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Impacts of Different Levels of RUP on Performance and Reproduction of Holstein Fresh Cows

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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 reproduction of early post-partum dairy cows determined. Forty wo multiparous and 16 primiparous Holstein cows were assigned to diets in a completely randomized block design immediately after parturition with 3 weeks experimental periods and then were fed one 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)] which rumen-degraded 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 (DM basis). Ingredients within 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 was linearly increased by treatment. Milk yield, FCM and protein content and yield all increased significantly when cows were fed diets with greater RUP but milk fat and lactose did not have different between treatments. BW changes was improved with intake of high RUP but BCS changes had significant difference and improved by increasing RUP in the diet. Number of breeding per cow, open days and first breeding conception rates had significantly increased with concuming HRUP diet ($p<0.05$). Also, plasma progesterone increased significantly ($p<0.05$) between days 11 and 21 after parturition. Plasma urea N concentrations were not statistically affected by diet. Plasma cholesterol concentrations increased significantly ($p<0.05$) by treatments.

Key words: Rumen undegraded protein, fresh cow, performance, reproductive traits

INTRODUCTION

It is widely recognized that 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 (Bell, 1995).

More ever, large increases in milk yield over the past 30 years were associated with declining fertility of dairy cows all over the world (Butler, 2003). This decline in fertility was attributed partly to unfavorable genetic correlations between milk yield and reproductive traits and partly to increasing imbalance of nutrients leading to metabolic stress (Pryce *et al*., 2004). Much emphasis has been placed on the strong association between Negative Energy Balance (NEB) in early lactation and length of the postpartum anovulatory period (Garnsworthy *et al*., 2008). Prolonged periods of NEB were associated with suppression of pulsatile LH secretion, reduced ovarian responsiveness to LH stimulation and reduced estradiol secretion by the dominant follicle, all of which influenced ovulation of the dominant follicle (Butler, 2003). Mobilization of body fat during NEB increased plasma concentrations of NEFA and BHBA, both of which were associated with reduced fertility (Garnsworthy *et al*., 2008). Negative energy balance resulted in loss of BCS as the cow mobilized body fat reserves to support milk production. Greater BCS loss was associated with delayed first ovulation postpartum and reduced conception rate (Butler, 2003). The magnitude and duration of BCS loss was directly related to BCS at calving, because dairy cows adjust their DMI in early lactation to move toward a biological target BCS at around 12 weeks postpartum (Garnsworthy and Topps, 1982). A recent review (Garnsworthy, 2007) suggested that biological BCS targets were defined by genetics and have reduced over the past 20 years therefore, modern dairy

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cows are more likely to suffer prolonged NEB. The most common strategy used to reduce the extent of NEB and BCS loss in early lactation is to increase dietary energy concentration by increasing the starch or fat components of the ration at the expense of forage components. Such changes in carbohydrate and fat supplies have implications for rumen function, milk composition, nutrient partition and metabolic hormones.

The contribution of AA to gluconeogenesis has been considered important during early lactation in the dairy cow (Bell, 1995) but supportive evidence has come from observations either *ex 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 DMI and rations formulated for lactation, these 2 demands create a negative protein balance for cows in early lactation.

Dairy cows in postpartum period have increasing demands to Metabolizable Protein (MP) to meet their requirements to milk production (NRC, 2001). Invariably, the early-lactating cow faces a glucose and amino acid deficit (Phillips *et al*., 2003). To ameliorate this nutrient deficit, body adipose and protein reserves are mobilized to support the energy requirements for high milk production in early lactation. Although, body fat depots are recognized as the major source of energy reserves, the catabolism of both body fat and protein contribute to nutrient requirements in early lactation (NRC, 2001). 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).

Excess CP that degraded to ammonia by ruminal microbes (id est RDP), absorbed into the portal blood and rapidly converted into urea by the liver. Concentrations of Plasma Urea Nitrogen (PUN) above 19 mg dL^{-1} have been associated with lowered pregnancy rates in dairy cows (Butler *et al*., 1996). Supplementing diets with RUP sources has been shown to lower PUN and improve reproductive indices (Butler *et al*., 1996). In addition, milk production increases have been observed in early lactation dairy cows fed TMR supplemented with RUP sources such as fish meal, blood meal, heat-treated soybean meal and corn gluten meal (Khorasani *et al*., 1996; Wheeler *et al*., 1995).

Nitrogen balance probably changes the most during the period from late gestation into the 1st few weeks of lactation. To optimize the amount of absorbable AA for high producing dairy cows, one of the diet formulation objectives is to provide adequate amounts of RUP (Schwab, 1995).

The primary objective of this experiment was to evaluate 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 losses. A second objective was to determine the effect of supplemental RUP on reproductive efficiency.

MATERIALS AND METHODS

Diets and cow management: Cows were assigned to a dietary treatment randomly 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 CGM and fish meal, partially substituted with SBM and barley, during early postpartum period (weeks 1-3). The amount of CGM and fish meal fed was designed to raise ration CP by 1.1-2.2% units. Cows received a similar diet after 3 weeks of lactation to 120 days of lactation and reproductive components were measured. Experimental diets has 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 and 1530.

Table 1: Ingredients and of experimental diets (DM%)

Items	LRUP	MRUP	HRUP
Alfalfa hay	26.300	26.300	26.300
Corn silage	12.600	12.600	12.600
Beet pulp	9.500	9.500	9.500
Barley and steam rolled	13.900	12.300	11.000
Corn grai and ground	9.700	9.700	9.700
Soybean meal	7.700	6.200	4.600
Roasted soybean	3.600	3.600	3.600
Whole cottonseed	6.700	6.700	6.700
Canola meal	0.510	0.510	0.510
Fish meal	2.000	3.600	5.150
Corn gluten meal	2.000	3.600	5.150
Fat	0.510	0.510	0.510
Salt	0.250	0.250	0.250
Sodium bicarbonate	1.000	1.000	0.920
Calcium carbonate	0.610	0.560	0.510
Magnesium oxide	0.150	0.150	0.130
Di-calcium phosphate	0.200	0.150	0.130
Min-Vit supplement[1]	0.825	0.825	0.825
Vitamin A[2]	0.050	0.050	0.050
Vitamin E[3]	0.500	0.500	0.500
Toxin binder	0.070	0.070	0.070
Glycoline[4]	1.290	1.290	1.290
Monensin	0.010	0.010	0.010
Availa 4[5]	0.010	0.010	0.010

[1]Contained 196 g Ca ; 96 g P; 71 g Na; 19 g Mg; 3 g Fe; 0.3 g Cu; 2 g Mn; 3 g Zn: 0.1 g Co: 0.1 g I: 0.001 g Se: 3 g antioxidant; 5000 IU Vit. A: 100000IU Vit. D_3 and 100 mg Vit. E; [2]Contained: 5000000IU Vit. A; [3]Contained: 4400 IU Vit. E; [4]Net energy = 1500 kcal; Ca 1.45%; EE 0.8%; CF 0.3%; 5. Zn not <5.15% ; Mn not <2.88%; Cu not <1.08%; Co not <0.18%

Sample collection: Orts were measured daily and feed offered was adjusted to allow for 5-10% 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 DM Intakes (DMI).

Milk weights were recorded daily throughout the trial. Milk samples were collected from milkings of the 3 sampling days. The Milko-Scan B-133 (Foss, Denmark) was used to determine milk fat, protein, lactose and SNF. Body weight was calculated as the average of measurements performed in the am of days 0, 11 and 21 before morning meal and after am milking. The body condition score of each animal was evaluated by the same person in the am of 0, 11 and 21 after milking.

Blood samples were also collected at days 11 and 21 after parturition from the coccygeal vein or artery of each cow into heparinized vacutainers at 1600 h and immediately cooled to 4°C. Plasma was separated by centrifugation of whole blood for 10 min at 2300×g at 4°C was separated in two 5 mL aliquots and frozen at -20°C until analyzed for urea N and cholesterole (by Pars Aamoon kits) and Progesterone (P4) and essradiole-17β by ELYSA Method using Diaplus kit.

Reproductve management: Estrus was determined by visually monitoring cows for 30 min am and pm and by tail chalking nonestrus cows on or near, predicted estrus. Cows exhibiting estrus prior to day 50 postpartum were not bred.

All cows were bred via artificial insemination from a single ejaculate of a single Holstein sire. Insemination was conducted approximately 12 h after cows were observed in standing estrus. A single inseminator was responsible for breeding all study cows. Reproductive data were recorded for days to first breeding, number of breedings, days nonpregnant, ovarian cysts and embryonic death. Pregnancy was determined by rectal palpation at d 40 after insemination (Table 2).

Table 2: Chemical composition of diets

Items	LRUP	MRUP	HRUP
Nel (Mcal kg^{-1})	1.65	1.67	1.68
CP (%)	17.90	19.00	20.10
RDP (CP%)	11.31	11.28	11.25
RUP (CP%)	6.65	7.72	8.79
Soluble protein (%)	23.00	22.30	21.40
Metabolizable protein (g day^{-1})	1893.00	2023.00	2149.00
Met (g day^{-1})	39.00	43.00	46.00
Lys (g day^{-1})	121.00	128.00	135.00
NDF (%)	33.20	32.70	32.20
PeNDF (%)	24.00	23.00	23.00
NFC (%)	36.50	35.70	35.00
EE (%)	4.70	4.90	5.10

Statistical analyses: The completely randomized block design were used. Data measured over time (DMI, milk yield and components and blood samples) within the period of interest were subjected to ANOVA by using the REPEATED statement MIXED procedure of SAS Institute (2003). BW and reproductive data (days to first breeding and number of breeding) were analyzed using GLM procedure of SA, Categorical variable (first service conception rate) was analysed by CATMOD 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.

RESULTS AND DISCUSSION

Dry matter intake: Least square means of DMI during experimental period for LRUP, MRUP and HRUP were 14.15, 14.40 and 15.04 kg day^{-1}, 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 has led to immunosuppression (Sejrsen *et al*., 2006; Le Floc'h *et al*., 2004) and incidence of metabolic disorders consisted of rapid loss of BCS, ketosis, fatty liver and displaced abomasum (Duffield *et al*., 2009; Drackley *et al*., 2001). Thus, diets that have higher levels of crude protein and RUP are effective in maintaining of production and BCS (NRC, 2001). Fresh cows in 1st 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 (NAN), Non Ammonia non Microbial Nitrogen (NANMN) and follow that entering of Essential Amino Acids (EAA) into small intestine (Ipharraguerre and Clark, 2005). Therefore, ratio of RUP supplements (corn gluten meal and fish meal) would be increased. The findings were in agreement with

Table 3: Least square means±SE of DMI, BW and BCS changes

				p-value		
Items	LRUP	MRUP	HRUP	Treatment	Block	Treat x Block
DMI (kg day^{-1})	14.15±0.170	14.40 ±0.17	15.04±0.170	0.0542	0.0002	0.6180
BW changes (kg day^{-1})	-53.89±10.29	-24.85±9.910	-37.12±11.62	0.1368	0.5335	0.1191
BCS changes	-0.76±0.060^b	-0.36±0.060^a	-0.43±0.070^a	0.0001	0.1215	0.1157
Initial BW (kg day^{-1})	668.51±11.94	653.25±11.50	662.65±13.49	0.6516	0.0001	0.9177
Initial BCS	3.30±0.070	3.30±0.070	3.31±0.080	0.9973	0.0239	0.9921

Law *et al*. (2009), Broderick and Radloff (2004) that 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).

BCS and body weight: Least square means of BW changes were -53.89, -24.85 and -37.12 kg day^{-1} for LRUP, MRUP and HRUP, respectively (Table 3). They did not have any significant differences between treatments but parity effect had significant difference. 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 and therefore, BCS might be used as indicator of energy balance during early lactation (De Vries and Veerkamp, 2000). Van Knegsel *et al*. (2007) suggest 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 the 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 in 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 role 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; Lal and Chugh, 1995). Leucine directly stimulate mRNA level of insulin in pancreas cells (Docherty and Clark, 1994). 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 the findings.

Milk production and composition: Least square means of whole milk production and FCM 4% were 35.42, 35.81, 38.54 kg day^{-1} and 29.89, 31.24, 33.0 kg day^{-1}, respectively (Table 4). Increasing level of RUP are accompanied by enhancing MP and supply of EAA to small intestine (Flis and Wattiaux, 2005). In a research by Schwab and Foster (2009) at Cornell University, they suggested that limiting factor for milk production in 1st week 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 16-21% however, this CP enhancement using RDP had less benefit.

Flis and Wattiaux (2005) indicated that diets contained over 10% CP than NRC recommendation had 1.5 kg more milk per day, this increase was due to RUP enhancement. In agreement with the findings Broderick (2003) reported 2.8 kg day^{-1} and Cunningham *et al*. (1996) reported 2.7 kg day^{-1} more milk production.

Heated SBM compare with row SBM, increase milk yield (Armentano *et al*., 1997), this response was due to high passage of ruminally undegradable protein to small intestine. This idea supported with Grummer *et al*. (1996) indicating higher milk production when animal by products added to diets contained SBM whereas RDP was fixed and RUP was increased. Diets in early lactation had high amounts of CP (17-19%) increased both milk yield and milk persistency (Armantano *et al*., 1997).

Least square means of milk fat content and yield were 3.01, 3.22, 3.17% and 1.048, 1.12, 1.17 kg day^{-1}, respectively (Table 4). There were no differences in the percentage and amount of fat in the milk between treatments.

Least square means of milk protein content and yield were 3.41, 3.53, 3.53% and 1.20, 1.26, 1.36 kg day^{-1} (Table 4). Milk protein significantly increased with

Table 4: Least square means±SE of milk yield and composition

				p-value		
Items	LRUP	MRUP	HRUP	Treatment	Block	Period
Milk yield (kg day^{-1})	35.42±0.920^{b}	35.81±0.85^{ab}	38.54±0.89^{a}	0.0280	0.0001	0.0001
FCM 4% (kg day^{-1})[1]	29.89±0.900^{b}	31.24±0.83^{ab}	33.0±0.870^{a}	0.0477	0.0001	0.0001
FCM 3.5 (kg day^{-1})[2]	32.20±0.980^{b}	33.68±0.91^{ab}	35.57±0.95^{a}	0.0477	0.0001	0.0001
Milk fat (%)	3.01±0.140	3.22±0.13	3.17±0.14	0.5464	0.0011	0.0001
Milk fat (kg day^{-1})	1.048±0.04	1.12±0.04	1.17±0.04	0.1995	0.0241	0.0001
Milk protein (%)	3.41±0.020^{b}	3.53±0.02^{a}	3.53±0.02^{a}	0.0008	0.0841	0.0001
Milk protein (kg day^{-1})	1.20±0.030^{b}	1.26±0.03^{ab}	1.36±0.03^{a}	0.0072	0.0001	0.0001
Milk lactose (%)	5.18±0.030	5.18±0.03	5.09±0.03	0.1282	0.4262	0.0001
Milk lactose (kg day^{-1})	1.83±0.040	1.85±0.04	1.95±0.04	0.0913	0.0001	0.0001
Milk SNF (%)	9.29±0.060	9.41±0.06	9.39±0.06	0.1172	0.4430	0.0001
Milk SNF (kg day^{-1})	3.29±0.080	3.36±0.07	3.61±0.07	0.0901	0.0001	0.0001
ECM (kg day^{-1})[3]	31.95±0.850^{b}	33.47±0.82^{ab}	35.35±0.83^{a}	0.0242	0.0001	0.0001
Milk Energy (kg day^{-1})[4]	0.67±0.010	0.69±0.01	0.68±0.01	0.3898	0.0001	0.0001
Mean Milk yield of 120 DIM (kg day^{-1})	42.94±1.470	41.99±1.36	43.97±1.42	0.8435	0.0001	0.0001

[1]FCM 4% = 0.4× milk (kg) +15× milk fat (kg); [2]FCM 3.5% = 0.4324× milk (kg) +16.216× milk fat (kg); [3]ECM = Milk (kg) × [383× f at (%) +242× protein (%) +165.4× lactose (%) +20.7]/3140; [4]Milk energy (Mcal kg^{-1}) = 0.0929× fat (%) +0.0547× protein (%) +0.0395× lactose (%) (NRC, 2001)

Table 5: Least square means±SE of reproduction and selected plasma constituents

				p-value		
Items	LRUP	MRUP	HRUP	Treat	Block	Treat x Block
Days to first estus	48.55±5.86	40.15±5.65	29.63±6.62	0.1100	0.610	0.6900
Breedings/Cow (all cows)	2.72±0.3^{a}	2.37±0.29^{a}	1.76±0.34^{b}	0.0100	0.560	0.0050
Open days	121.76±10.21^{a}	123.03±9.83^{a}	90.38±11.53^{b}	0.0400	0.560	0.0800
Days to first breeding	69.60±6.46	60.53±6.23	57.50±7.30	0.5900	0.330	0.9500
First breeding conception rate (%)	24.64^{b}	41.0^{a}	50.1^{a}	0.0300	0.670	0.2300
PUN (mg dL^{-1})	16.30±0.56	17.17±0.59	17.35±0.57	0.3900	0.006	0.6700
Cholesterole (mg dL^{-1})	78.30±3.9^{b}	106.73±4.07^{a}	110.14±3.9^{a}	0.0300	0.140	0.0100
Plasma progesterone at day 11 (ng mL^{-1})	0.96±0.27^{b}	2.06±0.29^{a}	1.90±0.29^{a}	0.0200	0.330	0.4600
Plasma progesterone at day 21 (ng mL^{-1})	1.02±0.68^{c}	2.18^{b}±0.72	3.42±0.77^{a}	0.0001	0.002	0.0400
Plasma estradiole at day 11 (p mL^{-1})	54.46±4.17	64.21±4.41	59.12±4.66	0.2900	0.005	0.0040
Plasma estradiole at day 21 (p mL^{-1})	45.33±4.47	51.60±4.73	57.54±5.0	0.2000	0.420	0.0006

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; Van Amburgh *et al*., 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 the findings, Broderick (2003) found that milk protein yield was improved by enhancing dietary CP from 15.3-16.7% but he did not any changes with 18.4% of CP.

Reproductive traits: Data of reproductive outcomes and plasma metabolites are shown in Table 5. Number of breeding per cow, open days and first breeding Conception Rates (CR) had significantly increased with concuming HRUP diet ($p<0.05$). Also, plasma progesterone increased significantly ($p<0.05$) between days 11 and 21 after parturition.

Several factors may contribute to extended days nonpregnant in cattle; the first limiting factor is the rapidity of return to normal ovarian activity following parturition and associated factors such as ova and Corpus Luteum (CL) development. Days to first breeding, a reproductive measure that is a function of the rate of return to normal postpartum estrous activity were 12.1 days longer ($p<0.05$) for HRUP-fed cows than for LRUP cows (Table 5). A delay in post-parturition to first CL activity among cows receiving excess RDP has been reported by NRC (2001). The interval from parturition to conception in cattle is also affected by the efficiency of estrus detection. These findings are in concert with those of Carroll *et al*. (1994) who noted similar reproductive responses in dairy cows fed 21% CP diets in which RUP as a percent of CP was increased from 34-40% by substituting fishmeal for soybean meal and increasing RUP from 27-36% in diets containing 16% CP had a positive impact on reproductive indices of super-ovulated dairy cows, increasing transferable and fertilized ova (Blanchard *et al*., 1990). Plasma Urea N (PUN) concentrations were not statistically affected by diet. Concentrations of Plasma Urea Nitrogen (PUN) above 19 mg dL^{-1} have been associated with lowered pregnancy rates in dairy cows (Butler *et al*. 1996). Supplementing diets with RUP sources has been shown to lower PUN and improve reproductive indices (Butler *et al*., 1996).

Increased concentrations of plasma progesterone have been associated with improved conception rates of lactating ruminants (Staples *et al*., 1998).

Plasma cholesterol concentrations increased significantly ($p<0.05$) by treatments. Plasma cholesterol concentrations increase between calving and weeks 6 postpartum in dairy cows (Spicer *et al*., 1993; Francisco *et al*., 2002) and are correlated with plasma P4 (Francisco *et al*., 2002), CR and number of recoverable embryos (Grummer and Carroll, 1988). The association of increased plasma cholesterol concentration with increased luteal-phase P4 secretion in early lactating dairy cows (Spicer *et al*., 1993) merits further investigation. Understanding what production and hormonal factors contribute to variation in plasma cholesterol may lead to insights that may help improve reproductive efficiency in dairy cattle. Early establishment of pregnancy (initial conception) was defined by an elevated progesterone concentration that persisted beyond day 24th after insemination.

Cows losing one unit or more BCS (five-point scale) during early lactation are at greatest risk for low fertility with conception rates of 17-38% reported in the various studies. Guidelines from recent studies indicated that cows with marked losses in BCS (1.25 unit) were only half as likely to conceive at first AI as cows with more modest loss (Gillund *et al*., 2001) and that conception rate increases 10% for every unit increase in BCS (Stevenson *et al*., 1999).

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 detrimental effects on reproduction, feeding high RUP indicate that did not increase PUN significantly and we suggest that high needs for MP in this period (0-3 weeks of lactation) have best benefits and after 21 DIM, CP concentration of diet could be lowered due to diminishing environmental and conception problems. However, reproductive traits include open days and number of breeding per conception were improved and plasma P4 and cholesterol increased.

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