Iranian Journal of Applied Animal Science

Effects of Rumen Undegradable Protein on Productive Performance and N Balance of Holstein Cows in Early Post-Partum Period

Research Article

M. Aboozar[1*] and F. Niazi[1]

[1] Department of Animal Science, Abhar Branch, Islamic Azad University, Abhar, Iran

Received on: 1 May 2012
Revised on: 1 Oct 2012
Accepted on: 1 Nov 2012
Online Published on: Dec 2013

*Correspondence E-mail: dr.mehranaboozar@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

Metabolizable protein (MP) supply and amino acid balance were manipulated through selection of highly digestible rumen-undegradable protein (RUP) sources. Effects on production efficiency and N utilization of early post-partum dairy cows were determined. Forty-two multiparous and 16 primiparous Holstein cows were assigned to the diets in a randomized complete block design immediately after parturition with 3-wk experimental periods, and then were fed a ration for 120 days in milk. Diets were formulated to provide 3 concentrations of dietary RUP (LRUP 6.65, MRUP 7.72 and HRUP 8.79% of dry matter (DM)) while rumen-degradable protein remained constant (11.3% of DM). Diets contained 26.30% alfalfa hay, 12.60% corn silage, 9.50% sugar beet pulp and 51.5% concentrate in DM basis. Ingredients within the diets were equal across treatments except for fish meal and corn gluten meal that partially replaced with steam rolled barley and soybean meal. Dry matter intake linearly increased by the treatments. Milk yield, Fat corrected milk (FCM) and protein content and yield increased significantly when cows were fed the diets with greater RUP, but milk fat and lactose was not different between treatments. Body weight (BW) changes was improved with intake of high RUP but Body condition score (BCS) changes had significant difference and improved by increasing RUP in the diet. The efficiency of N use increased linearly. Milk urea N and predicted urinary N increased linearly when cows were fed higher amounts of RUP, but differences between the control treatment and high RUP diets were not significant. Fecal N and N balance did not have significant difference. Total tract digestibility of DM and crude protein (CP) intakes increased significantly with greater RUP. In general, increasing amounts of MP and RUP improved productive performance and BCS status of fresh cows and enhanced digestibility of DM and CP.

KEY WORDS fresh cow, nitrogen balance, performance, rumen undegradable protein.

INTRODUCTION

It is widely recognized that feed intake in the immediate postpartum period lags behind that needed to support milk production such that the cow experiences negative energy and protein balance for several weeks following the initiation of lactation. To cope with the large increase in nutrient demand associated with milk production during this time, the cow experiences a multitude of metabolic adaptations. At the initiation of lactation, the demand for glucose in support of lactose production increases markedly and is partially met by an increase in gluconeogenesis, as well as a decrease in glucose oxidation (Doepel *et al.* 2009). The contribution of amino acid (AA) to gluconeogenesis has been considered important during early lactation in the dairy cow, but supportive evidence has come from observations either *in vivo* or *in vitro* (Drackley *et al.* 2001). The other important demand for AA is to support milk protein synthesis and this requirement increases greatly at the onset of lactation. Therefore, despite an increased supply of MP through increased DM intake (DMI) and rations formulated for lactation, these 2 demands create a negative protein balance for cows in early lactation (Doepel *et al.* 2009). Invariably, the early-lactating cow faces a glucose and amino acid deficiency (Phillips *et al.* 2003). To ameliorate this nutrient deficiency, body fat and protein reserves are mobilized to support the energy requirements for high milk production in early lactation. Although body fat deposits are recognized as the major source of energy reserves, the catabolism of both body fat and protein contribute to nutrient requirements at early lactation (NRC, 2001). During this period, body fat mobilization ranges from 41 to 90 kg and protein mobilization ranges from 21 to 24 kg (Komaragiri and Erdman, 1997; Komaragiri *et al.* 1998). Therefore, in addition to being in a negative energy balance, dairy cows experience a negative nitrogen (N) balance in early lactation (Plaizier *et al.* 2000).

Body protein mobilization is driven by the overwhelming need to supply amino acids for hepatic gluconeogenesis and for milk protein synthesis during early lactation. Propionate is the major precursor for gluconeogenesis (Drackley *et al.* 2001); however, limited feed intake during early lactation limits ruminal propionate supply to the liver, raising the requirement for alternative gluconeogenic precursors. Although skeletal muscle is the primary labile source of amino acids, only a few studies have investigated protein metabolism in this tissue during lactation (Komaragiri *et al.* 1998; Phillips *et al.* 2003; Chibisa *et al.* 2008). Skeletal muscle protein mass has been shown to decrease in early-lactating dairy cows (Komaragiri *et al.* 1998; Phillips *et al.* 2003).

Although the mobilization of protein reserves is necessary to augment the inadequate dietary supply of energy and protein, excessive mobilization can lead to an increased incidence of metabolic disorders, poor reproductive and lactational performance (Overton *et al.* 1998). Overton *et al.* (1998) used alanine as an indicator of gluconeogenesis from amino acids and found that propionate conversion to glucose at 1 and 21 d postpartum was 119 and 129% of that at 21 d prepartum, but that alanine conversion to glucose at 1 and 21 d postpartum was 198 and 150% of that at 21d prepartum. Various approaches to optimize postpartum nutrient supply and, thus, minimize the mobilization of body reserves, have been investigated.

The other important demand for AA is to support milk protein synthesis and this requirement increases greatly at the onset of lactation. Therefore, despite an increased supply of MP through increased DMI and rations formulated for lactation, these 2 demands create a negative protein balance for cows in early lactation (Doepel *et al.* 2009; Larsen and Kristensen, 2009).

Therefore, with proper balancing of RDP and RUP, some mobilization and repletion of body protein seems to help transition of cow to lactation. Ruminal microbial protein synthesis alone is insufficient to meet the protein needs of high producing cows therefore, it is important to include feeds in diets that have low protein degradabilities. Feeds such as fish meal and corn gluten meal, are low in ruminal degradability (NRC, 2001).

The objective of this study was to investigate whether consuming of different levels of RUP with fixed amounts of RDP, would affect performance of Holstein fresh cows and could decline the detrimental effects of negative protein and energy balance on milk production and BCS.

MATERIALS AND METHODS

Diets and cow management

Cows were randomly assigned to a dietary treatment within each block; Holstein cows (n=58; treatment 1=17, treatment 2=21, treatment 3=20) were blocked by parity (16 primiparous, 11 at second calving and 31 at third or higher lactation) and assigned randomly at calving in a completely randomized block design with unequal repeats, for 21 days of lactation to three experimental diet (LRUP: contained 17.1% CP with 6.65% RUP, MRUP: contained 19% CP with 7.72% RUP and HRUP: contained 20.1% CP with 8.79% RUP). RDP was constant between diets (11.3%, based on NRC recommendations). They received supplemental corn gluten meal (CGM) and fish meal, partially substituted with SBM and barley, during early postpartum period (wk1 to 3). The amount of CGM and fish meal fed was designed to raise ration CP by 1.1 to 2.2 percentage units. Experimental diets had been shown in Table 1. The diet administered throughout the trial *ad libitum* to achieve 5-10% orts as daily TMR that offered at 0830 h and 1530 h.

Sample collection

Orts were measured daily and feed offered was adjusted to allow for 5 to 10% orts. Because cows were housed in pens, it was not possible to measure individual feed intakes. Instead, the intake of each pen was recorded daily. Weekly samples of rations and orts were taken to determine DM content. These DM percentages were then used to calculate the pen average daily DMI intakes.

Milk weights were recorded daily throughout the trial. Milk samples were collected from milking of the 3 sampling days. The milko-scan B-133 (Foss, Denmark) was used to determine milk fat, protein, lactose and SNF (AOAC, 2000) and milk urea N measured using the chromatography method. Two fecal grap samples were collected on d 20 to 21. Duplicate samples were taken at each sampling time. One sample was dried at 60 ˚C for 36 h and the other was frozen at -20 ˚C for later analysis. Body weight was calculated as the average of measurements performed in the am of d 0, 11 and 21 before morning meal and after a.m. milking. The body condition score of each animal was evaluated by the same person in the am of 0, 11 and 21 after milking.

Table 1 Ingredients of experimental diets (%DM)

Feed stuffs	LRUP[1]	MRUP	HRUP
Alfalfa hay	26.30	26.30	26.30
Corn silage	12.60	12.60	12.60
Beet pulp	9.50	9.50	9.50
Barley and steam rolled	13.90	12.30	11.0
Corn grain and ground	9.70	9.70	9.70
Soybean meal	7.70	6.20	4.60
Roasted soybean	3.60	3.60	3.60
Whole cottonseed	6.70	6.70	6.70
Canola meal	0.51	0.51	0.51
Fish meal	2.0	3.60	5.15
Corn gluten meal	2.0	3.60	5.15
Fat	0.51	0.51	0.51
Salt	0.25	0.25	0.25
Sodium bicarbonate	1.0	1.0	0.92
Calcium carbonate	0.61	0.56	0.51
Magnesium oxide	0.15	0.15	0.13
Di-calcium phosphate	0.2	0.15	0.13
Min-vit supplement[2]	0.825	0.825	0.825
Vitamin A[3]	0.05	0.05	0.05
Vitamin E[4]	0.5	0.5	0.5
Toxin binder	0.07	0.07	0.07
Glycoline[5]	1.29	1.29	1.29
Monensin	0.01	0.01	0.01
Availa 4[6]	0.01	0.01	0.01

[1] LRUP: low rumen undegradable protein; MRUP: medium rumen undegradable protein and HRUP: high undegradable protein.
[2] Contained: Ca: 196 gr; P: 96 gr; Na: 71 gr; Mg: 19 gr; Fe: 3gr; Cu: 0.3 gr; Mn: 2 gr; Zn 3 gr; Co: 0.1 gr; I: 0.1 gr; Se: 0.001 gr; antioxidant: 3 gr; vit A: 5000 IU; vit D_3: 100000 IU and vit E: 100 mg.
[3] Vitamin A: 5000000 IU.
[4] Vitamin E: 4400 IU.
[5] Net energy: 1500 kcal; Ca 1.45%; EE 0.8% and CF 0.3%.
[6] Zn not less than 5.15%; Mn not less than 2.88%; Cu not less than 1.08% and Co not less than 0.18%.

Sample analysis

Orts samples, alfalfa hay, corn silage, beet pulp, concentrate mix (n=3 for each mix) and were dried at 105 ˚C for 24 h (except for corn silage that were dried at 60 ˚C for 72 h) and ground to pass a 1 mm screen (Wiley mill). Dry matter content of TMR was determined by drying at 60 ˚C for 72 h; CP was determined by micro Kjeldahl (AOAC, 2000). Total tract digestibility of DM and CP was calculated. Urinary nitrogen was predicted by using different equations (Table 6) and fecal N was calculated using digestibility of nitrogen and amount nitrogen intakes (Table 5).

Statistical analyses

The randomized complete block design was used. Data measured over time (DMI, milk yield and components) within the period of interest were subjected to ANOVA by using the Repeated Statement Mixed procedure of SAS (SAS Institute, 2004). N balance, digestibility and BW data were analyzed using GLM procedure of SAS. For all analysis, least squares means calculated. Means were evaluated by Tukey test. In this study differences among treatments were considered significant if P<0.05 whereas when 0.05<P<0.15, differences were considered to indicate a trend toward significant.

Table 2 Chemical composition of diets

Items	LRUP[1]	MRUP	HRUP
NE_L (Mcal/kg)	1.65	1.67	1.68
CP (%)	17.9	19	20.1
RDP[2] (% of CP)	11.31	11.28	11.25
RUP[3] (% of CP)	6.65	7.72	8.79
Soluble protein (%)	23	22.3	21.4
Metabolizable protein (g/d)	1893	2023	2149
Methionine (g/d)	39	43	46
Lysine (g/d)	121	128	135
NDF (%)[4]	33.2	32.7	32.2
PeNDF[5] (%)	24	23	23
NFC[6] (%)	36.5	35.7	35
Ether Extract (%)	4.7	4.9	5.1

[1] LRUP: low rumen undegradable protein; MRUP: medium rumen undegradable protein and HRUP: high undegradable protein.
[2] RDP: rumen degradable protein.
[3] RUP: rumen-undegradable protein.
[4] NDF: neutral detergent fiber.
[5] PeNDF: predicted neutral detergent fiber.
[6] NFC: non fibrous carbohydrates.
NFC (%): 100 - (% CP+% NDF+% EE+% Ash).

RESULTS AND DISCUSSION

Dry matter intake

Least square means of DMI during the experimental period for LRUP, MRUP and HRUP were 14.15, 14.40 and 15.04 kg/d, respectively (Table 3). By increasing level of RUP in diets of fresh cows, DMI in MRUP and HRUP in comparison with LRUP increased linearly and had trend (P=0.05). DMI has special importance to meet nutrient requirements of fresh cows to maintain their health and production. Low DMI and deficiency in nutrient supply, specially protein and amino acids, could led to immunosuppression (Nathalie *et al.* 2004) and incidence of metabolic disorders consisted of rapid loss of BCS, ketosis, fatty liver and displaced abomasum (Drackley *et al.* 2001). Thus, diets that have higher levels of CP and RUP are effective in maintaining of production and BCS (NRC, 2001). Fresh cows in first days of lactation period, specially immediately after parturition, faces with loss of appetite, because of increased level of estrogen in plasma (Ingvartsen, 2006) and since NRC (2001) recommended high concentration of CP for high levels of milk yield, therefore, because of low DMI in fresh cows, this amount of CP, must meet in the form of high concentrate of RDP and RUP in diets (Khorasani *et al.* 1996). Decreasing DMI in early postpartum period causes declining in passage rate and consequently protein degradability in the rumen increases, thus will decrease ruminal outflow of non ammonia nitrogen, non ammonia non microbial nitrogen and follow that entering of essential amino acids into small intestine (Ipharraguerre and Clark, 2005). Therefore, ratio of RUP supplements (corn gluten meal and fish meal) would be increased. Our findings were in agreement with Law *et al.* (2009), who reported higher DMI using RUP. Researchers reported that cows received excessive amounts of RUP (10%) than NRC (2001) recommendations had 2.1 kg higher DMI per day (Flis and Wattiaux, 2005). Least square means of BCS changes were -0.76, -0.36 and -0.43, respectively (Table 3) that refer to significant improvement (P=0.0001) in BCS by consuming high RUP diets. An indicator of energy balance status is BCS. Loss of BCS is correlated with fat mobilization, therefore, BCS might be used as indicator of energy balance during early lactation (DeVries and Veerkamp, 2000).

Van Knegsel (2007) suggested that glucogenic diets in comparison with lipogenic diets, resulted in deposition of energy in the body. These findings show that glucogenic nutrients such as RUP supplements in our study, lead to improve the BCS due to decreased body tissue mobilization by increasing DMI.

Santos *et al.* (1999) reported that replacement of RDP with RUP supplements in lactating cows, improved energy balance and led to 9% increased amount of NEl consuming. Furthermore, Leucine is effective in milk synthesis and BW changes in whole lactational period and infusion of branched chain amino acids (Leu, Ile and Val) has led to retention of nitrogen in the body (Langer and Fuller, 2004). However, using CGM as a rich source of Leu in diets of fresh cows could be an effective factor in maintaining protein reserves of body and consequently improve BCS changes. Likewise, branched chain amino acids have several roles in whole body metabolism and could influence insulin secretion. It has been suggested that these amino acids, could influence secretion of metabolic hormones, specially prolactin and insulin (Garnsworthy *et al.* 2008). Law *et al.* (2009) reported that increasing dietary CP from calving day to 150 DIM, had led to increased energy consumption and BW and BCS of cows had numerically increased that were in agreement of our findings.

NEFA and ßHBA

Least square means of NEFA of plasma of the treatments low RUP (LRUP), medium RUP (MRUP) and high RUP (HRUP) were 0.60, 0.55 and 0.48 m*M*/L (Table 4). By increasing RUP sources in the diet, concentration of NEFA had decreased significantly (P<0.05). Nydam *et al.* (2009) and Ospina *et al.* (2009), at Cornell university, studied on 104 herds with 2758 cows, that blood samples of 1440 cows prepartum and 1318 cows postpartum were collected. They found that high NEB (measured by NEFA and ßHBA) at transition period, led to clinical diseases and had negative effects on productive and reproductive efficiency in cows that housed in freestalls and fed TMR. Condensed management programs to minimizing risks of these diseases are: the cut point of NEFA at 14 to 2 days prepartum, should not be more than or equal 0.3 m*M*/L and for postpartum cows, should not be more than or equal 0.6 m*M*/L and concentration of ßHBA for 3 to 14 days after parturition, should not be more than or equal 0.96 m*M*/L.

These researchers (Nydam *et al.* 2009; Ospina *et al.* 2009; Duffield *et al.* 2009) reported that recognition of a target level for NEFA (non-esterified fatty acids) and ßHBA (ß-hydroxybutyrate acid) is difficult, because of variation between animals; however, critical threshold for metabolic disorders such as displaced abomasums, clinical ketosis, metritis or retained fetal membranes are 0.72, 0.57, and 0.36 m*M*/L and odds ratios are 9.7, 5 and 16, respectively. Le Blanc *et al.* (2005) and Van Saun, (2004) reported that if NEFA concentrations were higher than 0.4 m*M*/L for close-up period and were higher than 0.6 mM/L for fresh cows, they increase the risks of metabolic disorders by 4 to 5 fold. Drackley *et al.* (1999) suggested that one of the most important strategies for prevention of fatty liver, is to cause labor for the liver, such as ATP produced in beta-oxidation pathway and krebs cycles, do not shift to unuseful pathways. If produced ATP, did not used in useful metabolic pathways, fatty acids would be used to synthesis of tree acyl glycerol (TAG) or ketone bodies in liver. This was indicated that, by enhancing dietary protein in order to increasing gloconeogenesis and ureogenesis in hepatocyts, as an induction of labor for the liver, can reduce incidence of fatty liver, as suggested by Bobe *et al.* (2004). Least square means of plasma ßHBA were 0.65, 0.51, and 0.55 m*M*/L (Table 3) that treatments did not have any significant differences. Law *et al.* (2009) reported that from calving date to 150 DIM, increasing CP from 11.4 to 17.3% of DM, concentration of ßBHA decreased significantly. Cornell researchers (Nydam *et al.* 2009; Ospina *et al.* 2009) and Duffield *et al.* (2009) reported that critical threshold of ßHBA for metabolic disorders such as displaced abomasums, clinical ketosis, metritis or retained fetal membranes are 0.96, 0.96 and 0.67 m*M*/L and odds ratios are 6.9, 4.9 and 2.3, respectively. DMI and plasma NEFA are conversely correlated and accretion of TAG in liver resulted to hepatocyts dysfunction acetyle-coA converted to acetoacetate and beta hydroxyl butyrate. Elevated levels of these keton bodies in blood, milk and urine are primary indices of ketosis (Nydam *et al.* 2009; Ospina *et al.* 2009; Duffield *et al.* 2009), and since in this study, DMI increased by HRUP and NEFA concentration decreased, so it could be concluded, by increasing level of MP in early post partum period, to prevent incidence of fatty liver and ketosis.

Table 3 Least square (Means±SE) of DMI and milk yield and composition

Items	LRUP[8]	MRUP	HRUP	P-value		
				Treatment	Block	Period
DMI[1] (kg/d)	14.15±0.17	14.40±0.17	15.04±0.17	0.0542	0.0002	0.6180
Milk yield (kg/d)	35.42[b]±0.92	35.81[ab]±0.85	38.54[a]±0.89	0.028	0.0001	0.0001
FCM 4% (kg/d)[2]	29.89[b]±0.9	31.24[ab]±0.83	33.0[a]±0.87	0.0477	0.0001	0.0001
FCM 3.5 (kg/d)[3]	32.20[b]±0.98	33.68[ab]±0.91	35.57[a]±0.95	0.0477	0.0001	0.0001
Milk fat (%)	3.01±0.14	3.22±0.13	3.17±0.14	0.5464	0.0011	0.0001
Milk fat (kg/d)	1.048±0.04	1.12±0.04	1.17±0.04	0.1995	0.0241	0.0001
Milk protein (%)	3.41[b]±0.02	3.53[a]±0.02	3.53[a]±0.02	0.0008	0.0841	0.0001
Milk protein (kg/d)	1.20[b]±0.03	1.26[ab]±0.03	1.36[a]±0.03	0.0072	0.0001	0.0001
Milk lactose (%)	5.18±0.03	5.18±0.03	5.09±0.03	0.1282	0.4262	0.0001
Milk lactose (kg/d)	1.83±0.04	1.85±0.04	1.95±0.04	0.0913	0.0001	0.0001
Milk SNF (%)	9.29±0.06	9.41±0.06	9.39±0.06	0.1172	0.4430	0.0001
Milk SNF(kg/d)	3.29±0.08	3.36±0.07	3.61±0.07	0.0901	0.0001	0.0001
ECM (kg/d)[4]	31.95[b]±0.85	33.47[ab]±0.82	35.35[a]±0.83	0.0242	0.0001	0.0001
Milk energy (kg/d)[5]	0.67±0.01	0.69±0.01	0.68±0.01	0.3898	0.0001	0.0001
SCC (×1000/mL)[6]	293[a]±2.01	150[b]±2.31	142[b]±2.41	0.030	0.001	0.0001
Future milk yield[7] (kg/d)	42.94±1.47	41.99±1.36	43.97±1.42	0.8435	0.0001	0.0001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
[1] Dry matter intake.
[2] Fat corrected milk (FCM) 4%: [0.4 × milk (kg)] + [15 × milk fat (kg)].
[3] FCM 3.5%: [0.4324 × milk (kg)] + [16.216 × milk fat (kg)].
[4] Energy corrected milk (ECM): milk (kg) × [383 × fat (%) + 242 × protein (%) + 165.4×lactose (%) + 20.7] / 3140.
[5] Milk energy (Mcal/kg): (0.0929×fat %) + (0.0547×protein %) + (0.0395×lactose %) (NRC, 2001).
[6] Somatic cell counts.
[7] Milk production from day 21 to day 120 of lactation.
[8] LRUP: low rumen undegradable protein; MRUP: medium rumen undegradable protein and HRUP: high undegradable protein.

Table 4 Least square (Means±SE) of BW, BCS changes, and plasma NEFA and ßHBA

Items	LRU[1]	MRUP	HRUP	P-value		
				Treat	Block	Treat × Block
BW changes (kg/d)	-53.89±10.29	-24.85±9.91	-37.12±11.62	0.1368	0.5335	0.1191
BCS changes	-0.76[b]±0.06	-0.36[a]±0.06	-0.43[a]±0.07	0.0001	0.1215	0.1157
Initial BW (kg/d)	668.51±11.94	653.25±11.5	662.65±13.49	0.6516	0.0001	0.9177
Initial BCS	3.30±0.07	3.30±0.07	3.31±0.08	0.9973	0.0239	0.9921
NEFA (mmol/L)	0.60[a]±0.04	0.55[b]±0.05	0.48[b]±0.04	0.04	0.05	0.47
ßHBA (mmol/L)	0.65±0.08	0.51±0.09	0.54±0.09	0.5303	0.8544	0.57

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
[1] LRUP: low rumen undegradable protein; MRUP: medium rumen undegradable protein and HRUP: high undegradable protein.

Nutrient digestibility

Least square means of digestibility of DM and CP were 67.94, 70.63, 72.10% and 74.59, 76.58, 77.68%, respectively, that treatment effects on these data were statistically significant (P<0.05) (Table 7). Apparent digestibility of DM and N increased linearly as RUP supplement in diet, increased (Wright *et al.* 1998) that agreed with Flis and Wattiaux (2005) and our findings. These increases in digestibility were due to high postruminal digestibility of RUP supplements.

Milk production and composition

Least square means of whole milk production and FCM 4% were 35.42, 35.81, 38.54 kg/d, and 29.89, 31.24, 33.0 kg/d, respectively (Table 4). Increasing level of RUP accompanied by enhancing MP and supply of EAA to small intestine (Chen *et al.* 2009; Flis and Wattiaux, 2005). In a research by Schwab and Foster (2009) at Cornell university, they suggested that limiting factor for milk production in first weeks of lactation is MP not NEl, therefore enhancing RUP has beneficial effect. NRC (2001) indicated a quadratic relationship between milk production and dietary CP at the range of 16 to 21%, however this CP enhancement using RDP had less benefit. Flis and Wattiaux (2005) indicated that diets contained over 10% CP than NRC (2001) recommendation, had 1.5 kg more milk per day, this increase was due to RUP enhancement. In agreement with our findings Cunningham *et al.* (1996) reported 2.7 kg/d more milk production. Heated SBM compare with row SBM, increased milk yield (Armentano *et al.* 1997), this response was due to high passage of ruminally undegradable protein to small intestine.

This idea supported with indicating higher milk production when animal by-products added to diets contained SBM, whereas RDP was fixed and RUP was increased. Armentano *et al.* (1997) indicated diets in early lactation

Table 5 Least square (Means±SEM) of nitrogen input and output

Items	LRUP[10]	MRUP	HRUP	P-value		
				Treatment	Block	Treat × block
MUN (mg/dL)	15.13[b]±0.6	18.31[a]±0.66	17.21[ab]±0.74	0.0036	0.0583	0.3748
Predicted UN[1] (g/d)	189.78[b]±7.53	229.62[a]±8.31	215.9[ab]±9.29	0.0036	0.0583	0.3748
Milk N[2] (g/d)	190.32[b]±7.30	200.71[ab]±8.06	220.04[a]±9.01	0.0482	< 0.0001	0.4392
N intake[3] (g/d)	405.44[c]±0	437.81[b]±0	483.71[a]±0	< 0.0001	< 0.0001	< 0.0001
Predicted NI[4] (g/d)	574.82[b]±13.76	635.34[a]±15.18	642.1[a]±16.97	0.0043	< 0.0001	0.2628
Fecal N[5] (g/d)	102.94±2.07	102.52±2.28	107.95±2.55	0.2303	0.1367	0.5785
Predicted FN[6] (g/d)	25.33±11.42	7.47±12.60	47.76±14.08	0.1130	0.0464	0.8126
Predicted FN[7] (g/d)	194.72[b]±2.32	205.0[a]±2.58	206.15[a]±2.88	0.0041	< 0.0001	0.2628
NUE[8] (%)	32.82±0.77	31.46±0.85	34.09±0.96	0.1382	< 0.0001	0.5890
N balance[9] (g/d)	-77.61±11.45	-95.04±12.64	-60.18±14.13	0.1983	0.0737	0.8083

[1] Predicted UN (g/d): 12.54 × MUN (mg/dL) (Jonker *et al*. 1998).
[2] Milk nitrogen (g/d): milk protein (g/d) / 6.38 (NRC, 2001).
[3] NI (g/d): CP intake (g/d) / 6.25.
[4] NI predicted (g/d): (predicted UN+Milk N+97) / 0.83 (Jonker *et al.* 1998).
[5] Fecal N (g/d): [NI × (CP digestibility (%)-100)] / 100.
[6] Predicted FN (g/d): NI - predicted UN - milk N.
[7] Predicted FN (g/d): predicted NI - predicted UN - milk N.
[8] Nitrogen utilization efficiency (%): [milk N (g/d) × 100] / predicted NI (g/d) (Jonker *et al*. 1998).
[9] N balance: NI - (FN+MN+UN).
[10] LRUP: low rumen undegradable protein; MRUP: medium rumen undegradable protein and HRUP: high undegradable protein.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 6 Least squares (Means±SEM) of urinary nitrogen predictions and nitrogen balances with different equations

Items	LRUP[5]	MRUP	HRUP	P-value		
				Treatment	Block	Treat × block
Jonker *et al*. 1998[1] (g/d)	189.78[b]±7.53	229.62[a]±8.31	215.9[ab]±9.29	0.0036	0.0583	0.3748
Nitrogen balance (g/d)	-77.61±11.45	-95.04±12.64	-60.18±14.13	0.1983	0.0737	0.8083
Kohn *et al*. 2002[2] (g/d)	256.32[b]±9.07	304.30[a]±10.01	287.78[ab]±11.19	0.003	0.058	0.37
Nitrogen balance (g/d)	-144.15±12.68	-169.72±14.0	-132.06±15.65	0.18	0.13	0.77
Kauffman and St-pierre, 2001[3] (g/d)	266.97[b]±10.59	323.01[a]±11.69	303.71[ab]±13.07	0.003	0.058	0.37
Nitrogen balance (g/d)	-154.79±13.96	-188.43±15.41	-147.99±17.24	0.16	0.19	0.74
Kauffman and St-pierre, 2001[4] (g/d)	240.70[b]±12.52	298.84[a]±13.82	282.73[ab]±15.45	0.01	0.0001	0.14
Nitrogen balance (g/d)	-128.53±15.61	-164.26±17.22	-127.01±19.26	0.23	0.26	0.50

[1] PrUN: 12.54 × MUN.
[2] PrUN: 15.1 × MUN + 27.8.
[3] PrUN: 17.64 × MUN.
[4] PrUN: 0.0259 × MUN × BW.
[5] LRUP: low rumen undegradable protein; MRUP: medium rumen undegradable protein and HRUP: high undegradable protein.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

had high amounts of CP (17 to 19%) increased both milk yield and milk production persistency. Least square means of milk fat content and yield were 3.01, 3.22, 3.17%, and 1.048, 1.12, 1.17 kg/d, respectively (Table 4). There were no differences in the percentage and amount of fat in the milk between treatments.

Milk protein significantly increased with enhancing RUP (P<0.05). This increase, was probably due to providing good profiles of amino acids that were similar to milk amino acids profile, and enhancing RUP specially with FM could result optimal levels of Lys to Met ratios to the small intestine (Schwab and Foster, 2009), and since Lys and Met are limiting amino acids for milk production and milk protein, thus high levels of RUP causes increasing of milk protein. In agreement with our findings, Broderick (2003) found that milk protein yield was improved by enhancing dietary CP from 15.3 to 16.7%, but he did not any changes with 18.4% of CP.

Somatic cell count (SCC)

Least square means of SCC were 293000, 150000, and 142000 per mL (Table 4). Increasing levels of RUP caused that SCC of milk decreased significantly (P=0.03). Our findings were in agreement with Ellison Henson (1997) that increased RUP caused SCC linearly decreased. Houdijk *et al.* (2001) indicated that competition for metabolizable energy did not result to immuno suppression and it was correct only about MP. In other words, MP deficiency would make immunosuppression. These findings are based on this fact that immune systems have protein nature. Metabolic changes accompanied by inflammation and infectious diseases, increase needs for protein and amino acids. Incretement in cytokines (IL-6, IL-1, TNF-ɑ) had redound to protein metabolism changed and during immunological stress, amino acids go to inflammate tissues instead of protein production (lactation and growth) (Nathalie *et al.* 2004). On average, cows have 3500 neutrophil per microliter of blood

Table 7 Least square means±SE of digestibility of DM and CP

Items	LRUP[3]	MRUP	HRUP	P-value		
				Treatment	Block	Treat × Block
DCP[1] (%)	74.59[b]±0.47	76.58[a]±0.52	77.68[a]±0.58	0.0006	0.1439	0.5944
DDM[2] (%)	67.94[b]±0.78	70.63[ab]±0.86	72.10[a]±0.96	0.0052	0.0140	0.1184

[1] Digestibility of crude protein.
[2] Digestibility of dry matter.
[3] LRUP: low rumen undegradable protein; MRUP: medium rumen undegradable protein and HRUP: high undegradable protein.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

and half time of neutrophils are about 6 hours (Kehrli *et al.* 2006). Therefore amino acids requirements for proliferation of leukocytes and improving immune system, must be meet by adding protein supplements, and in this study by increasing RUP in the diet, SCC were decreased that probably indicating enhancing power of immune system and Consequently would be declined incidence of mastitis (Nathalie *et al.* 2004).

N Balance

Daily intake, digestion and excretion of N

In this trial, overall N intake from treatment 1 to 3 were 405.44, 437.81, and 483.71 g/d, milk N were 190.32, 200.71, and 220.04 g/d, fecal N were 102.94, 102.52, and 107.95 g/d, predicted urinary N were 189.78, 229.62, and 215.9 g/d based on Jonker *et al.* (1998) equation, and NB were, and -77.61, -95.04 and -60.18 g/d, respectively.

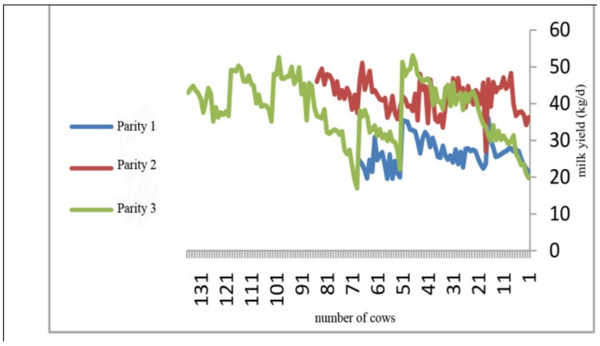

Figure 1 Variations of milk production in LRUP treatment during first 21 d of lactation

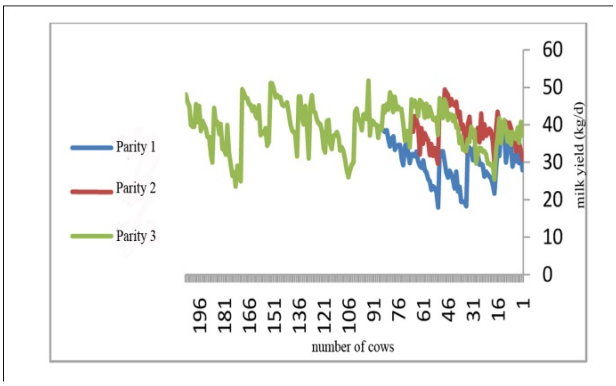

Figure 2 Variations of milk production in MRUP treatment during first 21 d of lactation

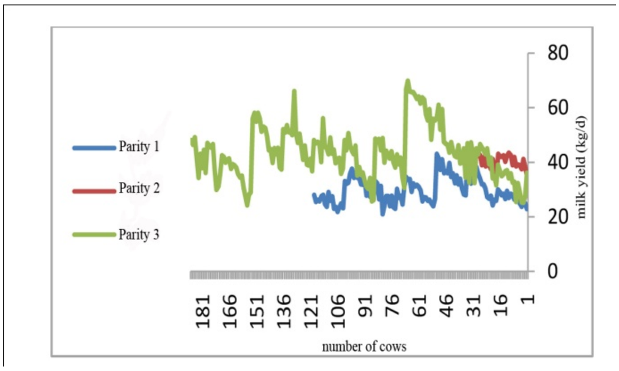

Figure 3 Variations of milk production in HRUP treatment during first 21 d of lactation

Daily excretion of N was significantly higher (P<0.05) in cows consuming the MRUP compared with LRUP, but there were no difference between HRUP and LRUP. Tamminga (1992) 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 inefficient conversion of absorbed AA into milk and proteins.

Fecal N primarily consisted of indigestible microbial protein produced in the GI tract, as well as endogenous protein, sloughed cells from the GI tract and undigested feed protein, because undigested feed protein is minor component of total fecal N, treatment differences in fecal N were not expected. Fecal N excretion increased linearly in response to increased RUP supplementation; however, this increase was quantitatively small compared with differences in N intake (Wright *et al.* 1998). Efficiency of milk N secretion declined quadratically as the concentration of RUP supplement in the diet was increased (Wright *et al.* 1998). Broderick *et al.* (2010) reported that improving in efficiency of microbial N synthesis and declining CP degradability in the rumen, are positively correlated with dietary N trap into milk N, however the reason of constant N utilization for milk production in our study would be related to enhancement of dietary RUP.

CONCLUSION

The results of this study shows that increasing the amounts of RUP in the diets of fresh cows, increased milk yield and milk protein and improved BCS and could be effective in prevention of mastitis. Furthermore, because of high CP intake caused environmental pollution, feeding high RUP indicate that predicted UN did not increase significantly and we suggest that high needs for MP in this period (0-3 wk of lactation) have best benefits and after 21 DIM, CP concentration of diet could be lowered due to diminishing environmental and conception problems.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Sharif-Abad agri-industrail Co. for feeding and care of the cows and would like to thank S. Ebrahimi, M. Mosavi and F. Niazi for their excellent technical assistance with all aspects of this work.

REFERENCES

AOAC. (2000). Official Methods of Analysis. 13th Ed. Association of Official Analytical Chemists, Arlington, VA.

Armentano L.E., Bertics S.J. and Ducharme G.A. (1997). Response of lactating cows to methionine or methionine plus lysine added to high protein diets based on alfalfa and heated soybeans. *J. Dairy Sci*. **80,** 1194-1199.

Bobe G., Young J.W. and Beitz D.C. (2004). Invited review: pathology, etiology, prevention and treatment of fatty liver in dairy cows. *J. Dairy Sci*. **87,** 3105-3124.

Broderick G.A. (2003). Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci*. **86,** 1370-1381.

Broderick G.A., Huhtanen P., Ahvenjärvi S., Reynal S.M. and Shingfield K.J. (2010). Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle. A meta-analysis. *J. Dairy Sci*. **93,** 3216-3230.

Chen J., Broderick G., Luchini D., Sloan B. and Devillard E. (2009). Effect of metabolizable lysine and methionine concentrations on milk production and N utilization in lactating dairy cows. *J. Dairy Sci*. **92,** (Suppl 1):171 (Abstr).

Chibisa G.E., Gozho G.N., Van Kessel A.G., Olkowski A.A. and Mutsvangwa T. (2008). Effects of peripartum propylene glycol supplementation on nitrogen metabolism, body composition and gene expression for the major protein degradation pathways in skeletal muscle in dairy cows. *J. Dairy Sci*. **91,** 3512-3527.

Cunningham K.D., Cecava M.J., Johnson T.R. and Ludden P.A. (1996). Influence of source and amount of dietary protein on milk yield by cows in early lactation. *J. Dairy Sci*. **79,** 620-630.

DeVries M.J. and Veerkamp R.F. (2000). Energy balance of dairy cattle in relation to milk production variables and fertility. *J. Dairy Sci*. **83,** 62-69.

Doepel L., Lobley G.E., Bernier J.F., Dubreuil P. and Lapierre H. (2009). Differences in splanchnic metabolism between late gestation and early lactation dairy cows. *J. Dairy Sci*. **92,** 3233-3243.

Drackley J.K., Overton T.R. and Douglas G.N. (2001). Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. *J. Dairy Sci*. **84,** 100-112.

Drackley J.K. (1999). Biology of dairy cows during the transition period: the final frontier? *J. Dairy Sci*. **82,** 2259- 2273.

Duffield T.F., Lissemore K.D., McBride B.W. and Leslie K.E. (2009). Impact of hyper ketonemia in early lactation dairy cows on health and production. *J. Dairy Sci*. **92,** 571-580.

Ellison Henson J., Schingoethe D.J. and Maiga H.A. (1997). Lactational evaluation of protein supplements of varying ruminal-degradabilities. *J. Dairy Sci*. **80,** 385-392.

Flis S.A. and Wattiaux M.A. (2005). Effects of parity and supply of rumen degraded and undegraded protein on production and nitrogen balance in Holsteins. *J. Dairy Sci*. **88,** 2096-2106.

Garnsworthy P.C., Gong J.G., Armstrong D.G., Newbold J.R., Marsden M., Richards S.E., Mann G.E., Sinclair K.D. and Webb R. (2008). Nutrition, metabolism and fertility in dairy cows: 3. amino acids and ovarian function. *J. Dairy Sci*. **91,** 4190-4197.

Houdijk J.G.M., Jessop N.S. and Kyriazakis I. (2001). Nutrient partitioning between reproductive and immune functions in animals. *Proc. Nutr. Soc.* **60,** 515-525.

Ingvartsen K.L. (2006). Feeding and management related diseases in the transition cow physiological adaptations around calving and strategies to reduce feeding related diseases. *Anim. Feed Sci. Tech.* **126,** 175-213.

Ipharraguerre I.R. and J.H. Clark. (2005). Impacts of the source and amount of crude protein on the intestinal supply of nitrogen fractions and performance of dairy cows. *J. Dairy Sci*. **88,** 22-37.

Jonker J.S., Kohn R.A. and Erdman R.A. (1998). Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cattle. *J. Dairy Sci*. **81,** 2681-2692.

Kauffman A.J. and N.R. St-pierre. (2001). The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. *J. Dairy Sci*. **84,** 2284-2294.

Kehrli M.E., Neill J.D., Burvenich C., Goff J.P., Lippolis J.D., Reinhardt T.A. and Nonnecke B.J. (2006). Energy and protein effects on the immune system. Pp. 460-461 in Ruminant Physiology. K. Sejrsen, T. Hvelplundand and M.O. Nielson, Eds. Wageningen Academic Publisher.

Khorasani G.R., Boer G.D.E. and Kennelly J. (1996). Response of early lactation cows to ruminallyundegradable protein in the diet. *J. Dairy Sci.* **79,** 446-453.

Kohn R.A., Kalscheur K.F. and Russek-Cohen E. (2002). Evaluation of models to estimate urinary nitrogen and expected milk urea nitrogen. *J. Dairy Sci.* **85,** 227-233.

Komaragiri M.V.S. and Erdman R.A. (1997). Factors affecting body tissue mobilization in early lactation dairy cows. I. Effect of dietary protein on mobilization of body fat and protein. *J. Dairy Sci.* **80,** 929- 937.

Komaragiri M.V.S., Casper D.P. and Erdman R.A. (1998). Factors affecting body tissue mobilization in early lactation dairy cows. II. Effect of dietary fat on mobilization of body fat and protein. *J. Dairy Sci.* **81,** 169-175.

Langer S. and Fuller M.F. (2004). Interactions among the branchedchain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization. *Br. J. Nutr.* **83,** 43-48.

Larsen M. and Kristensen N.B. (2009). Effect of abomasal glucose infusion on splanchnic amino acid metabolism in periparturient dairy cows. *J. Dairy Sci*. **92(7),** 3306-3318.

Law R.A., Young F.J., Patterson D.C., Kilpatrick D.J., Wylie A.R.G. and Mayne C.S. (2009). Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. *J. Dairy Sci.* **92,** 1001-1012.

Le Blanc S.J., Leslie K.E. and Duffield T.F. (2005). Metabolic predictors of displaced abomasum in dairy cattle. *J. Dairy Sci*. **88,** 159-170.

Nathalie L.F., Delphine M. and Christiane O. (2004). Modifications of protein and amino acid metabolism during inflammation and immune system activation. *Liv. Prod. Sci*. **87,** 37-45.

NRC (2001). Nutrient Requirements of Dairy Cattle, 7th Ed. National Academy Press, Washing ton, DC.

Nydam D.V., Ospina P.A., Stokol T. and Overton T.R. (2009). Evaluation of the effect of non-esterified fatty acids (NEFA) and ß-hydroxybutyrate (ßHB) concentrations on health, reproduction and production in transition dairy cattle from the Northeast USA. Pp. 97-103 in Proc. Cornell Nutr. Conf. Feed Manufac., Cornell Univ., Ithaca, NY.

Ospina P.A., Nydam D.V., Stokol T. and Overton T.R. (2009). Herd alarm levels for health, reproductive and production effects based on NEFA and ßHB concentrations in dairy herds. Proc. Am. Assoc. Bovine Pract. Annu.

Overton T.R., Drackley J.K., Douglas G.N., Emmert L.S. and Clark J.H. (1998). Hepatic gluconeogenesis and whole body protein metabolism of periparturient dairy cows as affected by source of energy and intake of the prepartum diet. *J. Dairy Sci*. **81,** 295.

Phillips G.J., Citron T.L., Sage J.S., Cummins K.A., Cecava M.J. and McNamara J.P. 2003. Adaptations in body muscle and fat in transition dairy cattle fed differing amounts of protein and methionine hydroxy analog. *J. Dairy Sci.* **86,** 3634-3647.

Plaizier J.C., Martin A., Duffield T.F., Bagg R., Dick P. and McBride B.W. 2000. Effect of prepartum administration of monensin in controlled release capsule on apparent digestibilities and nitrogen utilization in transition dairy cows. *J. Dairy Sci*. **83,** 2918-2925.

Santos J.E.P., Huber J.T., Theurer C.B., Nussio L.G., Tarazon M. and Santos F.A.P. (1999). Response of lactating dairy cow's tosteam-flaked sorghum, steam-flaked corn, or steam-rolled cornand protein sources of differing degradability. *J. Dairy Sci*. **82,** 728-737.

SAS Institute. (2004). SAS/STAT® User's Guide,Version 9.1 Edition. SAS Inst Inc, Cary, NC.

Schwab C.G. and Foster G.N. (2009). Maximizing milk components and metabolizable protein utilization through amino acid formulation. Pp. 1-15 in Proc. Cornell Nutr. Conf. Feed Manuf, Cornell Univ., Ithaca, NY.

Tamminga S. (1992). Nutrition management of dairy cows as a contribution to pollution control. *J. Dairy Sci.* **75,** 345-357.

Van Knegsel A.T.M., Vanden Brand H., Dijkstra J., Van Straalen W.M., Heetkamp M.J.W., Tamminga S. and Kemp B. (2007). Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. *J. Dairy Sci.* **90,** 1467-1476.

Van Saun R.J. (2004). Metabolic profiling and health risk in transition cows. Pp. 212-213 in Proc. Am. Assoc. Bov. Pract.

Wright T.C., Moscardini S., Luimes P.H., Susmel P. and McBride B.W. (1998). Effect of rumen undegradable protein and feed intake on nitrogen balance and milk protein production in dairy cows. *J. Dairy Sci.* **81,** 784-793.