentially RDP is not chemically determined but can be calculated as RDP minus soluble CP. Given degradation differences between fractions of RDP (AB_1 and B_2B_3), it is apparent the type and amount of each fraction may affect overall utilization of protein and other nutrients.

Interrelationships between dietary CP and energy in the form of carbohydrates within the rumen and ruminant body can have tremendous effects on overall

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nutrient use (Oldham, 1984). Gabler and Heinrichs (2003a), utilizing Holstein heifers 125 to 234 kg BW and fed for 0.80 kg/d gains, reported improved feed efficiency and increased structural growth as crude protein to metabolizable energy ratio (**CP:ME**) increased from 48.3, 59.1, 67.5, to 76.5 g/Mcal. Further elucidating effects of increasing CP:ME on nutrient utilization, Gabler and Heinrichs (2003b) reported feeding a CP:ME of 63.3 at 2.0% BW to Holstein heifers between 153 and 196 kg BW produced less calculated microbial N than higher CP:ME (69.4 and 77.3), but achieved a better overall relationship of dietary protein to energy. It can be hypothesized that greater calculated microbial N received from higher CP:ME (69.4 and 77.3) than 63.3 CP:ME occurred due to increased quantities of RDP or specific RDP fractions.

The objective of this study was to investigate a specific protein and energy level diet, with altered AB₁ and B₂B₃ protein fractions and the resulting rumen ammonia, volatile fatty acid (**VFA**) production, N balance, total tract apparent digestibility, and urinary excretion of purine derivatives (**PD**) in Holstein heifers between 148 and 190 kg of BW. In addition, phosphorus balance was measured for observational purposes.

MATERIALS AND METHODS

Four prepubertal Holstein heifers at 114.7 ± 2.1 d of age were fitted with rumen jarrets (2.54 cm i.d., Macam Rubber, Baulkham Hills, Australia). Surgery was performed under local anesthesia following standard surgical procedures of the Pennsylvania State University Institutional Animal Care and Use Committee. During a 30-d recovery period, before the experiment began, heifers were fed a ration meeting NRC (2001) recommendations for 0.80 kg/d gain with an estimated CP:ME of 63 g/Mcal. One week prior to the start of the experiment, heifers received larger rumen cannulae (3.81 cm i.d., Bar Diamond, Parma, ID). Heifers were housed individually in tie stalls inside an environmentally controlled barn, had free-choice access to water and were released to an outdoor paved exercise lot 3 h post feeding for approximately 2 h/d, except on intensive sampling days.

Heifers at 144.7 ± 2.1 d of age and 147.7 ± 13.5 kg BW were randomly assigned to one of four treatment rations in a 4 × 4 Latin square design. Each experimental period lasted 20 d, consisting of 10-d adaptation period and 10-d sampling period.

By design, treatment rations were formulated to contain 16% CP and 71.5% TDN, which was previously determined optimal for similar age and BW Holstein heifers fed at 2.0% of BW as DMI (Gabler and Heinrichs, 2003b). Diets were altered by adjusting the soluble CP

fraction (33.6 or 40.6% AB₁), and the potentially RDP fraction (20.9 or 28.2% B₂B₃), with similar calculated ME (2.6 Mcal/kg DM) across all rations. Treatment rations contained corn silage and grass hay; soybean meal, SoyPlus® (West Central Soy, Ralston, IA), and urea were major supplemental CP sources used to alter AB₁ and B₂B₃ protein fractions of diet CP. Rations were formulated to provide 60:40 forage to concentrate ratio, and DMI was maintained at 2.0% BW/d. Every 10 d BW was recorded two consecutive d, 3 h post feeding to determine quantity of treatment ration offered for the next 10 d.

Heifers were individually fed once daily at 0900 h and consumed the entire amount fed with no refusals. Rations and forages were sampled every other day, and every 10 d portions of ration and forage samples were dried for DM determination to adjust daily DMI. Remaining portions of ration samples were frozen (−20°C) until compositing at conclusion of each experimental period. Composited ration samples for each period were analyzed for particle size (Kononoff et al., 2003), DM, CP (AOAC, 1990), soluble CP (Krishnamoorthy et al., 1982), RDP (Krishnamoorthy et al., 1983), starch (Holm et al., 1986), sugar (Dubois et al., 1956), and NDF and ADF (Goering and Van Soest, 1970, with inclusion of alpha amylase). Analysis of ration calcium, phosphorus, magnesium, and potassium content was determined by AOAC (1990).

Sample Collection, Sample Analysis, and Calculations

The experiment utilized four 20-d periods, 10 d adaptation followed by 10 d sampling. Samples of ventral sac rumen fluid were obtained via hand vacuum pump just prior to feeding (0 h) and 1, 2, 3, 4, 6, 8, 12, 16, and 23.5 h post feeding on d 11 of each experimental period. Rumen fluid pH was recorded immediately (pH meter, model M90, Corning Inc., Corning, NY), and 15 ml subsample was preserved by addition of three ml 25% metaphosphoric acid and frozen (−20°C) until analysis. Samples were later centrifuged three times at $4000 \times g$ for 30 min at 4°C to obtain a clear supernatant. Supernatant was analyzed for rumen ammonia using a phenol-hypochlorite assay (Broderick and Kang, 1980) and molar concentrations of volatile fatty acids by gas chromatography.

Blood samples acquired through a left jugular vein catheter were obtained just prior to feeding (0 h) and 1, 2, 3, 4, 6, 8, 12, 16, and 23.5 h post feeding on d 13 of each experimental period. Blood samples were stored and cooled in tubes containing citrate as an anticoagulant. Plasma samples were aspirated after centrifugation ($4000 \times g$) and stored at 4.5°C for analysis that

day. Plasma samples were analyzed for urea N (procedure no. 0580; Standbio Laboratory Inc., San Antonio, TX).

Total collection of urine and feces took place from d 14 through d 18. Urinary catheters (#14 French balloons; Rusch Inc., Duluth, GA) were inserted prior to feeding on d 14 and removed at the conclusion of d 18. During one period, one heifer was unable to be fitted with a urinary catheter. For this heifer and period, urine and feces were collected manually for one 24-h period only. Urine samples were collected into a container with 120 ml of 12N hydrochloric acid to maintain the pH below 3. Every 24 h, total collection of urine and feces was weighed and recorded. A 1-L subsample of urine and a 1-kg subsample of feces were collected and stored each 24 h. Urine and feces subsamples were frozen (-20°C) until analysis. Fecal samples were dried at 55°C for 72 h to determine DM and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Dried fecal samples were analyzed for N (AOAC, 1990) and P (Fiske and Subbrow, 1925, Sigma Kit 670C). Urine samples were analyzed for N (AOAC, 1990), P (Fiske and Subbrow, 1925, Sigma Kit 670C), urea (Marsh et al., 1957), allantoin (Chen, 1989), and uric acid (Sigma Kit 686A). Microbial N was calculated from total urinary excretion of PD according to Valadares et al. (1999).

Statistical Analysis

Nutrient content of treatment rations were analyzed by the GLM procedure of SAS (1999). Least square means and standard errors were determined using the LSMEANS and STDERR statement in PROC GLM. Mean separations were determined using the PDIF statement in PROC GLM.

Dry matter intake, N intake, N excretion and related calculations, total tract apparent DM and N digestibility, P intake, P excretion and related calculations, urinary excretion of urea, allantoin, and uric acid, and microbial N calculated from urinary excretion of PD were analyzed using the SATTHERH model statement in MIXED procedure of SAS (Littell et al., 1996) for a 4×4 Latin square with a 2×2 arrangement of treatments. The model included effects for soluble and potentially RDP fractions of dietary CP and the interaction between these factors with experimental period repeated. Mean separations were determined using the PDIF statement in PROC MIXED.

The pH of rumen fluid, ammonia concentrations of rumen fluid, molar concentrations and proportions of VFA, and plasma urea nitrogen (PUN) concentrations were analyzed using MIXED procedure of SAS (Littell et al., 1996) with the model described above except the

repeated option was used for time after feeding instead of period. Ammonia concentrations of rumen fluid and PUN concentrations possessed an AB_1 by time point interaction, and molar proportion of propionate and PUN concentrations possessed a B_2B_3 by time point interaction, therefore both were included in the model.

Simple, autoregressive one, and compound symmetry covariance structures were utilized in the analysis depending on low values received for goodness of fit measures, Akaike's Information Criterion and Schwartz's Bayesian Criterion. Least square means and standard errors of the means were determined through the LSMEANS statement in MIXED procedure of SAS (Littell et al., 1996). Mean separations were determined using the PDIF statement in PROC MIXED with a $P \leq 0.05$ significance level.

RESULTS AND DISCUSSION

Treatment rations contained a constant $16.1 \pm 0.2\%$ CP and either 58.4 ± 0.9 or $64.9 \pm 0.5\%$ RDP as a percent of CP (Table 1). Soluble portion of CP in treatment rations determined by borate-phosphate solution was either 33.6 ± 0.2 or $40.6 \pm 1.1\%$ AB_1 as a percent of CP. Adjustment of RDP and the AB_1 fraction resulted in treatment containing either 20.9 ± 3.3 or $28.2 \pm 2.5\%$ B_2B_3 as a percent of CP. These protein fraction adjustments yielded four treatment rations: low AB_1 -low B_2B_3 (LL), high AB_1 -low B_2B_3 (HL), low AB_1 -high B_2B_3 (LH), and high AB_1 -high B_2B_3 (HH).

Dry matter intake was set at 2.0% of BW. Heifers were fed once daily and rapidly consumed the entire treatment ration prior to being let out to a paved exercise lot. We speculate that rumen pH, VFA production, and ammonia levels at certain hours could be influenced by this feeding/eating pattern.

Daily intake of DM, CP, protein fractions, ME, and CP:ME are presented in Table 2. Calculated CP:ME of treatment rations averaged 62.1 ± 0.8 . Intakes of RDP for rations LH and HH averaged 11.3% higher than rations LL and HL. By design, AB_1 intakes for rations HL and HH averaged 17.8% higher than rations LL and LH, while B_2B_3 intakes for rations LH and HH averaged 27.1% higher than rations LL and HL. Casper et al. (1994) emphasized synchronization of the non-structural carbohydrate to rumen degradable protein ratio (NSC:RDP) is critical for young, growing heifers because of limitations in DMI and fermentation capacity. In the current study, NSC:RDP (Table 2) averaged 3.86 g NSC/g RDP for LL and HL and 3.14 g NSC/g RDP for LH and HH.

An AB_1 by B_2B_3 interaction was determined for rumen pH ($P = 0.03$) (Table 3), however, no effects of AB_1 or B_2B_3 were observed with lowest recorded pH at 6 h.

Table 1. Ingredient and nutrient compositions of the treatment rations (DM basis).

	AB ₁ ¹ (High or Low intakes, g/d) and B ₂ B ₃ ² (High or Low intakes, g/d)				SE
	LL	HL	LH	HH	
Ingredients, %					
Corn silage ³	43.9	43.9	43.9	43.9	...
Grass hay ⁴	19.9	19.9	19.9	19.9	...
Wheat midds	3.3	...	5.3	4.9	...
Cottonseed hulls	5.9	5.7	6.0	4.0	...
Cracked corn	7.8	13.6	5.8	11.8	...
Soybean meal	7.1	...	13.3	3.3	...
SoyPLUS ⁵	10.4	14.3	4.2	9.7	...
Urea	0.3	1.0	0.1	0.9	...
Mineral mix ⁶	1.6	1.6	1.6	1.6	...
Nutrients ⁷					
DM, %	60.8	61.0	62.0	62.9	0.90
CP, %	15.9	15.9	16.1	16.4	0.60
Soluble CP, % of CP	33.4 ^a	41.7 ^b	33.8 ^a	39.7 ^b	1.10
RDP, % of CP	57.6 ^a	59.3 ^a	64.5 ^b	65.4 ^b	0.86
NSC ^{8,9} , %	35.9	35.8	32.8	33.5	.
Starch, %	21.0	19.9	21.9	20.3	0.53
Sugar, %	4.2	4.3	4.8	4.8	0.47
NDF, %	39.4	40.9	41.0	43.7	1.29
ADF, %	23.1	24.0	25.4	27.2	1.54
TDN ^{9,10} , %	71.6	71.5	71.4	71.6	.
ME ¹¹ , Mcal/kg DM	2.6	2.6	2.6	2.6	.
Ca, %	0.51	0.56	0.52	0.59	0.04
P, %	0.34	0.33	0.35	0.37	0.01
Mg, %	0.31	0.31	0.33	0.34	0.02
K, %	1.35	1.37	1.43	1.42	0.04
Particle size					
> 19 mm, %	19.8 ^a	23.7 ^b	20.3 ^a	24.4 ^b	1.20
19 mm – 8 mm, %	32.4	30.3	30.0	28.5	1.64
8 mm – 1.18 mm, %	30.9 ^a	30.0 ^a	32.0 ^b	30.0 ^a	0.65
< 1.18 mm, %	16.9 ^a	16.1 ^b	17.7 ^a	17.1 ^a	0.49

^{a,b}Superscripts that differ are significant, $P \leq 0.05$.

¹Soluble crude protein.

²Potentially rumen degradable protein.

³Corn silage contained 43.3 % DM, 46.4 % NDF, 24.7 % ADF, and 8.4 % CP on DM basis.

⁴Grass hay contained 92.2 % DM, 67.7 % NDF, 43.4 % ADF, and 10.5 % CP on DM basis.

⁵SoyPlus, West Central Soy, Ralston, IA. Contains: 88.0 % DM, 48.3 % CP, 10.0 % soluble CP as a % of CP, 61.8 % RUP as a % of CP, 23.6 % NDF, and 1.73 Mcal/kg DM of ME.

⁶Contained 46.2 aragonite, 19.3 salt, 16.8 magnesium oxide, 11.5 calcium sulfate, 2.9 selenium, 0.06% Se, 1.7 dairy TM II, and 1.6 vitamin ADE as a percent DM basis.

⁷ $n = 4$ composite samples representing 36 samples per treatment ration taken during the trial.

⁸Non-structural carbohydrates.

⁹Calculated from ingredients.

¹⁰Total digestible nutrients.

¹¹Estimated: Metabolizable energy (ME) = $\text{TDN} \times 0.04409 \times 0.82$.

Figure 1 shows only moderate fluctuations in rumen pH over 24 h, and no biologically significant differences between treatments are apparent. Increased intake of AB₁ decreased ($P = 0.01$) total VFA concentrations; significant decreases ($P < 0.02$) in molar proportions of isovalerate and isobutyrate accompanied decreased total VFA. Increased intake of B₂B₃ tended to increase ($P = 0.11$) total VFA concentrations and significantly increased ($P = 0.01$) molar proportions of propionate. The acetate to propionate ratio followed similar results as molar proportions of propionate with increased intake of AB₁ and B₂B₃.

Increased molar proportions of propionate and a corresponding trend toward increased total VFA concentrations resulting from increased intake of slower degrading B₂B₃ could be explained by propionate-producing rumen microbes having prolonged access to hydrolyzed peptides, ammonia, and branched chain fatty acids provided by other rumen microbes (Russell and Hespell, 1981). Conversely, decreased total VFA concentrations resulting from increased intake of AB₁ may not have provided enough of the proper protein substrate and end products or too much protein substrate too quickly, for full hydrolysis to required rumen

Table 2. Least square means for intakes of DM, crude protein, protein fractions, calculated metabolizable energy, calculated dietary protein to metabolizable energy ratios, calculated non-structural carbohydrate to rumen degradable protein ratios, beginning BW and ADG.

	AB ₁ ¹ (High or Low intakes, g/d) and B ₂ B ₃ ² (High or Low intakes, g/d)				
	NRC 2001 ³	LL	HL	LH	HH
DMI, kg/d	4.60	3.50	3.50	3.49	3.47
CP, g/d	699.20 ⁴	557.40	556.02	560.70	568.55
RDP ⁵ , g/d	442.00	320.83	329.42	361.65	371.65
Soluble CP ^{6,7} , g/d	...	186.43	231.60	189.42	225.47
Potentially RDP ^{6,8} , g/d	...	134.40	97.82	172.23	146.18
RUP ⁹ , g/d	253.00	236.57	226.60	199.05	196.90
ME ¹⁰ , Mcal/d	10.40	9.05	9.05	9.01	8.98
Ratio CP:ME ¹¹	67.23	61.59	61.43	62.23	63.31
Ratio NSC:RDP ¹²	...	3.92	3.80	3.17	3.12
Beginning BW, kg	...	163.4	164.5	161.9	162.4
ADG, g/d	...	603.9	577.3	516.0	544.3

¹Soluble crude protein.²Potentially rumen degradable protein.³Inputs into table generator for heifers were: mature BW 650 kg, BW 169 kg, ADG 800 g/d, BCS 2.80, age 6.2 mo, and days pregnant 0 (NRC, 2001).⁴NRC (2001) CP intake requirement (from heifer requirement table generator), the sum of RDP and RUP when perfectly balanced.⁵Rumen degradable protein of treatment rations g/d = RDP % × CP intake.⁶NRC 2001 soluble protein and potentially RDP requirements are included in the RDP requirement.⁷Soluble crude protein of treatment rations g/d = Soluble Protein % × CP intake.⁸Potentially RDP of treatment rations g/d = RDP g/d - Soluble Protein g/d.⁹Rumen undegradable protein of treatment rations g/d = RUP % × CP intake.¹⁰Estimated: Metabolizable energy Mcal/d = TDN × 0.04409 × 0.82.¹¹g of CP per Mcal of ME.¹²Ratio of non-structural carbohydrates to rumen degradable protein, g of NSC per g of RDP.**Table 3.** Rumen fermentation of Holstein heifers fed a dietary protein to metabolizable energy ratio with altered soluble and potentially rumen degradable protein fractions from 148 to 190 kg of BW.

	AB ₁ ¹ (High or Low intakes, g/d) and B ₂ B ₃ ² (High or Low intakes, g/d)				SEM	Contrasts		
	LL	HL	LH	HH		AB ₁ ³	B ₂ B ₃ ⁴	AB ₁ × B ₂ B ₃ ⁵
	<i>P</i>							
pH								
Mean	6.80	6.86	6.79	6.79	0.07	0.70	0.54	0.03
Low value	6.36	6.46	6.31	6.24	0.11	0.92	0.28	0.47
NH ₃ N								
Mean mg/dl	9.38 ^a	11.21 ^b	8.54 ^a	12.16 ^b	0.62	0.01	0.93	0.16
Total VFA, μmol/ml	80.25 ^a	68.87 ^b	83.84 ^a	76.64 ^{a,b}	3.48	0.01	0.11	0.55
VFA, molar proportions								
Acetate	66.98	66.32	66.61	65.12	0.90	0.25	0.40	0.65
Propionate	16.32 ^a	16.85 ^a	17.78 ^{a,b}	19.23 ^b	0.60	0.13	0.01	0.47
Butyrate	11.15	12.55	10.91	10.80	0.75	0.40	0.20	0.33
Isovalerate	2.53 ^a	1.73 ^b	2.18 ^c	2.06 ^c	0.09	0.01	0.91	0.01
Valerate	1.62	1.45	1.53	1.49	0.07	0.18	0.75	0.43
Isobutyrate	1.39 ^a	1.09 ^b	1.11 ^b	1.10 ^b	0.04	0.02	0.04	0.01
A:P Ratio	4.18 ^a	4.00 ^a	3.82 ^{a,b}	3.45 ^b	0.18	0.16	0.02	0.62
Plasma Urea N								
Mean mg/dl	13.85	13.67	13.21	14.49	0.71	0.45	0.90	0.33

^{a,b,c}Superscripts that differ are significant, $P \leq 0.05$.¹Soluble crude protein.²Potentially rumen degradable protein.³Soluble crude protein effect.⁴Potentially rumen degradable protein effect.⁵Soluble crude protein and potentially rumen degradable protein interaction.

Figure 1. Rumen pH of Holstein heifers fed a dietary protein to metabolizable energy ratio (g CP per Mcal ME) with altered soluble and potentially rumen degradable protein fractions (High or Low intakes, g/d) from 148 to 190 kg BW. Line highlighted by ▲ designates the LL treatment ration, ● designates the HL treatment ration, ◆ designates the LH treatment ration, and ■ designates the HH treatment ration.

microbial substrates before removal from the rumen (Roffler et al., 1975). Additionally, treatment rations with increased AB₁ may not have contained enough NSC for full utilization of RDP, resulting in decreased total VFA concentrations. It should also be noted that feed energy values were estimated from NRC (2001) and not analyzed.

Mean rumen ammonia concentrations (Table 3) were increased ($P = 0.01$) through increased intake of AB₁. Rumen ammonia concentrations peaked between 1 and 2 h post feeding (Figure 2) and those values less than

Figure 2. Rumen ammonia concentrations (mg/dl) of Holstein heifers fed a dietary protein to metabolizable energy ratio (g CP per Mcal ME) with altered soluble and potentially rumen degradable protein fractions (High or Low intakes, g/d) from 148 to 190 kg BW. Line highlighted by ▲ designates the LL treatment ration, ● designates the HL treatment ration, ◆ designates the LH treatment ration, and ■ designates the HH treatment ration.

5 mg/dl may be indicative of suboptimal availability of ammonia for microbial growth (Satter and Slyter, 1974). However, little data exists linking rumen ammonia concentrations and microbial growth in growing Holstein heifers, and the use of urea to alter dietary AB₁ fractions may have resulted in inefficient CP utilization. Rumen ammonia concentrations of 5 mg/dl or less were observed with the HL ration for approximately 12 h of the 24-h sampling day, while LH, LL, and HH treatments had concentrations less than 5 mg/dl for 4, 6, and 8 h, respectively. Rumen ammonia concentrations for ration LH, approximately 4 h suboptimal (< 5 mg/dl), disagree with a similar treatment ration reported by Gabler and Heinrichs (2003b), which reported approximately 18 h of suboptimal availability of ammonia for microbial growth. In the current study, from 6 h post feeding until the next feeding, rumen ammonia concentrations were maintained at low levels. It is likely little rumen microbial activity was occurring (consistent with total VFA concentrations) during this time period, which may have influenced other parameters such as urea recycling, digestion, and nutrient utilization.

Excess ammonia in rumen fluid is absorbed through the rumen wall and transported to the liver where it is metabolized to urea. Urea then can be reabsorbed and utilized by tissue or excreted in urine. Increased intake of AB₁ or B₂B₃ did not affect mean PUN concentrations (Table 3) ($P > 0.33$). Even though increased intake of AB₁ increased rumen ammonia concentrations, it appears ammonia concentrations were not high enough to elevate PUN. However, lack of PUN response may be due to the experimental design used in this experiment.

Total tract apparent DM digestibility (Table 4) was not affected by increased intake of AB₁ or B₂B₃. Total tract apparent DM digestibility values in the current study are lower than values reported by Gabler and Heinrichs (2003b) utilizing heifers with similar BW, DMI, and ration composition. However, corn silage in the current study was more mature, higher in NDF, and comprised more of the ration, which may account for decreased digestibility.

Nitrogen intakes (Table 4) remained constant across treatments by design. Fecal N excretion, total tract apparent N digestibility, urinary N excretion, total N excretion, N retained, and N retained as a percent of N intake showed no response to increasing intake of AB₁ and B₂B₃. Total tract apparent N digestibility and N retained as a percent of N intake were also lower than values reported by Gabler and Heinrichs (2003b), again likely due to lower quality corn silage.

Formulating rations with constant CP intakes in such a manner that permitted investigation into effects

Table 4. Digestibility of treatment rations, N balance, and P excretion of Holstein heifers fed a dietary protein to metabolizable energy ratio with altered soluble and potentially rumen degradable protein fractions from 148 to 190 kg of BW.

	AB ₁ ¹ (High or Low intakes, g/d) and B ₂ B ₃ ² (High or Low intakes, g/d)					Contrasts		
	LL	HL	LH	HH	SEM	AB ₁ ³	B ₂ B ₃ ⁴	AB ₁ × B ₂ B ₃ ⁵
						<i>P</i>		
Apparent DM Digestibility, ⁶ %	65.52	65.24	68.35	67.48	1.67	0.74	0.16	0.86
N intake, g/d	85.98	86.83	89.71	87.78	4.88	0.91	0.64	0.78
Fecal N excretion, g/d	24.17	25.13	25.33	25.99	2.19	0.72	0.66	0.95
Urinary N excretion, g/d	36.86	38.06	37.31	38.52	2.40	0.63	0.85	0.99
Total N excretion, g/d	61.04	63.19	62.64	64.51	3.16	0.54	0.65	0.97
Apparent N Digestibility, ⁶ %	71.99	70.94	71.52	70.49	1.54	0.52	0.77	0.99
N retained, % N intake	28.18	26.67	30.21	26.50	3.25	0.44	0.78	0.74
Urine Urea excretion g/d	25.52	27.64	25.64	28.59	2.22	0.14	0.67	0.70
P intake, g/d	12.24	12.58	12.04	12.41	0.28	0.58	0.62	0.93
Fecal P excretion, g/d	7.51	5.69	8.45	7.33	1.16	0.24	0.30	0.77
Urine P excretion, g/d	0.33	0.95	0.78	0.88	0.59	0.56	0.75	0.68
Total P excretion, g/d	7.85	6.63	9.23	8.21	0.97	0.28	0.16	0.92
P excretion, % P intake	64.99 ^{a,b}	52.97 ^a	83.26 ^b	67.14 ^{a,b}	7.41	0.04	0.03	0.87

^{a,b}Superscripts that differ are significant, $P \leq 0.05$.

¹Soluble crude protein.

²Potentially rumen degradable protein.

³Soluble crude protein effect.

⁴Potentially rumen degradable protein effect.

⁵Soluble crude protein and potentially rumen degradable protein interaction.

⁶Total tract apparent digestibility.

of protein fractions A, B₁, B₂, and B₃ required alteration of RDP concentrations of treatment rations, resulting in RUP concentrations averaging either $35.1 \pm 0.5\%$ RUP as a percent of CP for rations LH and HH or $41.6 \pm 0.8\%$ RUP as a percent of CP for rations LL and HL. Devant et al. (2000) reported increased fecal N excretion by crossbred heifers, 101 ± 4.5 kg of BW, fed diets containing either 58 or 42% RUP as a percent of CP. Lack of fecal N excretion response in the present study compared to observed effects reported by Devant et al. (2000) might be explained by RUP concentrations in the present study not high or different enough to elicit a similar response.

Urinary excretion of urea (Table 4) tended to increase ($P = 0.14$) with increasing intakes of AB₁. Increases in rumen ammonia concentrations and urinary excretion of urea with increased intakes of AB₁ suggest excess rumen ammonia was excreted as urea by way of urine, even though effects were not great enough to be detected in PUN analysis with this experimental design. Increased urea excretion was observed with the diets containing high levels of feed grade urea and may indicate a decreased utilization of ammonia by rumen microbes (Lana et al., 1997).

Phosphorus intakes, by design, were constant across treatment rations (Table 4) and met requirements established by NRC (2001). Fecal and urinary P excretion were not affected by increasing intake of AB₁ and B₂B₃.

Increased intake of AB₁ decreased ($P = 0.04$) total excretion of P as a percent of P intake, while increased intake of B₂B₃ decreased ($P = 0.03$) total excretion of P as a percent of P intake. Challa et al. (1989) calculated regression equations relating P intake to endogenous fecal P excretion utilizing bull calves between 100 and 150 kg BW consuming between 18.4 and 70.5 g/d P. The current study's results averaged $46.5 \pm 8.4\%$ higher than values calculated through equations provided by Challa et al. (1989). However, regression equations calculated by Challa et al. (1989) did not include P intakes as low as the present study.

The current study's total P excretion as a percent of P intake across all diets averaged 25.1% higher (treatment ration LH) than results reported for similar BW heifers receiving a ration with similar CP:ME (Gabler and Heinrichs, 2003b). A majority of P consumed in the current study was of feed origin (organic), however, in the previous study (Gabler and Heinrichs, 2003b) approximately 20% of P consumed was from mineral supplements (inorganic). Since inorganic P is absorbed more readily in ruminants than organic P (NRC, 2001), this difference in P sources may account for higher P excretion relative to the intake in the current study.

Topps and Elliott (1965) showed that urinary excretion of PD provided a valid indicator and noninvasive method of predicting amount of synthesized microbial protein that leaves the rumen and is digested in the

Table 5. Urinary excretion of purine derivatives and calculated microbial N of Holstein heifers fed a dietary protein to metabolizable energy ratio with altered soluble and potentially rumen degradable protein fractions from 148 to 190 kg of BW.

	AB ₁ ¹ (High or Low intakes, g/d) and B ₂ B ₃ ² (High or Low intakes, g/d)				SEM	Contrasts		
	LL	HL	LH	HH		AB ₁ ³	B ₂ B ₃ ⁴	AB ₁ × B ₂ B ₃ ⁵
						<i>P</i>		
Allantoin, g/d	8.01	7.16	8.43	9.08	1.01	0.86	0.15	0.33
Uric acid, g/d	0.55	0.51	0.47	0.60	0.18	0.68	0.79	0.77
Total PD, ⁶ g/d	8.55	7.69	8.88	9.68	1.12	0.96	0.16	0.29
Microbial N, ⁷ g/d	30.46	25.98	28.31	31.79	5.67	0.96	0.16	0.29

¹Soluble crude protein.²Potentially rumen degradable protein.³Soluble crude protein effect.⁴Potentially rumen degradable protein effect.⁵Soluble crude protein and potentially rumen degradable protein interaction.⁶Total urinary excretion of purine derivatives, which is the sum of allantoin and uric acid excretion.⁷Calculated from Valadares et al., 1999.

ruminant intestine. Allantoin and uric acid are the only PD present in cattle urine because high xanthine oxidase activity in blood and tissue converts xanthine and hypoxanthine into uric acid prior to excretion (Valadares et al., 1999). Martin-Orue et al. (2000) reported urinary PD closely reflects microbial N production as determined by digesta flow measurements in growing Holstein heifers (initial BW 306 kg).

Urinary excretion of allantoin and uric acid was not affected by increased intake of AB₁ and B₂B₃ (Table 5). Urinary excretion of allantoin constituted 94% of total PD excreted in the present study, which is consistent with previous studies (Devant et al., 2000; Gabler and Heinrichs, 2003b). Microbial N absorbed in the intestine was calculated from total urinary excretion of PD using equations described by Valadares et al. (1999). Calculated microbial N (Table 5) showed no response to increased intake of AB₁ or B₂B₃.

CONCLUSIONS

Increasing solubility (40.6 versus 33.6% AB₁ as a percent of CP) of CP in a ration containing 62.1 ± 0.8 g/Mcal CP:ME decreased total VFA concentrations and molar proportions of isovalerate and isobutyrate. Results with increased intake of AB₁ signal a possible lack of proper protein substrates and products, too much protein substrate too quickly for full utilization by rumen microbes, too low of NSC intake and NSC:RDP, or a deficiency of other nutrients from metabolism of excess ammonia to urea as indicated by trends in urinary excretion of urea. Increased intake of B₂B₃ tended to increase total VFA concentrations and significantly increased molar proportions of propionate. In Holstein

heifers between 148 and 190 kg BW with DMI restricted to 2% BW, consumption of a diet containing 62.1 ± 0.8 g/Mcal CP:ME and increased potentially RDP (28.2 versus 20.9% B₂B₃ as a percent of CP) altered rumen fermentation but had no overall effect on N utilization.

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