Effects of Rumen-Undegradable Protein and Feed Intake on Nitrogen Balance and Milk Protein Production in Dairy Cows

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ABSTRACT

An experiment was designed to determine the response of milk protein production and N utilization in dairy cows to supplementation of a predominantly rumen-undegradable protein (RUP) mixture with a fixed amino acid (AA) pattern and the response to the amount of feed intake. The experiment was designed as a 6×6 Latin square with a 3×2 factorial arrangement of treatments. The factors were three concentrations of RUP supplement (4.5, 14.9, and 29.1% of dry matter intake) and two levels of feed intake restriction (10 and 20%) of the basal diet. The supplement was designed to approximate a postruminal AA pattern that was similar to bovine caseins for Met, Lys, Phe, His, and Thr. Measurements were made during the last 5 d of each 21-d period.

Milk protein production responded linearly as the concentration of RUP supplement in the treatment diet increased within the given range. The difference in feed intake restriction did not affect milk protein production. Efficiency of N utilization for milk production exceeded 30% for cows fed the lowest RUP supplement. Results indicated that there is an opportunity to increase milk protein production by using RUP formulations that are balanced for AA while minimizing waste N excretion.
(**Key words**: dairy cows, milk protein, nitrogen balance, rumen-undegradable protein)

INTRODUCTION

A variety of experiments have been conducted in an attempt to enhance milk protein production using several methods, including supplemental RUP, rumen-protected AA, and postruminal infusions of individual AA and groups of AA (16, 24). The collective results of those experiments and others demonstrate that essential AA above those provided from microbial protein are necessary to support high milk production. The use of RUP is a common and practical approach to the supplementation of essential AA in the diet (14). However, the positive responses of milk protein production that are observed after dietary changes are often difficult to relate to dietary AA inputs. Bacterial protein production in the rumen or differences in the AA content of RUP sources can induce changes in the AA of digesta postruminally. Dietary changes caused by the use of an RUP supplement with a fixed essential AA content administered while microbial protein changes are held to a minimum could effect a response in milk protein production that would be attributable to the intended RUP dietary perturbation. After feeding diets that were deficient in CP (10.7 to 11.5%, DM basis) and infusing various AA into the abomasum, Schwab et al. (24), concluded that Met and Lys were the first two limiting AA for milk protein production in cows that consumed low protein diets based on corn. In their review, Murphy and O'Mara (20) indicated the AA that were limiting for milk protein production were likely one or more of the following essential AA: Met, Lys, Phe, His, or Thr. The RUP supplement was designed to have a postruminal AA profile similar to bovine caseins (22) for Met, Lys, Phe, His, and Thr.

A feed restriction difference of 10% was intended to provide a constraint to milk protein production by limiting dietary energy intake and minimizing dietary induced changes to milk composition. This experimental design allowed response curves to be developed similar to those of Campbell et al. (7), who tested the influence of feeding level on the protein requirements of growing pigs. When a fixed pattern of AA at different levels of intake is tested, conclusions can be made as to the effectiveness of the supplementation of increased percentages of RUP and the role feed intake has on milk protein production. In the development of the ideal AA pattern for pigs, Wang

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and Fuller (31) used N balance experiments, which allowed them to trace the fate of consumed N and evaluate the dietary AA balance. In the present experiment, a similar goal to determine the fate of N when cows consumed different percentages of an RUP supplement necessitated N balance measures.

Therefore, the present experiment was designed to test the null hypothesis that milk protein production in dairy cows would not have a dose-dependent response to an RUP supplement with a balanced AA content or to the amount of feed intake.

MATERIALS AND METHODS

Experimental Design and Cows

Six mature Holstein cows (5.1 ± 1.4 yr of age; 618 ± 65 kg of BW; 101 to 133 DIM) that were past peak lactation were used in a 6 × 6 Latin square design with 21-d periods and a 3 × 2 factorial arrangement of treatments. The experimental factors were three concentrations of an RUP supplement and two levels of feed intake restriction of the common basal diet. The use of cows for this experiment was approved by the University of Guelph Animal Care Committee.

RUP Formulation

The RUP supplement was formulated based on the results of a prior study (T. C. Wright and B. W. McBride, 1994, unpublished data) in which AA determinations were conducted on many feed residues from nylon bags that were incubated in the rumen for 14 h. Different combinations of residues were evaluated for AA composition. The combination selected as the best approximation to caseins for the selected essential AA included herring, feather, and blood meals (Ralston Purina Canada Ltd., Woodstock, ON, Canada). These ingredients were pelleted (0.5 cm diameter) with wheat to reduce sorting of the individual ingredients by the cows. Rumen-protected Met and Lys (Smartamine ML®; Rhône-Poulenc, Mississauga, ON, Canada) were added to the RUP supplement to avoid a calculated deficiency in their balance relative to their concentrations in caseins using only herring, feather, and blood meals (Table 1). The rumen-protected AA product was not included in the pellet because the pressure used in the pelleting process would damage the copolymer coating of the product and reduce protection from the rumen. The rumen-protected AA were weighed and mixed by hand into the ration of pellets for each diet.

The AA composition of the original material of the pellets in the present experiment (pellets were ground through a 1-mm screen) was determined after a 12-h rumen incubation period (Table 2) as part of

TABLE 1. Ingredients and chemical analysis of pelleted RUP supplement.[1]

Component		
	(%, as-fed basis)	
Ingredient		
Soft white wheat	25.0	
Herring meal	42.7	
Feather meal	22.8	
Blood meal	9.5	
	$\bar{X}$	SE
Chemical		
DM, %	92.8	0.2
	(%, DM basis)	
CP	63.7	0.7
ADIN	4.3	0.2
Soluble protein	7.2	0.4
NDICP[2]	23.2	1.3
Crude fat	7.5	0.2
Ca	2.1	0.1
P	1.3	0.03
Ash	7.5	0.2

[1]Analysis of six samples.

[2]Neutral detergent insoluble CP.

TABLE 2. The AA composition of the RUP supplement residues following 12 h of incubation in the rumen of the original material and reference caseins.[1]

AA	Supplement		Caseins
	(%)		
	$\bar{X}$	SE	
Asp	7.42	0.03	4.24
Glu	10.05	0.19	8.98
Ser	5.26	0.08	3.67
Gly	4.48	0.00	1.28
His	2.44	0.14	2.35
Arg	4.88	0.06	3.46
Thr	3.82	0.00	4.84
Ala	4.97	0.00	3.11
Pro	4.80	0.04	8.92
Tyr	3.15	0.06	5.99
Val	5.09	0.03	6.09
Ile	3.10	0.01	5.58
Leu	7.21	0.01	7.44
Phe	4.34	0.01	4.21
Lys	6.64[2]	0.47	8.01
Cys	1.63	0.04	0.46
Met	1.96[2]	0.04	2.40

[1]Amino acids in the incubated residues of the RUP supplement are percentages of the DM. Amino acids in the reference caseins are molar percentages.

[2]Includes calculated contribution from rumen-protected Met and Lys. Addition to Lys was 0.903% and to Met was 0.31%. Both calculated additions assumed 90% rumen protection.

another rumen degradation study, which did not include the previous 14-h incubation time. Rumen-cannulated cows were offered the low CP basal diet used in the present experiment for ad libitum intake.

Diets

The basal diet in this experiment was intentionally designed to be low in CP (Table 3). Changes to the CP content of the treatment diets were accomplished by altering the concentration of the RUP supplement. During each period, cows were offered feed for ad libitum intake for the first 7 d. Ad libitum intake was characterized as follows: allotment of the basal diet to allow at least 10% orts but fixed quantities of RUP supplement that were fed according to treatment. On d 8 of each period, mean feed consumption was determined for each cow based on intake over the previous 7 d. The RUP supplement then constituted a fixed proportion of intake according to treatment: low, 4.5%; medium, 14.9%; and high, 29.1% of DMI. Allocation of the basal component of the diet was restricted by either 10 or 20% of the remainder of the dietary allotment, depending on treatment. Therefore, feed intake restriction was accomplished by limiting only the basal portion of the diet. During the experiment, the pellet and rumen-protected AA were included at the following mean concentrations in the diet (as-fed basis): low, 0.865 ± 0.066 kg/d (rumen-protected AA = 17 ± 2.0 g/d); medium, 3.019 ± 0.314 kg/d (rumen-protected AA = 59 ± 6.0 g/d); and high, 6.076 ± 0.641 kg/d (rumen-protected AA = 117 ± 12.0 (g/d). The entire supplement was top-dressed onto the basal diet at feeding, which was at 0700 and 1230 h.

TABLE 3. Ingredients and chemical analysis of basal diet.[1]

Component		
	— (%, as fed basis) —	
Ingredient		
Mixed straw	4.04	
Corn silage (36.2% DM)	60.56	
High moisture corn (75.1% DM)	33.60	
Mineral and vitamin mix[2]	0.83	
NaCl	0.19	
Ground limestone	0.67	
KCl	0.09	
	$\bar{X}$	SE
Chemical		
DM, %	51.2	0.9
	— (%, DM basis) —	
CP	8.0	0.1
ADIN	0.5	0.1
Soluble protein	36.7	1.1
ADF	20.2	1.0
NDF	36.2	1.8
Crude fat	3.1	0.1
NFC[3]	48.1	1.7
NE_L,[4] Mcal/kg	1.6	0.0
Ca	0.8	0.1
P	0.3	0.1

[1]Analysis of six samples.

[2]Mineral and vitamin mix contained (per kilogram): 16% Ca, 14% P, 7.5% Mg, 1.5% K, 3200 mg of Mn, 1500 mg of Cu, 5000 mg of Zn, 36 mg of Co, 100 mg of I, 2% S, 600,000 IU of vitamin A, 250,000 IU of vitamin D, 3200 IU of vitamin E, and 22 mg of Se (F0438-01; Floradale Feed Mill Ltd., Floradale, ON, Canada).

[3]Nonfiber carbohydrates = (100 − NDF − CP − fat − ash).

[4]Estimated $NE_L = 2.2 \times [0.866 - 0.007 \times \text{ADF (percentage)}]$.

Sample Collection

Data were collected during the last 5 d of each period. Milk samples were collected from consecutive morning and afternoon milkings and pooled daily based on production. A fresh sample of milk was analyzed daily for fat, protein, and lactose. Milk samples for subsequent analyses of N and true protein were frozen at –20°C.

Urine was collected using indwelling bladder catheters (26 French, 75 ml; C. R. Bard, Inc., Covington, GA), which was similar to the method described by Crutchfield (13). A modification of this technique included leaving the catheter in place, unconnected to collection tubing, for 24 h before collection. Cows were administered 40 ml of penicillin (Ethacilin®; Rogar/STB, London, ON, Canada) intramuscularly for 3 d following removal of the catheters. Urine was collected under acidic conditions, and 150 ml of concentrated hydrochloric acid (Fisher Scientific, Toronto, ON, Canada) were added daily to the empty polyethylene containers. A 5% subsample of urine was taken each day during the collection period. Daily urine samples were also collected, diluted five times with distilled water, and frozen at –20°C. The frozen samples were used to determine urea content. Fecal samples were collected into large steel trays positioned over the gutter behind each stall. Daily collections were placed into a plastic container, weighed, and mixed; then, grab samples were taken. Samples were frozen at –20°C for later analysis.

Feed samples were taken twice during collections and frozen at –20°C. Orts were weighed every morning, when present, and a representative sample was collected and frozen. Dry matter determinations for feeds and feces were done by lyophilization (50 SRC; The Virtis Co., Gardiner, NY). Feeds and feces were ground using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England) equipped with a 1-mm screen. Feces were proportioned on a DM basis

each day to obtain a composite sample for each cow. Feeds were ground and combined in equal proportions from each sampling to determine N and feed chemistry.

Blood samples were taken during the afternoon on d 21 from the coccygeal vein between 1530 and 1630 h. Samples were collected using 2.5-cm, 20-gauge needles (Becton Dickinson, Rutherford, NJ) into 10-ml vacuum tubes for serum collection (Becton Dickinson). Blood was allowed to clot for 90 min at ambient temperature before centrifugation at $1500 \times g$ for 15 min. Serum was transferred from the vacuum tubes by disposable transfer pipet in 1-ml aliquots and frozen at –70°C until analysis.

Analytical Determinations

Milk fat, protein, and lactose were analyzed by near infrared analysis (Foss System 4000; Foss Electric, Hillerød, Denmark) at the Ontario Ministry of Agriculture Food and Rural Affairs Central Milk Test Laboratory (Guelph, ON, Canada). Nitrogen determinations were made on all feed, orts, feces, urine, and milk samples using the macro-Kjeldahl procedure of the AOAC (3). Milk samples were thawed in a water bath at 38°C and mixed according to AOAC method 925.21 (3) prior to determinations of N and true protein. True protein was determined according to AOAC method 991.22 (4). Urea in urine and milk was determined using a kit (no. 542946; Boehringer-Mannheim Montreal, QC, Canada). Feed samples were analyzed for input into the Cornell Net Carbohydrate and Protein System by wet chemistry at a commercial laboratory (Northeast DHIA, Ithaca, NY). All analytical determinations were corrected when appropriate by DM determination [AOAC method 930.15; (3)] following analysis.

Blood samples were analyzed at the Department of Clinical Pathology, Ontario Veterinary College (Guelph, ON, Canada). Samples were tested using a Coulter Dacos biochemistry analyzer (Coulter Electronics, Hialeah, FL) for total protein and urea N. The respective blood constituents were analyzed using prepared kits: total protein (Coulter no. 7546061; Coulter Electronics) and urea N (Coulter no. 7546773; Coulter Electronics).

Statistical Analysis

All dependent variables were analyzed using the general linear models procedure of SAS (23). The model used for this experiment was

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \tau_l + (\gamma\tau)_{kl} + \epsilon_{ijkl}$$

where

μ = grand true mean,
α_i = effect of cow (i = 1, 2, 3, 4, 5, or 6),
β_j = effect of period (j = 1, 2, 3, 4, 5, or 6),
γ_k = effect of RUP supplement (k = 2.5, 8.8, or 18.5%),
τ_l = effect of feed intake restriction (l = 10 or 20%),
$(\gamma\tau)_{kl}$ = interaction term, and
ϵ_{ijkl} = random residual error.

The residual effect was initially included in the model but was removed because it had no significant effect. The associated degrees of freedom were added to the error term. Sources of variation from the main effects of RUP supplement and intake are reported with their interaction. When the main effect of protein was significant, orthogonal contrasts with a single degree of freedom were used to test for linear or quadratic effects. Significant effects were declared at $P < 0.05$.

RESULTS AND DISCUSSION

This study was designed such that the manipulation of dietary protein from the RUP supplement balanced for a specific AA pattern was the only change to the amount of true protein that reached the small intestine. In a companion study conducted by Moscardini (19), purine derivatives were measured in urine from the present experiment to quantify microbial protein production in the rumen. Moscardini (19) found no difference from either the main effect of RUP supplement or the main effect of feed intake restriction on the urinary excretion of purine derivatives. Excretion of purine derivative N from cows fed the low, medium, and high RUP concentrations were 17.5, 19.6, and 18.3 (±0.7) g/d, respectively. The 20% restriction and 10% feed restriction levels resulted in purine derivative N concentrations of 17.8 and 19.2 (±0.5) g/d, respectively. This measure has been related to rumen microbial N yield (30). Analysis of the six treatment diets using the Cornell Net Carbohydrate and Protein System (11) indicated that there was an increase in the metabolizable protein from RUP sources when RUP supplements were fed. Metabolizable protein from RUP (grams per day) was estimated to be 529, 1240, and 2050 for the low, medium, and high RUP concentrations, respectively, for cows fed at the 20% feed restriction level. Cows fed at the 10% feed restriction level had metabolizable protein concentrations (grams per day) of 580, 1228, and 2159 for the low, medium, and high RUP concentrations, respectively,

according to the Cornell Net Protein and Carbohydrate System (11). Therefore, differing protein concentrations delivered to the small intestine were probably due to increased supply from the RUP source and not to additional microbial production.

DMI and Digestibility

As planned, DMI was different (P = 0.002) between the two feed restriction levels (Table 4). Intake of N increased linearly (P = 0.0001) as the concentration of RUP supplement in the diet increased (Table 4). Consumed CP concentrations were approximately (DM basis) 10.5, 17.0, and 23.6% for cows fed the low, medium, and high RUP concentrations, respectively. Apparent digestibility of DM and N (Table 4) increased linearly as RUP supplement in the present experiment increased. Improved (18) and impaired (21) DM digestibility has been reported as dietary protein increased. The linear increase in apparent N digestibility as dietary protein increased has been reported previously (12, 15). The increase in both DM and N digestibility as the RUP supplement was elevated in the present experiment was likely due to a high postruminal digestibility of the RUP supplement, which progressively replaced some of the basal diet.

The protein supplied by the high RUP treatment diets exceeded that supplied in an experiment by Sloan et al. (25), who investigated the effects of a formulated excess of RUP and found no advantage to the administration of RUP at concentrations above those proposed by the Agricultural Research Council (2). The deleterious effect of feed intake restriction on milk production demonstrated by Cohick et al. (10) over 7-d periods was not evident in this experiment. However, the DMI difference in this experiment was only 10.1% between the two intake levels, considerably smaller than the approximately 30% difference in the study of Cohick et al. (10). The absence of a main effect of feed intake restriction on many variables measured in this experiment reflected the small difference in feed intake. Cows fed at 20% feed intake restriction might have been in short-term energy deficit for the level of milk production in this experiment, but the magnitude of that deficiency would be small and, when necessary, was probably met by mobilization of body stores of energy.

N Balance

The N balance measurements were useful to determine the partitioning of N in the present experiment, including N concentrations that were both deficient and in excess of the cow needs. Urinary N excretion increased linearly (P = 0.0001) as the concentration of RUP supplement in the diet increased (Table 4). The amount of N that was excreted in the urine of cows fed the high RUP supplement was approximately fivefold higher than that excreted by cows fed the low RUP supplement, but N intake increased by a factor of only approximately 2.3. Urinary N excretion

TABLE 4. Nitrogen balance measurements.

	Feed restriction level									
	20%			10%				Effect		
Measurement	Low[1]	Medium	High	Low	Medium	High	SE	Protein (P)	Intake (I)	P × I
	(kg/d)									
DMI[2]	16.1	17.0	16.9	18.5	18.4	18.7	0.6	0.72	0.002	0.77
	(g/d)									
N Intake	275	492	665	302	468	672	28	0.0001[3]	0.87	0.65
Fecal N	134	173	178	157	186	173	12	0.02[3]	0.29	0.52
Urinary N	70	185	331	59	154	303	14	0.0001[3]	0.06	0.77
Milk N	95	112	118	98	111	121	5	0.0002[3]	0.74	0.92
N Retention	–24	22	38	–11	16	75	18	0.0004[3]	0.33	0.51
	(%)									
Apparent digestibility										
DM	64.4	64.4	71.6	61.2	66.8	72.1	1.0	0.001[3]	0.15	0.20
N	51	66	73	48	61	75	1	0.0001[3]	0.11	0.15

[1]The RUP supplement constituted a fixed proportion of intake according to treatment: low, 4.5%; medium, 14.9%; and high, 29.1% of DMI.

[2]Total DMI (basal diet and supplement).

[3]Linear effect.

as a percentage of N intake increased linearly (P = 0.0001), and, at the high RUP concentration, urinary N excretion was the route of excretion for approximately 50% of total consumed N (Table 5). Intake of the basal diet tended (P = 0.06) to alter urinary N excretion (Table 4), and the percentage of N intake excreted via urine was different between feed intake restriction levels (Table 5). Cows fed at 20% feed intake restriction had greater urinary N excretion. This result might be indicative of insufficient energy substrates available in the rumen for productive use of the N. Literature (26, 28) supports the suggestion that, as protein in the diet increases, urinary N increases linearly and becomes the primary route for N excretion from cattle. Tamminga (28) identified that N losses in urine originated from many sources: rumen loss, replacement of metabolic losses in the gut, incorporation of dietary protein into microbial nucleic acids, and losses caused by the inefficient conversion of absorbed AA into milk and body proteins.

Fecal N excretion increased (P = 0.02) linearly in response to increased RUP supplementation; however, this increase was quantitatively small compared with differences in N intake (Table 4). Fecal N excretion, expressed as a percentage of N intake, declined (P = 0.001) linearly as protein concentration in the diet increased (Table 5). The significant effect of RUP supplementation on fecal N excretion disagrees with the results of a study by Aarts et al. (1). Those researchers found that fecal N yield was relatively constant over a very wide range of dietary protein concentrations. However, the concentration of protein in the diet has a greater impact on urinary N excretion than on fecal N excretion.

Nitrogen in milk increased linearly (P = 0.0002) as additional RUP supplement was included in the diet. Nitrogen retention increased linearly as additional RUP supplement was included in the diet (P = 0.0004; Table 4). No significant interaction of the concentration of RUP supplement and feed intake restriction was detected, however. Numerically, N retention increased for cows with higher intakes of the basal diet. Nitrogen balance for cows fed the low RUP treatment diet was negative at both feed restriction levels. This result indicated that the cows were deficient in N at a dietary CP concentration of 10.5% when they produced approximately 600 g/d of true milk protein.

Efficiency for N utilization in milk (Table 5), expressed as a percentage of N intake, was similar to the percentages reported by Tyrrell et al. (29) for the low RUP treatment diet in this experiment. As the concentration of the RUP supplement increased, returns for N utilization in milk diminished. Efficiency of milk N secretion declined quadratically (P = 0.01) as the concentration of RUP supplement in the diet was increased; the effect was greater between low and medium concentrations. Cant and McBride (8) reported that, although a positive correlation between the output of N in milk and the input of N from the gut could be detected, efficiency for N use ranged from 0.28 to 0.73 (SD = 0.09). Efficiency was defined as the percentage of apparently absorbed N that was excreted as milk N. Van Vuuren and Meijs, as cited by Aarts et al. (1), reported that a maximum of 43% of N ingested by lactating cows could be converted into milk and live weight gain, but typical utilization was only 15 to 25%. The efficiency for N utilization for cows fed the low RUP treatment diets in the present experiment were 0.35 and 0.33 for the 20 and 10% feed restriction levels, respectively. These values were

TABLE 5. Utilization of N as a percentage of N intake.

	Feed restriction level									
	20%			10%				Effect		
Measurement	Low[1]	Medium	High	Low	Medium	High	SE	Protein (P)	Intake (I)	P × I
Fecal N	48.6	34.9	27.0	52.1	39.5	25.2	1.6	0.001[2]	0.11	0.15
Urinary N	25.6	38.4	50.3	19.5	33.5	45.5	2.1	0.0001[2]	0.0068	0.94
Productive N[3]	25.7	26.7	22.7	28.5	27.0	29.0	2.9	0.90	0.21	0.60
Milk N	34.8	22.8	17.8	32.7	23.3	17.6	1.3	0.01[4]	0.55	0.59
Retained N[5]	–9.1	3.9	4.9	–4.2	3.8	11.4	3.4	0.0002[2]	0.19	0.60

[1]The RUP supplement constituted a fixed proportion of intake according to treatment: low, 4.5%; medium, 14.9%; and high, 29.1% of DMI.

[2]Linear effect.

[3]Calculated as 100 – (fecal N + urinary N).

[4]Quadratic effect.

[5]Calculated as productive N – milk N.

higher than the reported value of 0.27 for cows consuming approximately 14% CP diets (15) or the 0.31 efficiency reported by Cressman et al. (12) for cows fed approximately 12% CP diets. Low protein diets resulted in a more efficient conversion of feed N to milk N. The higher efficiency reported in the present experiment may be related to diets with a lower CP content and improved AA balance delivered by the RUP supplement. The quadratic response for the efficiency of milk N secretion indicated that there is a potential benefit of reducing overall dietary protein when using balanced AA supplements. Efficiency for retained N, expressed as a percentage of N intake, increased linearly as concentration of the RUP supplement increased (Table 5). Lower dietary CP concentrations reduce N waste, particulary in the form of urinary N.

Excretion of urine increased linearly ($P = 0.001$) as RUP concentration increased in this experiment. Urine yields were 12.7, 20.7, and 27.3 kg/d (±2.2) for low, medium, and high concentrations of RUP, respectively, for cows fed at the 20% feed restriction level. Urine was 12.2, 18.4, and 24.8 kg/d (±2.2) for low, medium, and high concentrations of RUP, respectively, for cows fed at the 10% feed restriction level. A direct relationship between dietary CP concentration and urine volume has been documented by Holter and Urban (17), who indicated that urine volume increased about 0.5 L/d ($r^2 = 0.42$) for each unit of percentage increase in dietary CP.

Urea concentrations in urine increased quadratically ($P = 0.01$) as RUP supplement increased: 6.1, 16.0, and 17.5 (±1.5 g/L) for the low, medium, and high concentrations of RUP, respectively, for cows fed at the 20% feed restriction level. Urine urea concentrations were 3.0, 13.1, and 17.8 (±1.5 g/L) for low, medium, and high concentrations of RUP, respectively, for cows fed at the 10% feed restriction level, which falls within the range of concentrations reported previously (5). The increase in urinary urea from cows fed diets with higher dietary CP is in agreement with the relationship between dietary protein and urinary urea production presented by Susmel et al. (26). Urinary N constituted 51.5, 83.5, and 67.1% of total urinary N for the low, medium, and high concentrations of RUP, respectively, for cows fed at the 20% feed restriction level. The low percentage of urea N for cows fed the low RUP treatment diet supports a theory for higher efficiency of N recycling for cows fed the low RUP treatment diet and reflects the low N intake.

Milk urea concentration increased linearly ($P = 0.0001$) in response to RUP supplementation. Milk urea concentration was 1.5, 4.0, and 6.0 (±0.4) mmol/L for cows fed the low, medium, and high RUP treatment diets, respectively, at the 20% feed restriction level. Milk urea concentration at the 10% feed restriction level was 1.1, 3.6, and 5.5 (±0.4) mmol/L for cows fed the low, medium, and high RUP treatment diets, respectively. This response is similar to the increase reported by Susmel et al. (26). In the present experiment, milk urea concentrations were defined ($r^2 = 0.86$) by the linear relationship: milk urea (millimoles per liter) = –2.48 + 0.37[dietary CP percentage (DM basis)].

Blood Serum Chemistry

No significant effects of basal diet restriction on the measured blood variables were detected. Serum urea increased ($P = 0.0001$) linearly as the percentage of RUP in the supplement increased. Serum total protein concentration increased in a linear manner ($P = 0.001$) as RUP supplementation increased (Table 6). Increased concentrations of both total protein and urea agree with data from Cressman et al. (12), who observed linear increases in plasma protein and urea concentrations when dietary CP percentages of 12, 15, and 18% were fed in an experiment that measured milk production responses to dietary protein.

TABLE 6. Blood measures.

	Feed restriction level									
	20%			10%				Effect		
Component	Low[1]	Medium	High	Low	Medium	High	SE	Protein (P)	Intake (I)	P × I
Urea, mmol/L	0.72	4.55	7.53	0.60	3.87	6.85	0.49	0.0001[2]	0.21	0.79
Total protein, g/L	77.3	78.2	80.7	77.8	80.3	82.7	1.07	0.001	0.09	0.70

[1]The RUP supplement constituted a fixed proportion of intake according to treatment: low, 4.5%; medium, 14.9%; and high, 29.1% of DMI.

[2]Linear effect.

TABLE 7. Milk and milk solids production.

	Feed restriction level									
	20%			10%				Effect		
Production	Low[1]	Medium	High	Low	Medium	High	SE	Protein (P)	Intake (I)	P × I
	(kg/d)									
Milk	22.4	26.4	26.3	23.4	25.0	26.7	1.12	0.003[2]	0.99	0.54
Protein	0.69	0.81	0.83	0.71	0.79	0.85	0.04	0.0006[2]	0.87	0.85
True protein	0.59	0.70	0.72	0.61	0.68	0.72	0.03	0.003[2]	0.99	0.91
Fat	0.79	0.71	0.62	0.77	0.71	0.62	0.03	0.0001[2]	0.81	0.97
Lactose	0.97	1.19	1.16	1.03	1.09	1.19	0.05	0.003[2]	0.87	0.31

[1]The RUP supplement constituted a fixed proportion of intake according to treatment: low, 4.5%; medium, 14.9%; and high, 29.1% of DMI.

[2]Linear effect.

Production of Milk and Milk Solids

Milk production responded linearly ($P = 0.003$) to RUP supplement concentration (Table 7). Linear increases also occurred in milk protein, measured by near infrared spectroscopy and acid precipitation ($P = 0.006$ and $P = 0.003$, respectively) in response to the RUP supplement (Table 7; Figure 1). At the 20% feed intake restriction, true protein production increased 22% from the low RUP treatment diet to the high RUP treatment diet. A similar increase (18%) was observed at the 10% feed intake restriction (Table 7).

Wu and Huber (32) noted that an increase in blood concentrations of AA by dietary means should increase the quantity of those AA transferred to the mammary gland. Schwab et al. (24) postruminally infused AA over 9 d in five separate experiments. In an isonitrogenous and isocaloric comparison with control infusions (e.g., diammonium hydrogen citrate), abomasal infusion of 10 essential AA and sodium caseinate increased milk production by 5.5% and increased protein content by 5.9% (24). Milk protein secretion increased 12% compared with that of the negative controls. Clark (9) reviewed the effects of an increase in the postruminal delivery of essential AA. Abomasal casein infusion increased milk production by 1 to 4 kg/d and increased milk protein production by 10 to 15% when cows were fed diets that were designed to meet their requirements for protein and energy. In the present experiment, delivery of RUP AA to the small intestine increased as dietary RUP increased, which resulted in greater milk protein production. The RUP fraction in the digesta delivered to the small intestine increased milk and protein production by supplying essential AA but did not change microbial protein production as validated by measurements of urinary purine derivatives (19).

Milk fat production declined linearly ($P = 0.0001$) when a greater concentration of the RUP supplement was fed (Table 7). The decline in milk fat production when unsaturated fat is fed (as is commonly found in fish meal) has been described by Sutton (27). The amount of fat in the herring meal used (10.5%, DM basis) would likely be sufficient to promote milk fat depression given the amount fed in the medium and high RUP treatment diets. The calculated amount of fat in the low, medium, and high RUP treatment diets that originated from the herring meal was 39, 132, and 267 g/d, respectively. Calsamiglia et al. (6) reported a decline in milk fat production when fish meal was administered into the rumen or into the duodenum of lactating Holstein cows. The mechanism of the milk fat depression is unclear.

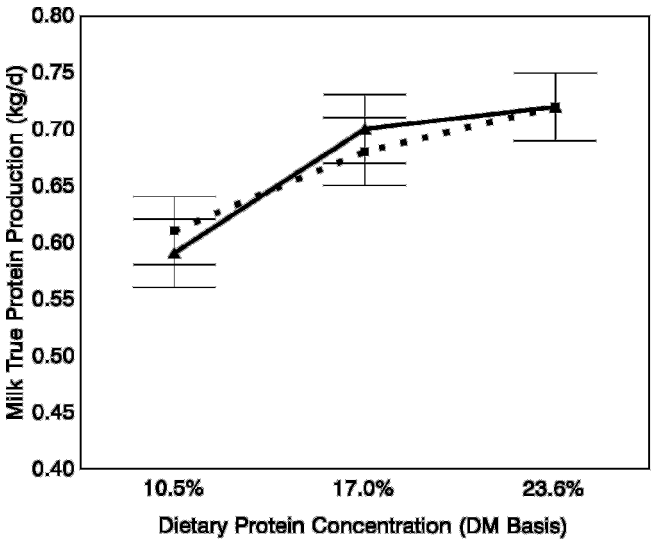

Figure 1. Dose-response curves of milk true protein production as the concentration of dietary CP increased from an RUP supplement balanced for AA. Feed restriction of 20% (▲) and 10% (■) is shown. Production increased ($P < 0.05$) linearly as RUP supplementation increased, but no significant effect of feed intake restriction was observed.

Lactose production increased linearly ($P = 0.003$) as the concentration of RUP supplement in the diet increased (Table 7). This result corresponded to the linear increase in protein in this experiment and agrees with the summary by Sutton (27), who indicated that the lactose content in milk was relatively constant. The milk and lactose production responses to increased concentrations of RUP supplement complemented each other.

CONCLUSIONS

The linear response (Figure 1) of milk protein production observed in the present experiment supports previous studies (9, 16, 24) that reported positive effects of the postruminal supply of AA that were thought to limit milk protein synthesis. The difference in DMI in the present experiment was approximately 10%, which was insufficient to demonstrate the role of an intake effect on milk protein production at different concentrations of dietary protein. The lack of a significant interaction between concentration of RUP supplement and feed restriction level in this experiment indicated that milk protein production responded similarly at a 10% difference in DMI during short-term restriction across a wide range of dietary protein. Experiments that clearly examine the effects of individual AA or groups of AA on milk protein production are integral to the determination of which AA limit milk protein synthesis and will lead to diets that minimize N wastage. Our experiment demonstrated that an RUP supplement balanced for five AA can increase milk protein production linearly in a dose-dependent manner over the defined range of dietary CP.

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