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Nutrient digestibility, nitrogen excretion, and milk production of mid-lactation Jersey × Friesian cows fed diets containing different proportions of rumen-undegradable protein

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Abstract: The present study was planned to test the hypothesis that feeding lactating dairy cattle with varying levels of rumen-undegradable protein (RUP) can enhance protein utilization, milk production, milk protein, and nitrogen (N) excretion. Forty mid-lactating crossbred (Jersey × Friesian) cattle were randomly divided into four groups. Four treatment diets were formulated to contain 30%, 40%, 50%, and 60% RUP of crude protein. Dry matter (DM) and crude protein intakes were significantly reduced with increasing dietary RUP levels. Crude protein digestibility increased linearly with incremental increases in dietary RUP levels. Cattle fed 60% RUP showed a linear decrease in N intake compared to that in the other groups. A linear decrease in urinary N and linear increases in net N, milk N, and N-use efficiency were observed with increasing dietary RUP levels. Actual milk, energy-corrected milk, and 4% fat-corrected milk yields (kg/day) increased linearly with an increasing degradability of protein. However, milk protein, solids not fat and total solids, as well as the yields of protein, fat, and lactose, showed significant increases with increased RUP supplementation. Collectively, the results indicate that formulating dairy cow diets to contain 60% RUP results in better lactating performance and N-use efficiency and lower N excretion.

Key words: rumen-undegradable protein, nitrogen excretion, nutrient digestibility, milk production, milk composition.

INTRODUCTION

In the dairy sector, protein, which plays a vital role in body functions, is among the limiting nutrients in the diet (Nisa et al. 2008). Although the efficiency of crude protein (CP) consumption in dairy cattle is higher than that in other ruminants, nitrogen (N) excretion in their milk and feces is approximately two to three times greater (Bahrami-Yekdangi et al. 2014, Broderick 2003). This N excretion increases the costs of milk production and exacerbates environmental pollution (Savari et al. 2018). Accordingly, research is needed to identify means of enhancing N-utilization efficiency and optimizing milk production in dairy cattle (Wang et al. 2008, Alsaht et al. 2014).

Absorbed amino acids are essential for protein synthesis to the maintenance, reproduction, growth, and milk production of dairy cattle. In the small intestine, the absorbed amino acids could be provided from two types of protein. The first is rumen-degradable protein (RDP) in the rumen, which offers peptides, amino acids, and ammonia for microbial growth to generate microbial protein. The former synthesized microbial protein provides approximately 70–80% of the required amino acids passing to the small intestine. The second is rumen-undegradable protein (RUP), which is digested in the small intestine by the animals to produce amino acids (Chumpawadee et al. 2006, Laudadio & Tufarelli 2010). Ruminal degradation can be protected by several means, including the identification of naturally protected protein, chemical treatment/modification, inhibition of proteolytic activity, and heat treatment (Nisa et al. 2008). Energy loses associated with protein loses and fermentation incurred in transformation of dietary protein to microbial protein can be eliminated by feeding nutrients post-ruminally.

Many studies have demonstrated that the efficiency of N utilization can be improved when diets contain appropriate proportions of RDP and RUP. An imbalance in the proportions of both RDP and RUP can cause substantial increases in N defecation (Kauffman & St-Pierre 2001); conversely, N retention can be enhanced by increasing the levels of RUP in feed (Paengkoum et al. 2004).

The biological value of bacterial protein is low and so there is no need to offer high sources of RDP (Sarwar et al. 2005, Tufarelli et al. 2009, Ayyat et al. 2019). Using more RUP instead of RDP will provide a higher proportion of absorbable amino acids at the intestinal level (Eastridge 2006). Nevertheless, an appropriate ratio of RUP to RDP should be maintained in dairy rations, as both of these protein types have their own specific importance. Rumen-degradable protein is required for ruminal fermentation and is also a source of bacterial protein for the host animal; however, if this is provided in excess, it will represent a considerable drain of energy via the degradation process that could otherwise be used in various productive processes in the animal body. Thus, RDP is supplied to animals in a ratio that maintains an appropriate fermentation and N balance. If there is an inappropriate ratio of RDP to RUP in the dairy ration, this may have detrimental effects on the productive potential of animals and may also resulting in reduced dry matter (DM) intake and digestibility (NRC 2001) and poor reproductive performance (Butler 1998).

Most previous studies related to N excretion into the environment (feces and urine) focused on decreasing the crude protein or supplying of protein at the duodenum content, but the information about the linkage between RUP relative to RDP and total N excretion still limited. We hypothesize that increasing dietary RUP relative to RDP, such that the RDP supplies necessary for microorganisms are not limiting, will enhance lactation performance, reduce N excretion, and promote greater overall N-use efficiency by cattle. Accordingly, in the present study, we sought to examine the effect of different ratios of RUP relative to RDP in the diet of mid-lactating animals on feed intake, nutrient digestibility, milk production, milk composition, N-use efficiency, and N excretion.

MATERIALS AND METHODS

The present research was conducted at "Mian Dairy Farm" which is located 1.5 km from the University College of Agriculture, Sargodha Pakistan. All experimental procedures were carried out in accordance with the guidelines of the Local Experimental Animal Care Committee and were approved by the institutional ethics committee of the College of Agriculture, University of Sargodha.

For this trail, 40 mid-lactating crossbred (Friesian × Jersey) cows (12.09±0.26 kg DIM, 16.0±1.87 kg milk yield, 4.11± 0.28% fat, 2.77±0.06% protein, 378.1±4.18 kg BW) were randomly divided into four groups (each containing 10 animals). All animals were examined for parasites before the start of the trail, and were maintained under the same housing and environmental conditions. Animals were reared on concrete floor in separate pens. Appropriate hygiene measures were instigated to ensure that the animals remained in a healthy condition. Daily cleaning of sheds and feed mangers was also undertaken.

The experimental diets were prepared in such a way that they contained similar levels of CP and other nutrients, as recommended by the National Research Council (NRC 2001). Formulation and nutrient content of the tested rations are given in Table I. The ration contained levels of RUP of 30%, 40%, 50% and 60% of crude protein. All animals were fed three times a day on an *ad libitum* basis. Fresh water availability was ensured at all times during the trail. The experiment lasted for 75 days, with the first 15 days being allocated for adaptation to the respective diets and the remaining period (60 days) used for data collection.

All animals were weighed at the start of the trail and thereafter at fortnightly intervals. Each morning prior to feeding, the previous day's uneaten feed (orts) was weighed, recorded, and discarded before offering fresh rations. Milking was performed twice daily (at 5 a.m. and 5 p.m.) using a milking machine, and the individual milk yield was recorded. Milk samples were collected twice a week to determine milk composition. Samples were preserved with bronopol and kept at -5°C. Milk fat, protein, lactose, total solids, and solids-not-fat (SNF) were estimated as described by (Gupta et al. 1992).

Table I. Ingredient and nutrient composition of the diets.

Ingredients %	**RUP (%) in diet**			
	30%	**40%**	**50%**	**60%**
Berseem fodder	37.00	36.10	35.00	30.20
Rhode grass hay	7.70	4.20	9.95	8.75
beat pulp dry	8.00	8.00	10.00	15.00
Wheat straw	7.00	8.00	6.90	5.60
Concentrate (Wanda)	18.00	16.00	14.00	14.00
Corn gluten 60	0.20	9.00	12.48	13.00
Canola meal	17.50	9.00	9.33	1.40
Vegetable oil	1.00	0.50	0.00	2.00
Molasses	2.10	7.00	1.00	1.00
Soya pass®	0.00	1.00	0.80	9.00
Mineral Mix	0.30	0.40	0.40	0.05
Urea	1.20	0.80	0.14	0.00
Nutrients composition %				
Dry matter	60.00	60.01	60.03	60.01
Crude protein	15.98	16.04	15.90	16.00
Rumen un-degradable proteins	29.91	40.07	50.03	59.97
Rumen degradable protein	70.09	59.93	49.97	40.03
Neutral detergent fiber	42.5	41.91	42.90	42.57
Acid detergent fiber	25.04	25.6	24.96	25.08

Fat-corrected milk (4%FCM) was calculated using the Gaines (1928) equation as follows: FCM = 0.4 × milk yield + 15 × fat yield. Milk energy content (Mcal kg^{-1}) as milk NEL and energy-corrected milk (ECM) were calculated according to the NRC (2001) using the following equations:

$$NEL = 0.0929 \times \%\ fat + 0.0547 \times \%\ protein + 0.0395 \times \%\ lactose.$$

$$ECM = 0.323 \times milk\ (kg) + 12.82 \times milk\ fat\ (kg) + 7.13 \times milk\ protein\ (kg).$$

Feed efficiency was calculated by dividing the 4%FCM yield (kg/d) by DM intake (kg/d).

From the 70th to 75th day of the experiment, nutrient digestibility and balance trial determinations were performed using the total collection method (Arif et al. 2019). Refused feed was weighed and sampled daily. Likewise, total feces and urine voided by individual animals was weighed and sampled daily and stored at -20°C for further analysis. Urine was collected using locally made catheters and was acidified with 50% H_2SO_4 to prevent any N losses. Milk samples were collected for analysis of milk proteins. At the end of the collection period, the samples were thawed and composited for each animal. Fecal samples were dried at 55°C, ground, and passed through a 1-mm screen. Feed and fecal samples were analyzed for acid detergent fiber (ADF) and neutral detergent fiber (NDF) by following the procedure of Van Soest et al. (1991), and DM and CP (N × 6.25) content (methods 930.15, 925.04) were determined using the Kjeldahl method by following the procedures specified by the AOAC (2000). N balance was calculated using an equation recommended by the NRC (1989).

All data on nutrient intake and digestibility, N balance, and milk production were statistically analyzed using a general linear model procedure (SPSS 21 software, Chicago, IL). Means were compared for differences using Duncan's Multiple Range Test. Orthogonal polynomial contrasts were determined using the same SPSS model as used to test the linear and quadratic effects of increasing dietary RUP.

RESULTS

The nutrient intakes of lactating cows fed diets with different levels of RUP are shown in Table II. Dry matter, CP, NDF, and ADF intakes were linearly and quadratically reduced ($P < 0.05$) with increasing dietary RUP levels. The highest values were observed in cattle fed 30%

Table II. Daily nutrients intake of cross bred (Friesian×Jersey) dairy cattle fed diets with various levels of rumen undegradable protein (RUP).

	RUP (%) in diet				SEM	L[1]	Q[2]
	30	40	50	60			
Dry matter	12.75 [a]	12.72 [a]	12.89 [a]	11.85 [b]	0.14	0.021	0.036
Crude protein	2.04 [a]	2.04 [a]	2.05 [a]	1.90 [b]	0.02	0.018	0.043
Neutral detergent fiber	5.42 [a]	5.33 [a]	5.53 [a]	5.05 [b]	0.06	0.041	0.051
Acid detergent fiber	3.19 [a]	3.26 [a]	3.22 [a]	2.97 [b]	0.04	0.012	0.014
Rumen un-degradable proteins	0.61 [d]	0.82 [c]	1.02 [b]	1.14 [a]	0.05	<0.001	0.010
Rumen degradable protein	1.43 [a]	1.23 [b]	1.02 [c]	0.76 [d]	0.06	<0.001	0.142

[a,b,c and d]Means with different superscripts in the same row differ significantly (P<0.05). [1]P value for linear effect. [2] P value for quadratic effect.

RUP, whereas the lowest values were observed for the 60% RUP group. Moreover, RDP intake was significantly diminished (linear, P < 0.001) with increasing RUP levels. In contrast, a linear increase (P < 0.001) in RUP intake was recorded with increasing dietary RUP levels. The highest RUP intake was observed in cattle fed 60% RUP, whereas the lowest values were observed for the 30% group.

As shown in Table III, there was no effect of different RUP levels on DM digestibility. CP digestibility increased linearly (P < 0.001) with incremental increases in dietary RUP levels. The highest CP digestibility was observed in cattle fed 60% RUP, whereas the lowest values were observed for the 30% group. However, there was no significant difference regarding NDF digestibility among the different RUP levels. In contrast, ADF digestibility differed linearly and quadratically (P = 0.003) as dietary RUP levels increased. The highest ADF digestibility was observed in cattle fed 40% RUP, whereas the lowest values were observed for the 60% group.

Regarding the effect of different dietary RUP proportions on N balance, cattle fed 60% RUP showed significantly decreased (linear, P = 0.018) N intake compared with the other groups (Table IV). Fecal N decreased linearly (P < 0.001) with increasing dietary RUP levels. A linear decrease (P = 0.004) in urinary N was also observed with increasing dietary RUP levels. Cattle consuming 30% RUP had the highest urinary N, whereas the lowest values were observed for the 60% group. Increasing the proportion of dietary RUP resulted in a linear increase (P < 0.001) in net N (g/day and % of N intake), milk N, and N-use efficiency. Nitrogen retention was significantly (P < 0.001) affected by varying dietary RUP levels. The highest N retention was recorded in cattle fed 50% RUP, whereas the lowest values were observed for the 60% group.

Table V presents information on milk production and its composition as affected by varying levels of dietary RUP. Actual milk, energy-corrected milk (ECM), and 4%FCM yields (kg/day) increased linearly (P < 0.001) with the increasing degradability of protein. The different proportions of RUP in the experimental diets did not significantly alter milk fat and lactose. However, milk protein, SNF and total solids, as well as the yields of protein, fat, and lactose, showed significant increases (P < 0.05) with increasing RUP levels. Linear (P < 0.001) and quadratic (P = 0.008) reductions in the milk fat to protein ratio were detected with increasing dietary RUP levels. In contrast, we observed no significant effect of different RUP levels on milk energy content among the experimental diets. Feed efficiency [i.e., milk yield/ DM intake and CP, g/kg FCM) was improved with increasing RUP

Table III. Nutrient digestibility (%) of cross bred (Friesian×Jersey) dairy cattle fed diets with various levels of rumen undegradable protein (RUP).

	RUP (%) in diet				SEM	L[1]	Q[2]
	30	40	50	60			
Dry matter	73.99	74.39	73.55	72.22	0.55	0.251	0.461
Crude protein	70.56[c]	73.38[bc]	76.20[ab]	78.13[a]	0.90	<0.001	0.707
Neutral detergent fiber	64.59	66.66	67.82	64.99	0.80	0.753	0.161
Acid detergent fiber	48.24[a]	50.36[a]	46.77[a]	40.90[b]	1.14	0.003	0.023

[a,b and c]Means with different superscripts in the same row differ significantly (P<0.05). [1]P value for linear effect. [2]P value for quadratic effect.

Table IV. Nitrogen balance (g/day) of cross bred (Friesian×Jersey) dairy cattle fed diets with various levels of rumen undegradable protein (RUP).

	RUP (%) in diet				SEM	L[1]	Q[2]
	30	40	50	60			
N Intake	326.10 [a]	326.45 [a]	327.80 [a]	303.36 [b]	3.59	0.018	0.043
Fecal N	96.00 [a]	86.90 [b]	78.02 [c]	66.35 [d]	2.90	<0.001	0.382
N absorb	230.10 [b]	239.55 [ab]	249.78 [a]	237.02 [ab]	2.58	0.114	0.018
Urinary N	141.11 [a]	135.85 [ab]	138.72 [a]	131.51[b]	1.18	0.004	0.566
Net N	88.98[b]	103.70 [a]	111.06 [a]	105.51 [a]	2.44	0.001	0.003
Net N (% of intake)	27.28 [c]	31.75 [b]	33.86 [a]	34.78 [a]	0.77	<0.001	0.002
Milk N	63.31[c]	73.48[b]	79.80 [b]	99.93 [a]	3.62	<0.001	0.063
Apparent N efficiency, %	19.42 [c]	22.57 [b]	24.34 [b]	32.92 [a]	1.33	<0.001	0.002
N Retained	25.67 [a]	30.23 [a]	31.26 [a]	5.58 [b]	3.02	<0.001	0.001

[a, b and c]**Means with different superscripts in the same row differ significantly (P<0.05).** [1]**P value for linear effect.** [2] **P value for quadratic effect. Apparent N efficiency, % = milk N (g/d)/N intake (g/d) × 100.**

levels in the cow diet. The highest improvement in feed efficiency was recorded in cattle fed with 60% RUP, whereas the lowest enhancement was observed in cattle fed with the 30% RUP diet.

In this study, the relationships among the examined variables were confirmed using principal component analysis (PCA). Figure 1 shows that the first two components of the loading plot accounted for 73.06% of the total variation. The variables grouped narrowly with each other within the loading plot (<90°) and are positively correlated. RUP intake, milk production, ECM, 4%FCM, milk N, protein yield, fat yield, N-use efficiency, and feed efficiency variables clustered together and were strongly correlated with the first component. This cluster was highly negative correlated with fecal N, milk fat to protein ratio, and milk fat variables. Overall, the loading plot results illustrated that increasing dietary RUP contents had positive effects on lactation performance, N-use efficiency, and feed efficiency.

DISCUSSION

The decreases in DM, NDF, and CP intake with increasing levels of RUP observed in the present study are consistent with the findings of other researchers (Henson et al. 1997), who observed decreases in DM and CP intake in animals fed with high levels of RUP. These responses might be attributable to the presence of less ruminally fermentable N in the diet. In the rumen, dietary N is converted to ammonia, which is conducive to the proliferation of microflora, and if the amount of dietary fermentable N is reduced, ruminal ammonia remains at a lower level. Also, the diminished intake in high RUP diets can be because of its less palatability (Hayirli et al. 2002). However, the results of the present study, are in contrast with the findings of other studies (Kridli et al. 2001, Lee et al. 2001), who have reported increases in DM intake with increasing levels of RUP. This discrepancy might be explained by the fact that in the present study we used 15% beat pulp in the 60% RUP diet, which has a high dietary fiber content (48%) that tends to contribute to a low DM intake (Firkins et al. 1984).

Table V. Feed efficiency, milk yield and its composition of cross bred (Friesian×Jersey) dairy cattle fed diets with various levels of rumen undegradable protein (RUP).

	RUP (%) in diet				SEM	L[1]	Q[2]
	30	40	50	60			
Milk yield, kg/d							
Actual milk	14.06 [c]	16.06 [bc]	16.68 [b]	19.07 [a]	0.57	0.001	0.798
4% FCM	14.39 [c]	16.51 [b]	16.63 [b]	18.97 [a]	0.49	<0.001	0.851
ECM	14.85 [c]	17.09 [b]	17.46 [b]	20.31 [a]	0.56	<0.001	0.590
Milk composition, %							
Fat	4.16	4.20	4.00	3.98	0.05	0.079	0.749
Protein	2.82 [b]	2.87 [b]	3.00 [b]	3.29 [a]	0.05	<0.001	0.065
Fat: protein	1.48 [a]	1.47 [a]	1.33 [b]	1.21 [c]	0.03	<0.001	0.008
Lactose	4.99	4.98	4.95	4.94	0.04	0.660	0.980
Solid not fat	8.59 [b]	9.64 [a]	8.61 [b]	8.57 [b]	0.13	0.086	0.001
Total solids	12.75 [b]	13.84 [a]	12.60 [b]	12.55 [b]	0.15	0.010	0.001
Milk composition, g/d							
Fat	584.06 [c]	672.56 [b]	664.02 [b]	756.04 [a]	17.42	<0.001	0.918
Protein	395.68 [c]	459.24 [b]	498.72 [b]	624.54 [a]	22.60	<0.001	0.060
Lactose	701.06 [b]	798.87 [b]	826.49 [ab]	942.04 [a]	29.20	0.002	0.838
Milk energy content, Mcal/kg	0.74	0.74	0.73	0.74	0.01	0.927	0.745
Feed efficiency							
FCM/DMI	1.13[c]	1.30[b]	1.29 [b]	1.60 [a]	0.05	<0.001	0.145
CP, g /kg FCM	141.88[a]	124.08[b]	123.45[b]	100.25 [c]	4.19	<0.001	0.499
Mean BW, kg	384.0	377.6	379.8	371.0	4.79	0.401	0.217

[a,b and c]**Means with different superscripts in the same row differ significantly (P<0.05).**

Our finding that DM digestibility remained unaltered with an increasing level of RUP is consistent with the findings of other researchers (Castillo et al. 2001, Noftsger & St-Pierre 2003, Sun et al. 2009), who have also reported no change in the digestibility of DM with increasing RUP levels. This might be related to the narrow range of RUP used. In contrast, Nisa et al. (2008) and Griswold et al. (2003) observed linear increases in the digestibility of DM. These contradictory results might be related to the source of RUP used. In the present study, the RUP used was of plant origin, whereas in previous studies animal protein has been used as a source of the RUP supplement. The increase in CP digestibility with increasing levels of RUP observed in the present study is consistent with the findings of other studies (Kridli et al. 2001, Paengkoum et al. 2004,

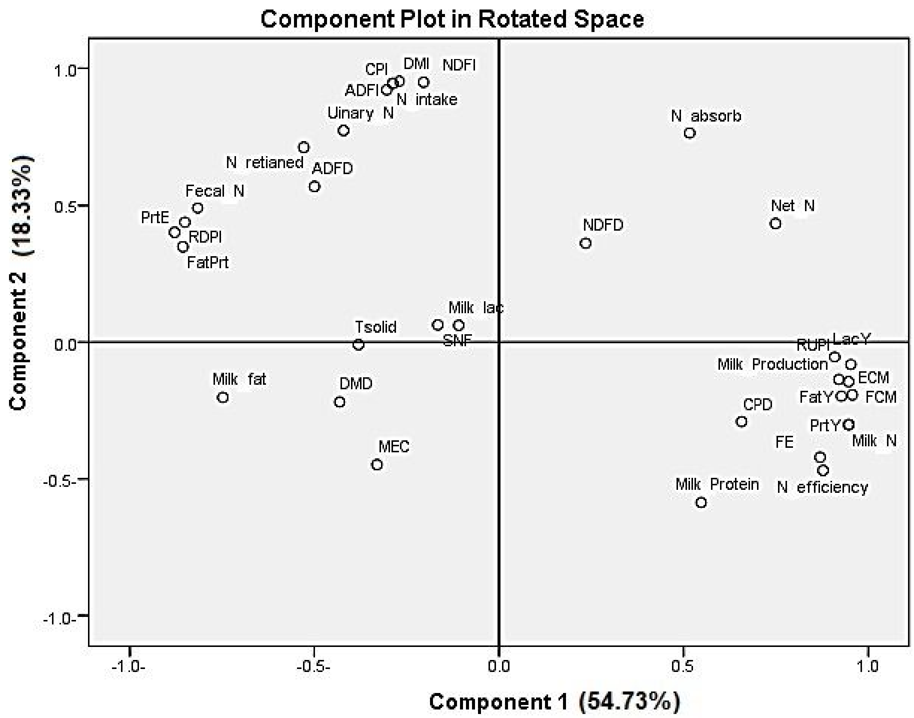

Figure 1. A plot illustrating the relationships among rumen-undegradable protein (RUP) intake, nutrient digestibility, nitrogen (N) use, and lactation performance variables. The variables are as follows: DMI, dry matter intake; CPI, crude protein intake; NDFI, neutral detergent fiber intake; ADFI, acid detergent fiber intake; RUPI, RUP intake; RDPI, rumen-degradable protein intake; DMD, dry matter digestibility; CPD, crude protein digestibility; NDFD, neutral detergent fiber digestibility; ADFD, acid detergent fiber digestibility; N intake; Fecal N; N absorbed; Urinary N; Net N; Milk N; N retained; N-use efficiency; ECM, energy-corrected milk; Milk Production; FCM, fat-corrected milk; Milk fat; Milk Protein; FatPrt, fat to protein ratio; Milk lac, milk lactose; SNF, solids not fat; Tsolid, total solids; PrtY, protein yield; FatY, fat yield; LacY, lactose yield; MEC, milk energy content (Mcal kg^{-1}); FE, feed efficiency; PrtE, protein efficiency.

Wankhede & Kalbande 2001), which have also reported higher digestibility of CP in animals fed with higher levels of RUP. The enhancement of CP post-ruminal digestibility and essential amino acids availability could improve the efficiency of N utilization, resulting in higher absorption rate of amino acids that cover the requirements of amino acids for milk synthesis (Noftsger & St-Pierre 2003).

Our observation that fiber digestibility decreased with increasing dietary RUP levels is also consistent with the findings of other studies (Bodine et al. 2000, Javaid et al. 2008, Koster et al. 1996), which have reported reduced fiber digestibility when diets containing higher RUP were fed to animals. This response might be related to the unavailability of a specific amount of N at the ruminal level, which is necessary for maintaining ruminal pH (Mould et al. 1983). If the ruminal pH is not maintained at an appropriate value, cellulolytic activity is affected. Javaid et al. (2008) have similarly reported decreased CF digestion with a reduction in ruminal pH.

We found that lower RUP levels led to an increase in fecal N, which is consistent with the findings of Burgos et al. (2007), who found that manure ammonia emissions decreased linearly when RDP in feed was decreased from 18% to 12% of DM. This might be related to the fact that N from diets having high levels of RDP will be converted as a part of microbial proteins, which, in contrast to ruminally undegradable amino acids, will be absorbed slowly through the intestinal wall (Burgos et al. 2007). An increase in urinary N with a decrease in RUP is in line with the findings of Broderick et al. (1991), who reported that excess ruminal NH_3-N derived from degradable proteins is absorbed across the rumen wall, converted to urea in the liver, and mostly excreted in urine.

The increase in milk N with increasing levels of RUP observed in the present study is consistent with the findings of Ipharraguerre & Clark (2005), who also reported a linear increase in milk N with increasing dietary RUP. This finding might be related to the availability of large quantities of absorbable amino acids at the duodenum level, leading to an increase in milk protein. The results of present study are, however, inconsistent with the findings of Kalscheur et al. (2006) who reported a linear decline in milk nitrogen. This response might be related to the nature of the feed used, as the content of RDP was reduced with an increase in RUP content. It might also be related to the level of milk production, as a major portion of the N consumed is secreted in milk when animals are fed with high levels of RUP (Baker & Silverton 1985).

Our observation that animals fed a diet containing the highest RUP level showed the lowest retention of N is consistent with the findings of Firkins et al. (2006). These authors reported that in animals fed diets containing higher levels of RDP, a portion of the ruminally fermentable N is transferred to the blood stream and subsequently recycled in the rumen, and thus the N is retained by the animal. However, in the case of higher RUP levels, higher amounts of absorbable amino acids are available, which are mainly consumed in milk production. As most of these amino acids are lost to milk, very low amounts of N are transferred to the blood stream, and thus N retention by animals is reduced. In this regard, the results of the present study were not in agreement with the findings of Pattanaik et al. (2003), who reported higher N retention in calves fed higher levels of RUP. These observed differences might be attributable to the types of animals used. Although Pattanaik et al. (2003) used calves and the main objective of their study was to assess weight gain, in the present study, we focused on milk production under conditions in which a major portion of N is drained in milk.

The increase in milk yield with increasing levels of dietary RUP observed in the present study is consistent with the findings of other researchers (Christensen et al. 1993, 1994, Santos et al. 1998, Tamminga 1992), who observed increases in milk yield with increasing dietary RUP levels. RUP may increase milk yield either directly or indirectly (Clark 1975). The direct route may be through either an increased supply of limiting amino acids to the mammary glands for protein synthesis or through enhanced gluconeogenesis in the liver, resulting in an increased supply of glucose to the mammary glands for lactose synthesis. The indirect route may be mediated through altered hormonal status, particularly increased concentrations of plasma growth hormone, which promotes nutrient partitioning in favor of growth and milk production and away from fat deposition (Sartin et al. 1985). In this respect, the results of the present study contrast with the findings of Kalscheur et al. (2006), who reported a decrease in the milk yield of cattle. This difference might be related to a reduction in the level of CP, as these authors reduced the level of CP in the diet from 17.1% to 12.3%. These discrepancies highlight the need to ensure that RUP supplements are of consistent quality with respect to rumen protection and the bio-availability and digestibility of essential amino acids in the small intestine. Such characteristics are essential with respect to improving milk yield and protein content, and decreasing N excretion (Santos et al. 1998). Although responses to the various protein supplements are variable, chemically treated soybean meal and fish meal are the most effective in increasing milk yield. With regards to milk composition in lactating animals, amino acid balance is more important in attaining a positive response than total RUP supplementation (Noftsger & St-Pierre 2003).

The increase in milk protein with increasing RUP levels we observed is also consistent with the findings of Santos et al. (1998), who described an increase in milk protein with decreasing levels of RDP. This finding might be attributable to the accessibility of large amounts of amino acids at the intestinal level for absorption and milk production (Santos et al. 1998). The results of the present study, however, contrast with the findings of Kalscheur et al. (2006), who reported a decrease in milk production and milk protein. These conflicting results might be related to differences in the protein contents of the provided diets. In the study reported by Kalscheur et al. (2006), both RDP and RUP were decreased, and so it was not clear which was responsible for the decrease in milk protein.

Our observations of unaltered milk fat (%) and increased milk fat yield with increasing RUP levels are consistent with Maiga & Schingoethe (1997) who detected no effect of RUP level on milk fat composition. Chaturvedi & Walli (2001) reported that the percentage of milk fat increased linearly when the lactating crossbred cows were fed diets containing an increasing amount of RUP (29%, 42%, and 56% of CP). In the current experiment the increase in milk yield, fat yield because of increase in RUP level in the feeding regimen may be due to enhanced use of supplements with diminishing in the blood urea nitrogen and increase in blood alpha amino nitrogen fixation (Chaturvedi & Walli 2000) required for tissue protein amalgamation. In contrast to our findings, Wright et al. (1998) reported a decline in milk fat when greater concentrations of RUP were supplemented. These conflicting results might be due to differences in the lactation stage of the experimental animals. A decrease in total milk solids with increasing RUP level is consistent with the findings of Sutton (1989), who similarly reported a decrease in milk fat when fish meal was used as RUP. This might be related to Richmond's formula in which total solids are 1.21% of fat. If milk fat decreases, then total solids will also decrease.

Conclusively, from production and environmental perspectives, formulating the diets of mid-lactation dairy cows to contain 60% RUP of crude protein results in enhanced milk production, milk protein, and N-utilization efficiency through reduced N excretion in feces and urine.

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