The Effects of Substituting True Protein with Non-Protein Nitrogen in Holstein Dairy Heifers Precision-Fed Different Forage to Concentrate Ratios

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ABSTRACT

Understanding the efficiency and digestibility of dietary nutrients in dairy heifers is essential for minimizing costs and environmental impacts of the dairy industry. The objective of this study was to interpret the effects of manipulating rumen-degradable protein (RDP) in a precision feeding system. Eight Holstein ruminally cannulated heifers (14.6 ± 0.1 mo of age, and 386 ± 9.1 kg of weight) were randomly assigned to 2 forage levels: HC (25% forage) and LC (75% forage) and to a degradable protein sequence [0% degradable protein from urea **U**, 100% casein **C** (no urea treatment **U0**); 33% U, 67% C (low urea treatment **U2**); 67% U, 33% C (high urea treatment **U3**); 100% U 0% C (all urea treatment **U4**)] within forage level administered according to a split-plot, 4 × 4 Latin square design with 21 d periods. Heifers fed HC had greater total apparent digestibility for dry matter (DM) digestibility and organic matter (OM) digestibility. Interactions were noted for neutral detergent factor and acid detergent factor(NDF & ADF) digestibilities with linear interactions for the LC forage level and quadratic interactions for the HC forage level. Similar results were noted for nitrogen (N) parameters including: digestibility, fecal N (g/d), urine N (g/d), total excreted N (g/d), retained N (g/d) and retained N (%). These results indicated linear interactions in LC forage diets with quadratic interactions in HC diets. The LCU0 treatment offered the highest utilization/retention of N within LC diets while the HCU2 diet offered the highest utilization/retention of N within HC diets. Overall, the HCU2 treatment offered the highest N efficiency among all treatments.

INTRODUCTION

The ruminant animal is unique in its ability to survive on a diet consisting entirely of non-protein nitrogen (Dewhurst et al., 2000), but its growth cannot be sustained solely by rumen microbial protein synthesis. Microbial protein contribution can represent from 50% to 80% of the amino acids absorbed (Clark et al., 1992; Storm and Orskov, 1983). Microbial protein supply must be maximized to take advantage of this unique characteristic (Johnson et al., 1998; Bach et al., 2005). Several studies have demonstrated that infusing greater amounts of readily fermentable carbohydrates decreased NH_3 N concentration in the rumen due to an increased N intake by the ruminal microbes (Henning et al., 1993; Cameron et al., 1991; Casper and Schingoethe, 1999).

Research on impact of protein degradability in dairy heifer diets is limited. Gabler and Heinrichs (2003) and Zanton et al. (2007) studied the effect of manipulating the soluble fraction of RDP in dairy heifers fed high forage diets, and observed no difference in N utilization when soluble protein was increased as portion of RDP. Both studies were cautious with their conclusions because statistical evidence was insufficient and effects seen were diet dependent. Also, it has been suggested that a CP:ME of around 55 g/Mcal per d maximizes ADG and nutrient utilization (Lammers and Heinrichs, 2000; Gabler and Heinrichs, 2003). Recently, Zanton and Heinrichs (2009) suggested an optimal level of N intake for heifers limit-fed either high or low concentrate diets is 1.67 g of N/kg $BW^{0.75}$. Casper et al. (1999) reported increased ADG with an **NSC:**RDP of 3.30 and hypothesized that synchronizing NSC:RDP may increase **AA** flow to the small intestine and maximize efficiency of protein use for growth. Rapid fermentation rate of non-structural carbohydrates can provide carbon skeletons for microbial

protein synthesis from NPN (Heldt et al., 1999) decreasing the loss of more biologically valuable protein.

It is important to note that the synchrony at which nutrients are utilized in growing animals differs from mature dairy cows (Van Soest, 1994). In dairy heifers passage rates (**kp**) are higher due to a lower rumen capacity, but rates of passage are lower when diets are being precision-fed compared to ad libitum, with high concentrates contributing a reduced kp and increased digestibility (Lascano et al., 2012). When sources of rumen degradable protein (RDP) such as barley or soy bean meal are incorporated, which are 80% and 85 % degradable in the rumen respectively (NRC, 2001), the majority of this protein is converted to microbial protein available for absorption in the small intestine (Storm, and Orskov. 1983). This suggests that NPN supplementation can replace the true protein fraction of the RDP. The rapid fermentation rate of NSC can provide carbon skeletons for microbial protein synthesis from NPN decreasing the loss of more biologically valuable protein. To what extent this happens in a precision-fed scenario is not known. Altogether, these results suggest the degradable protein fraction of the diet can be manipulated with different responses expected as the forage level of the diet changes. Therefore, the objective of this experiment was to determine the effects of manipulating the degradable protein fraction in precision-fed heifers containing different NPN and true protein combinations when two levels of forage were fed.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures involving the use of animals were approved by the California Polytechnic State University Institutional Animal Care and Use Committee. Eight Holstein ruminally cannulated heifers (14.6 ± 0.1 mo of age, and 386 ± 9.1 kg of weight) were randomly assigned to 2 forage levels: HC (25% forage) and LC (75% forage) and to a degradable protein sequence [0% degradable protein from urea **U**, 100% casein **C** (no urea treatment **U0**); 33% U, 67% C (low urea treatment **U2**); 67% U, 33% C (high urea treatment **U3**); 100% U 0% C (all urea treatment **U4**)] within forage level administered according to a split-plot, 4 × 4 Latin square design with 21 d periods. The whole plot factor was the proportion of forage in the diet and the subplot was the ratio of U to C. Specifically, heifers were offered a basal diet containing HC or LC with 40% of the protein concentration coming from U0, U2, U3 or U4 combinations. Similar N intake and RDP were provided to supply 1.70 g N/kg $BW^{0.75}$, which has been observed to maximize N utilization in dairy heifers (Zanton and Heinrichs, 2009). Diets were provided as a TMR at a level calculated to provide equal intakes of ME and to allow for 800 g/d of ADG. Adaptation to treatment rations (U:C combinations) were made over the first 15 d of each period and on d 16 sample collection began. Basal diets are presented in Table 1. Heifers were weighed weekly 2 h before feeding; amount of feed offered for the next 8 d was based on the weighted weekly averages and not changed. Rations were mixed daily at 1200 h by preparing each diet individually and the U and C (sodium caseinate; American Casein Company, Burlington, NJ) combinations were top dressed accordingly. Heifers were fed daily at 1400 h. Heifers were housed in individual stalls (117 × 302 cm) in a naturally ventilated tie-stall barn with rubber mattress bedding and were allowed access to an exercise lot for 2 h before the 1400 h feeding on

non-sampling days. Time (min) required to finish a meal was recorded, and water was available ad libitum.

Fecal, Urine, and Feed Sample Collection and Analysis

Feces and urine were collected from d 16 to 20 (4 d of total collection). Urine was collected via modified urine device (Lascano et al., 2010), weighed, and sub-sampled daily after feeding. A 250-mL subsample was frozen at -20ºC for further analysis. Urine pH was monitored and acidified to pH < 2 by the addition of 12 *N* HCl as required to minimize NH_3 volatilization (Zanton and Heinrichs, 2009). Feces were collected hourly and stored in airtight containers; every 24 h total collection of feces was mixed, weighed, recorded, and sub-sampled. Feedstuffs, TMR, feces (dry basis), and urine (wet basis) were composited by period. Samples were dried in a 65ºC forced air oven for 4 d, ground through a 1-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA), and analyzed for DM, OM, ash, N (AOAC, 2000), ADF, sulfuric acid detergent lignin (ADL), and NDF (Van Soest et al., 1991) using an ANKOM^{200} Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY) with heat resistant α-amylase and sodium sulfite utilized in the NDF procedure. Frozen fecal and urine subsamples were thawed and analyzed for N using the Kjeldahl method (AOAC, 2000). Metabolizable energy intake was calculated for each heifer within each period using observed digestible OM intake × 4.409 × 0.82 (NRC, 2001).

Ruminal Sampling

Rumen fluid (15 mL) was sampled on d 20 to 21 at -2, 0, 2, 4, 6, 8, 12, 16, 20, and 24 h after the 1400-h feeding to determine the profile of rumen ammonia and VFA as described by Moody et al. (2007). An aliquot of 4 mL was mixed with 1 mL of methyl green formalin-saline solution (35% formaldehyde solution, 100 mL; distilled water, 900 mL; methyl green, 0.6 g;

sodium chloride, 8.0 g). At the end of each 21-d period rumen contents were evacuated 4h after the 1400-h feeding, which was expected to represent the times rumen contents reach maximum level. Mass and volume of total contents as well as solid and fluid fractions (SP and FP, respectively) were recorded. The FP was defined as material not retained on 2 layers of cheesecloth. The proportional composition of the 2 fractions was determined gravimetrically and frozen for later analysis. Samples were freeze dried and analyzed for chemical composition as indicated previously for feed and fecal samples.

Statistical Analysis

Statistical analyses were conducted in Statistical Analysis System version 9.2 for windows (SAS Institute, Cary, NY) using the mixed procedure. All dependent variables were analyzed as a 4×4 Latin square design. A split plot design was used with forage level as the whole plot and U:C combinations as the sub-plot. Sources of variation associated with fixed design effect of period and fixed treatment effects of forage level, U:C, and their interaction were used with heifer within forage level included as a random effect. The sequences of U:C were balanced for carryover with respect to previous U:C combination such that all treatments followed every other treatment once; therefore, fixed effect of previous treatment also was included in statistical analysis. For observations where multiple measures occurred in a period, fixed effect of time and its interaction with other fixed effects was included in the model. Repeated measurements (Littell et al., 1998) including 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. Forage level effect was assessed with denominator degrees of freedom and error term as associated with whole plot error of heifer within forage, and the effects of U:C and the

interaction were evaluated against the pooled residual error. Normality of residuals was evaluated using the Shapiro-Wilk test for normality. Differential responses between F:C and U:C combination were assessed for some variables through mixed model regression analysis; output from this analysis is displayed as the adjusted (for random effect of heifer) response against U:C combination. Least squares means are presented in tables, and evidence for statistical significance was declared at $P < 0.05$ and trends were indicated at $P < 0.10$.

RESULTS

Nutrient Intake

Diet ingredients and chemical composition values are shown on Table 1. Diets were formulated to be isoenergetic and isonitrogenous. Two forage levels served as whole plot factors with subplot factors designed to offer different combinations of true protein and non-protein N replacing 40% of RDP (Table 1). Nitrogen intake was similar among all treatments with a targeted value of 1.67 g of N/kg $BW^{0.75}$ to maximize N intake for precision-fed heifers as suggested by Zanton and Heinrichs (2009). Furthermore, total mixed rations allowed for 800g/d ADG with metabolizable energy for the HC diets at 2.75Mcal/kg$\pm$0.01 and for the LC group at 2.47Mcal/kg$\pm$0.02. The time period required to consume a meal differed between the forage treatments from 80 to 87 min for the LC group and 40 to 44 min for the HC group. Whole plot intake treatments differed when providing kg/d of intake for: as fed basis, DM, OM, Ash, NDF, ADF, CP and NFC but were similar between subplot groups. Urea treatments amounts differed linearly for NFC values from 2.44 to 2.69 kg/d.

Diet Digestibility

Total tract apparent digestibilities are shown in Table 3. The total tract digestibility of DM and OM differed between forage treatments with higher digestibilities noted for the HC treatment. Interaction was noted for NDF and ADF digestibility between the concentrate and urea levels. In the LC treatment, the NDF apparent digestibility was the highest at the U0 (no urea) level, whereas for the HC diet, apparent digestibility was highest at the U2 (67% urea) level. ADF digestibility yielded similar results, with the LC treatment having its highest apparent digestibility at the U0 level and the HC diet at the U2 level. A similar linear interaction is seen

with regards to N apparent digestibility; the LCU0 and HCU2 treatments represent the groups with highest N digestibility between whole-plot factors.

N Dynamics

Retention and dynamics for N are shown in Table 3. With similar intake amounts of N across all treatments, differing values for the outputs of N show interesting results. Fecal N and Urine N resulted in higher total N excretion for LC compared to HC diets. Taking subplot effects into account, total fecal N excretion was the lowest in the LCU0 and HCU2 treatments with values of 48.38 g/d and 39.33g/d, respectively. Urinary N excretion has similar treatment results between LCU0 and HCU2 groups with respective Urine N, g/d values at 50.34 and 37.45 indicating a higher urinary excretion amount of N for the LC treatment. With regards to retained N, we see the LCU0 and HCU2 groups retain the most amounts of N among the different U levels respectively. Looking at the % retained N from intake values, the interaction between test parameters render the LCU0 and HCU2 combinations as the ones with the highest values. The LCU0 treatment retained as much as 35.13% of dietary N while the HCU2 treatment retained as much as 50% of dietary N.

DISCUSSION

Results indicated interactions between forage and urea treatments with regards to apparent diet digestibilities and N parameters. Two points of interest include: dietary fiber digestibility, and the protein sources and their utilization.

Dietary Fiber Digestibility

The high quantities of readily fermentable carbohydrates present in the HC forage diets yielded much higher apparent digestibilities for DM and OM treatment properties. Urea amount appeared to have an opposite effect on these digestibilities whether it was increased as part of the degradable fraction in the HC or the LC diet. This differing effect in N utilization resulted in similar fiber degradability; the NDF and ADF apparent digestibilities of the diet followed similar as N utilization. There are two limiting factors for microbial protein synthesis: energy from carbohydrates, and an N source. This is important to understand because too little N could decrease MCP while too much N could yield ammonia toxicity in the animal. Also, rates of passage (kp) differ between mature cows and growing heifers; a heifer's lower rumen capacity increases kp. However, kp is lowered when diets are precision-fed with reduction in kp due to high concentrate proportions (Lascano et al., 2012). The quick enzymatic reaction breaking down urea into carbon dioxide and ammonia provides the rumen microbial population quick access to readily useable N. Understanding these rates of N provision are crucial to achieving correlation with the rates of carbohydrate digestion by ruminal microbes. As seen in Table 3, the LC diets appeared to attain higher digestibility for both NDF and ADF at a U0 urea treatment. Alternatively, HC diets demonstrated higher NDF and ADF digestibility at a urea level of 67%. The slower digesting, high fiber LC diets might supply a CHO backbone in unison to the rate of N provision by true protein degradation while in the HC forage diets, the readily fermentable

carbohydrates might be digested at a rate optimizing the utilization of N provided from the quickly degraded urea. Therefore, the synergy seen in both treatments might be due to the well balanced proportions of energy from CHOs and N availability.

Protein Sources and their Utilization

Casper et al. (1999) noted an increase in ADG with a NFC:RDP ratio of 3.30. They hypothesized that proper synchronization of NFC:RDP could possibly increase efficiency in utilization of N and carbohydrates for microbial protein synthesis. This, in turn, was noticed when analyzing biological values of N in our treatments, as seen in Table 3. Fecal and urinary N excretion demonstrated to have strong interactions between and within treatments. In diets with higher quantities of readily fermentable carbohydrates (HC), lower quantities of N were excreted when urea represented 27% of the RDP (Table 3). Meanwhile, with a LC diet, the lowest quantities of N excretion were observed for the U0 group. Nonetheless, the HCU2 treatment demonstrated to have the lowest total excretion of N, g/d at 76.98. This data goes hand in hand with the quantities of retained nitrogen, once again indicating the most retention occurring for the HCU2 treatment.

These data support previous findings indicating that feeding greater amounts of readily fermentable carbohydrates decreased NH_3 N concentration in the rumen due to an increased N intake by ruminal microorganisms (Henning et al., 1993; Cameron et al., 1991; Casper and Schingoethe, 1999). It becomes important to note the quadratic effects seen with the effects of urea combinations on HC forage diets. Optimization occurred at U2, where urea represented 67% of the 40% RDP that the combination of urea and casein provided in this experiment. Diets with 100% urea demonstrated to have the lowest efficiency rates in either of the two forage treatments supporting the data from Dewhurst et al. (2000) which highlighted that the ruminant

animal is unique in its ability to survive on a diet consisting entirely of non-protein nitrogen, but does not necessarily entail efficient practice. Overall, these results advocate previous findings indicating that DM digestibility and feed efficiency are preferential with the use of HC diets compared to control fed LC diets(Moody et al., 2007; Lascano et al. 2009b). Furthermore, such comparisons have indicated no perturbation of metabolic pathways and the health of the animals (Moody et al., 2007; Lascano and Heinrichs, 2009; Lascano et al., 2009b).

By manipulating passage rates of feed with different carbohydrate sources, we were able to demonstrate a high retention of N in heifers with a NPN level of 27% and true protein level of 13% of RDP with HC diets. Further studies analyzing the effects of varying RUP characteristics will help elucidate further understandings of nutrient utilization, rumen fermentation, and microbial protein synthesis aimed at improving whole-animal efficiency and sustainability.

CONCLUSIONS

Manipulation of the degradable protein fraction in precision-fed heifer's diets was done to study the synchrony at which nutrients are utilized in growing dairy heifers. Understanding how rates of passage differ between mature cows and growing heifers, two forage levels (HC and LC) were utilized as whole plot parameters to test within differing dietary RDP characteristics. As expected, HC forage levels yielded higher N utilization and retention between whole plot factors. The HCU2 treatment proved to be the most efficient combination as a whole for optimizing dietary N in growing heifers. Therefore, it appears that a lower kp, due in part to the precision-feeding style and high amount of readily fermentable carbohydrates, along with the quickly catalyzed urea composing 27% of RDP, resulted in the most efficient usage of dietary N. It becomes clear that dietary ingredients and their chemical compositions are strongly interrelated with whole-animal digestive kinetics and the synergy under which interaction occurs with its digestive tract microorganisms. While this experiment sought to understand the effects of manipulating the composition of RDP in a ruminant's diet, it's only half of the story. Investigating of the effects of rumen undegradable protein on whole-animal production is the next step.

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APPENDIX

Table 1. Ingredient and diet chemical composition of dairy heifers fed differing forage to concentrate ratios (F:C) as low (LC) or high (HC) concentrate diets containing 4 Combinations of true protein and non-protein N replacing 40% of RDP (U0; no urea, U1; 33% as urea; U2 67% as urea; U4 100% as urea)

	Treatment				Treatment			
	HC				LC			
	U0	**U1**	**U2**	**U4**	**U0**	**U1**	**U2**	**U4**
Ingredients, %DM								
Oat Hay	2.50	2.50	2.50	2.50	18.00	14.67	11.33	8.00
Alfalfa Hay	2.50	2.50	2.50	2.50	7.00	7.00	7.00	7.00
Corn Silage	20.00	20.00	20.00	20.00	50.00	53.33	56.67	60.00
Cracked Corn	42.25	41.78	41.31	40.84	6.80	6.62	6.44	6.26
SBM	0.99	0.99	0.99	1.00	0.00	0.00	0.00	0.00
[1]Soy Best	2.46	2.46	2.46	2.46	4.81	4.68	4.54	4.41
Molasses	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
SB hulls	20.00	20.00	20.00	20.00	4.20	4.40	4.60	4.80
Starch	0.00	1.54	3.09	4.63	0.25	1.35	2.46	3.56
CASEIN	4.90	3.27	1.63	0.00	4.54	3.03	1.51	0.00
Urea	0.00	0.49	0.99	1.48	0.00	0.46	0.91	1.37
Sodium Bicarb	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ammoniun Sulfate	0.00	0.06	0.13	0.19	0.00	0.07	0.13	0.20
[2]Heifer Mineral	2.95	2.95	2.95	2.95	2.95	2.95	2.95	2.95
Chemical Composition								
CP, %	15.93	15.83	15.74	15.65	14.22	14.16	14.09	14.02
Soluble, % of CP	6.30	6.25	6.20	6.15	5.69	5.67	5.65	5.63
SP, %CP	0.40	0.39	0.39	0.39	0.40	0.41	0.40	0.41
RDP, % of CP	11.37	11.22	11.07	10.92	10.47	10.38	10.29	10.20
RDP, %CP	71.00	71.00	70.00	70.00	74.00	73.00	73.00	73.00
Casein+Urea, %RDP	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Casein, %RDP	40.00	27.00	14.00	0.00	40.00	27.00	14.00	0.00
Urea, %RDP	0.00	13.00	27.00	40.00	0.00	13.00	27.00	40.00
Urea, % C+U	0.00	33.00	67.00	100.00	0.00	33.00	67.00	100.00
RUP, % of CP	4.56	4.54	4.51	4.49	3.77	3.71	3.64	3.58
NFC[3], %	47.62	48.78	49.94	51.10	34.76	35.83	36.90	37.97
NDF, %	28.75	28.71	28.66	28.61	40.07	40.02	39.96	39.91
ADF, %	17.81	17.79	17.77	17.75	22.93	22.78	22.63	22.48
EE, %	4.22	4.19	4.16	4.13	3.47	3.47	3.47	3.47
ME[4], Mcal/kg	2.76	2.75	2.74	2.74	2.47	2.46	2.45	2.44

[1] Soy Best. Contains: 87.04% DM, 46.9% Protein, 4.03% soluble CP, 21.9% RDP, 21.7% NDF, 10.4% ADF, 1.0% Fat.

[2] Mineral mix, Contains: 11.44% calcium, 0.41% Phosphorus, 9.03% salt, 2.65% magnesium, 0.48% potassium, 0.48% sulfur, 9.20 ppm cobalt, 542.71 ppm copper, 22.20 ppm iodine, 235.12 ppm iron, 1628.87 ppm manganese, 10 ppm selenium, 1639.37 ppm zinc, 16.73 mg/lb niacin, 320.84 mg/lb choline, 70746.82 IU/lb vitamin A, 17637.9 IU/lb vitamin D, 1228.7 IU/lb vitamin E.

[3]NFC: non-fiber carbohydrates = 100 - CP - ether extract - crude fat - NDF – Ash

[4]ME: calculated as TDN× 0.04409 × 0.82

Table 2. Feed intake of dairy heifers fed differing forage to concentrate ratios (F:C) as low (LC) or high (HC) concentrate diets containing 4 Combinations of true protein and non-protein N replacing 40% of RDP (U0; no urea, U1; 33%as urea; U2 67% as urea; U4 100% as urea)

Item		Urea				SEM	Concentrate	Contrast, P – value[1]			
								Urea		Interaction	
		U0	U1	U2	U4			L	Q	L	Q
BW, kg	LC	395	391	390	394	9.1	0.72	0.95	0.65	0.76	0.78
	HC	392	386	396	389						
Time to	LC	81	87	80	85	9	<0.01	0.34	0.56	0.67	0.5
complete a meal, min	HC	44	40	43	42						
N Intake											
N, g/d	LC	152.4	150.36	150.07	149.45	5.7	0.5	0.8	0.45	0.76	0.87
	HC	152.41	145.43	154.46	150.66						
N Intake, g/kg of $BW^{0.75}$	LC	1.72	1.71	1.71	1.69	6.06	0.78	0.87	0.75	0.86	0.32
	HC	1.73	1.67	1.74	1.72						
Intake, kg/d											
As fed	LC	12.47	13.62	12.57	12.92	0.37	0.02	0.55	0.56	0.45	0.78
	HC	10.57	10.55	10.55	10.95						
DM	LC	6.86	6.81	6.79	6.85	0.26	0.04	0.67	0.78	0.6	0.8
	HC	6.45	6.54	6.51	6.57						
OM	LC	6.17	6.2	6.25	6.3	0.14	0.02	0.26	0.34	0.58	0.71
	HC	5.87	5.95	6.05	6.04						
Ash	LC	0.7	0.64	0.57	0.58	0.06	0.05	0.84	0.98	0.89	0.88
	HC	0.61	0.62	0.49	0.56						
NDF	LC	2.81	2.8	2.8	2.83	0.14	<0.01	0.73	0.63	0.67	0.45
	HC	1.8	1.78	1.82	1.79						
ADF	LC	1.61	1.59	1.59	1.59	0.1	<0.01	0.6	0.23	0.56	0.87
	HC	1.12	1.1	1.13	1.11						
CP	LC	1	0.99	0.99	0.99	0.06	<0.01	0.37	0.62	0.61	0.87
	HC	1	0.98	1	0.98						
CP:MEg	LC	57.47	57.44	57.42	57.39	0.4	0.82	0.81	0.49	0.67	0.82
CP:Mcal/kg	HC	57.81	57.59	57.37	57.15						
RDP	LC	0.73	0.73	0.72	0.72	0.5	0.78	0.61	0.45	0.34	0.45
	HC	0.71	0.7	0.7	0.68						
NFC	LC	2.44	2.5	2.59	2.69	0.1	<0.01	0.04	0.45	0.67	0.34
	HC	2.98	3.03	3.17	3.2						
ME,[3] Mcal/d	LC	17.36	17.23	17.19	17.32	0.24	0.25	0.45	0.32	0.45	0.34
	HC	17.2	17.06	17.39	17.16						

[1]L = linear; Q = quadratic.
[2] ME: calculated as digestible OM × 0.04409 × 0.82

Table 3. Nutrient apparent digestibility and N dynamics of dairy heifers fed differing forage to concentrate ratios (F:C) as low (LC) or high (HC) concentrate diets containing 4 Combinations of true protein and nonprotein N replacing 40% of RDP (U0; no urea, U1; 33% as urea; U2 67% as urea; U4 100% as urea)

Item	For age	Urea				SEM	Concentrate	Contrast, P – value[1]			
								Urea		Interaction	
		U0	U1	U2	U4			L	Q	L	Q
Apparent digestibility, %											
DM	LC	63.33	62.71	63.62	63.99	1.42	<0.01	0.69	0.34	0.45	0.80
	HC	69.08	69.34	69.00	68.42						
OM	LC	67.06	66.14	67.18	67.29	1.29	<0.01	0.56	0.45	0.33	0.39
	HC	72.33	73.01	72.23	72.03						
NDF	LC	58.02	56.67	56.05	54.03	1.96	<0.01	0.80	0.45	0.05	0.03
	HC	60.44	62.33	64.90	60.49						
ADF	LC	48.56	46.89	46.67	44.89	1.68	<0.01	0.23	0.22	0.03	<0.01
	HC	49.21	50.40	54.58	52.88						
Ash	LC	45.61	45.58	45.85	47.31	2.27	0.33	0.92	0.62	0.63	0.67
	HC	42.56	37.67	45.89	32.82						
N											
Digestibility, %	LC	68.27	63.58	62.02	60.28	0.85	<0.01	0.15	0.88	0.05	0.03
	HC	70.32	72.67	74.55	69.43						
Intake, g/d	LC	152.40	150.36	150.07	149.45	5.70	0.50	0.80	0.45	0.76	0.87
	HC	152.41	145.43	154.46	150.66						
Fecal N, g/d	LC	48.38	54.78	57.02	59.38	1.84	0.02	0.1	0.76	0.04	0.02
	HC	45.25	39.77	39.33	46.08						
Urine N, g/d	LC	50.34	54.45	56.02	58.21	3.74	0.03	0.33	0.45	0.05	0.02
	HC	45.54	42.23	37.45	42.44						
Total excreted N, g/d	LC	98.92	109.43	113.24	117.79	5.72	0.02	0.16	0.19	0.04	<0.01
	HC	90.99	82.20	76.98	88.72						
Retained N, g/d	LC	53.53	40.98	36.88	31.71	3.42	<0.01	0.13	0.49	0.02	0.03
	HC	61.46	63.28	77.53	61.99						
Retained N, % Intake	LC	35.13	27.29	24.38	21.25	2.74	<0.01	0.24	0.28	0.05	0.05
	HC	40.34	43.56	50.17	41.15						

[1]L = linear; Q = quadra