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Influence of Protein Sources with Different Degradability on Performance, Ruminal Fermentation, Blood Metabolites and Protozoal Population in Lactating Dairy Cows

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Abstract: An experiment was carried out to evaluate protein degradability on animal performance, ruminal and blood metabolites and ruminal ecosystem. Eight multiparous Holstein cows were used in a change over design in four period. Supplemental protein which provided via Soybean Mal (SBM), Meat Meal (MM), urea were added to basal diet based on the providing same CP protein. Treatments were provided via Soybean Mal (SBM), Meat Meal (MM), urea and with flowing rumen degradability (T_1: 74.5, T_2: 70.4, T_3: 68.7 and T_4: 63.7%). Each experimental period was conducted for 21 days (14 days for adaptation and 7 days for sampling). There were no significant difference between treatments for DMI. However, trend indicates that by reduction of RDP in diet milk production decreases. No difference were observed for milk composition, body weight, pH and blood metabolites. Ruminal ammonia nitrogen was numerically higher for does fed T_1 (9.25 vs 7.81, 6.94 and 7.98) and this trend was observed for blood urea nitrogen as well. Protozoa number was higher in does fed SBM with Urea.

Key words: Degradability, meat meal, protozoa, soybean meal, urea, ruminal ammonia

INTRODUCTION

Increasing the CP content of dairy cow diets may result not only in greater milk yeild (Armentano *et al*., 1993; Wu and Satter, 2000) but also in increased concentrations of ruminal ammonia and blood urea N and consequently greater urinary N losses (Armentano *et al*., 1993; Christensen *et al*., 1993; Castillo *et al*., 2001). Although, as exemplified by Tamminga (1992), ruminal N loss is the greatest single contributor to urinary N losses, metabolic losses, indigestible microbial N, losses in maintenance and inefficient conversion of absorbed AA into milk protein may comprise up to 72% of the urinary N losses in the dairy cow.

With few exceptions in studies investigating effects of CP level, diets supplied different amounts of Metabolizable (MP), Ruminally Degradable (RDP) or Ruminally Undegradable Protein (RUP). Thus, the individual contributions of RDP, RUP or MP to urinary or overall N losses cannot be readily distinguished. Dietary RDP can be used for Microbial Protein Synthesis (MPS) if enough energy provided.

However, If RDP not used for MPS, it can converted to ammonia and subsequently absorbed through the ruminal wall, detoxified to urea in the liver (Lobley *et al*., 1995) most of unused urea will lost via urine; some RDP may bypass the rumen and contribute to the duodenal AA and peptide flow (Choi *et al*., 2002). Therefore, the efficiency of RDP use in the rumen is a central factor determining the economic cost and environmental impact of ruminant production.

It is hypothesized that if provided energy is not limiting in the rumen, excess ammonia from feed RDP will enhance MPS and its use for milk protein synthesis by the dairy cow.

Thus, the objectives of this study were to investigate the effect of dietary RDP and RUP level in diets with similar and presumably adequate CP concentrations on ruminal fermentation, protozoal population, blood matabolites, milk production and performance in lactating dairy cows.

MATERIALS AND METHODS

Eight ruminally multiparous Holstein (656±15 kg) were used in a change over design. Four isonitrogenous and isocaloric diets with different degradability were formulated based on NRC recommendation (NRC, 2001).

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Table 1: Diets composition

Item	SBM + Urea	SBM	SBM + MBM	MBM + Urea
Corn silage	22.160	22.04	22.16	22.39
Ground barley	28.810	28.65	28.81	29.11
Alfalfa	22.160	24.24	22.16	22.39
Cottonseed meal	4.430	5.29	4.43	4.48
Soybean meal	10.200	5.73	5.76	-
Meat and bone meal	-	-	4.43	8.73
Wheat barn	10.200	11.02	10.20	10.30
Urea	-	1.01	-	0.54
Vitamin permix	1.020	1.01	1.02	1.03
$Caco_3$	1.020	1.01	1.02	1.03
Analyzed dietary nutrient content (DM CP, %) calculated with NRC 2001	19.300	20.80	19.50	20.60
CP (%), analyzed	19.100	20.27	19.20	20.30
RDP[1], CP (%)	74.500	70.40	68.70	63.70
RUP[2], CP (%)	25.500	29.60	31.30	36.30
NEL (Mcal kg^{-1} DM)	1.560	1.56	1.56	1.56
NDF	52.300	47.70	45.75	53.25
ADF	30.950	29.15	25.10	31.10
Ash	7.250	5.65	9.40	9.10

[1]RDP = Rumen Degradable Protein, [2]RUP = Rumen Undegradable Protein

The basal diet contained 22.16% Corn silage, 22.16% Alfalfa, 22.81% ground barley, 10/20% wheat barn, 4/43% Cottonseed meal and 2% Minerals and Vitamins. Treatments were SBM with Urea (74.5% RDP-25.4% RUP% CP), SBM (70.4% RDP-29.6% RUP% CP), SBM with MBM (68.7% RDP-31.3% RUP) and MBM with Urea (63.7% RDP-36.3% RUP% CP).

The fertilizer grade urea was used to attain this desired RDP and RUP (Table 1). The cows were individually housed on concrete floor in separate pens and fed twice a day (morning at 7.00 am and evening at 7.00 pm). Fresh water was provided *ad libitum*. The cows were fed for four periods and each period was of 3 weeks. The first 2 weeks of each period served as adaptation time while the 3rd week used for collection.

Ruminal samples were taken via stomach tubes from the rumen at 3 h after morning feeding for determination of pH, NH_3-N, protozoal population. The first 20-30 mL of rumen fluid were discarded to prevent salivary contamination. pH was measured immediately after sampling.

The samples thereafter was squeezed through 4 layers of cheesecloth and about 20 mL of the liquid was acidified with 20 mL of 0.2 N HCl to terminate the fermentation and was frozen at -20°C. Ruminal NH_3-N was steam distilled using kjeldahl equipment and titrated against sulphuric acid (Giri *et al*., 2005).

Rumen liquor was collected in sterilized plastic bottles at 3 h post feeding for the determination of microbial count. The protozoal counts were determined at 3 h post feeding. About 1 mL of rumen liquor was added with 9 mL of MFS solution (100 mL 35% formaldehyde solution, 900 mL distilled water, 0.6 g Methyl green and 8.0 g Sodium chloride (Lab grade)] to fix and staining the nuclei of the protozoa. From final dilution, 0.1 mL fluid was transferred to slide which was covered with cover slip of 24×50 cm^2. The counts were made from thirty microscopic fields and calculations were made according to following equation.

$$\text{Protozoa/mL} = \left(\frac{\text{Total number counted}}{\text{Number of blocks counted)}}\right)\times \frac{}{\text{dilution (1m in 1 m)}\times(5\times10^5)}$$

Body weight were measured on 3 consecutive days at the start of the trial and at the end of each period to compute BW change. Milk yield was recorded for all daily am and pm milking times. Milk samples were taken at 2 consecutive milking days (am and pm) on day 19 and 20 each period. Samples for milk composition were prepared from each cow in each sampling day by proportion of milk yield for ach time. Composite samples were analyzed for fat, true protein, lactose, TS and SNF. Blood samples were collected from the jugular vein of each animal at 3 h post feeding. Blood samples were also collected via each cow without anticoagulant to harvest blood serum which were stored in aliquots at -20°C awaiting analysis for biochemical parameters. All biochemical parameters was determined by using the analytical kits. Feed offered and samples were analyzed for Dry Matter (DM), Organic Matter (OM), Nitrogen (N) content (AOAC, 1990) and Neutral Detergent Fiber and Acid Detergent Fiber (NDF and ADF; Van Soest *et al*., 1991).

Statistical analysis: The data collected on ruminal pH, ruminal ammonia N, bacterial count, protozoal count, digestibility, blood pH, BUN and N balance was subjcted to ANOVA using general linear model procedure of SAS (1999). The means were compared by Duncan's Multiple Range Test. The samples taken from each cow in each period were considered change over and therefore, correlated on time and were analyzed together using the following model:

$$Y_{ijkL} = \mu + S_{K+}R_{ik} + C_{jk} + T_L + (T_L \times S_K) + e_{ijLk}$$

Where:

Y_{ijkl} = Dependent variable
μ = Overall mean
S_K = Effect of group k
R_{ik} = Effect of cow I in group k
C_{jk} = Effect of period j in group k
T_L = Effect of treatment l
$(T_L \times S_K)$ = Interaction between treatment l and group k
e_{ijLk} = Residual error

RESULTS AND DISCUSSION

Dry matter consumption and body weight: Treatment had not significant effect on amount of daily dry matter consumption ($p>0.05$), data were shown in Table 2. However, highest amount of Dry Matter Consumption (DMC) was attributed to SBM+urea and the lowest was observed in SBM. This data indicated that with adding RUP level in ration and making less RDP level by using of meat meal from 5.3-6.8%, amount of DMC is decreased too.

Period had significant effect on amount of DMC ($p<0.0001$). It was observed that DMC has had upward trend during first period to forth period. Treatments didn't affect on body weight changes ($p>0.05$). Highest amount of BWC was observed in treatment of SBM and lowest amount of BW was related to SBM+Urea (639.22 kg). Studies has established that different levels of RUP and CP did not significant effects on BWC and DMC (Davidson *et al*., 2003).

It is worthy of mention that treatments were regulated with usage of MBM and SBM. Reynal and Broderick (2005) decreased amount of ration's RDP (from 13.2-10.6%) and they didn't observed significant differences among treatments of course with a view to DMC and BWC. Knaus *et al.* (1998) increased ration's UIP (0-7.8%) by means of SBM and composition of MBM, Fish Meal (FM), feather meal and Blood Meal (BM), According to their findings no significant effect was observed in amount of DMC and changes of BW of cows. Similarly Lu *et al.* (1990) didn't observe differences among treatments of MBM, SBM and MBM+Urea with a view to amount of DMC and changes of BW.

Reed *et al.* (2007) increased ration's UIP (8-40.6%) and noticed that basic treatment's (2.5%) Low, (8%) average (19.6%) and high (40.6%) didn't has significant effect on DMC and BWC. In another trial Dyer and Fletcher (1958) carried out an experiment in different proportions of MBM and SBM (MBM 6.75%-SBM 0%), (MBM 4.36%-SBM 2.85%), (MBM 2.18%-SBM 5.72%) and (MBM 0%-SBM 8.58%) and found out that there weren't significant differences among treatments with view to changes of BW and DMC.

This data is same with results were found by (Grummer and Luck, 1994) by using SBM, roasted SBM and SBM+animal by product meal which had different RDP. He and collaborators reported that there weren't significant differences in view to BW and amount of DMC among treatments of SBM with 16% CP, MBM with 18% CP, fish meal 16% CP and SBM with 18% CP. Similarly highest amount of DMC and changes of BW were related to treatment of SBM. The deduction of DMC in rations of MBM+Urea and MBM+SBM observed in the experiment maybe due to bad smell of MBM and not eating well. Urea also can cause deduction of consumption of DM (Chalupa, 1968). In present experiment, Urea was used 1.01 and 0.54% and in regarded to Shine *et al.* (1995) making supplemental urea to level 1.5 and 2% didn't has influence on amount of DMC. Linear increase and significant differences among periods can be result of improvement of enviromental conditions and better adaptation. This experiment began middle of summer and ended middle of autumn, decreasing heat stress can be one of reasons which DMC went up from first period to forth period.

Table 2: Influence of supplemental protein sources with different degradability on intake and BW

	Treatments					
Item	SBM+ Urea	SBM	SBM+ MBM	MBM+ Urea	SEM	p-value
Dry matter intake (kg day^{-1})	23.24	23.20	23.01	22.70	0.27	0.36
BW change (kg day^{-1})	0.51	0.58	0.43	0.52	0.13	0.85

Means in the same row without a common superscript differ ($p<0.05$)

Table 3: Influence of supplemental protein sources with different degradability on N-NH_3 and pH in rumen

	Treatments					
Item	SBM+ Urea	SBM	SBM+ MBM	MBM+ Urea	SEM	p-value
Ammonia (mg L^{-1})	9.25	7.81	6.94	7.98	0.94	0.71
Ruminal pH	6.78	6.72	6.74	6.62	0.05	0.42

Means in the same row without a common superscript differ ($p<0.05$)

Ammonia nitrogen and ruminal pH: The degradation of nitrogen fro different source of protein and ruminal pH were shown in Table 3. Analysed data indicated the ruminal N-NH_3 did not differ among treatments ($p>0.05$). However, the highest density of N-NH_3 was attributed to treatment of SBM+Urea and lowest density of N-NH_3 observed in treatment of SBM+MBM. Ruminal pH didn't affect largely by treatments ($p>0.05$) (Table 3). As shown in Table 3 with increasing RUP in ration, pH was decreased.

Reed *et al.* (2007) have raised the degree of ration's UIP from 8-40.6% and noticed that there wasn't significant differences among treatments, out of consideration for density of N-NH_3. They expected such a result because they equalized the degree of consumption crude protein. They did the best to equalize amount of CP among treatments, so the results show the fact that it isn't necessary to be the existence of significant diversity among treatments of course in regard to density of N-NH_3. This issue is in conformity with findings of present experiment. Lu *et al.* (1990) reported that There won't be significant differences among treatments of SBM, MBM+Urea and MBM which including 43.1, 40.8 and 36.2% RDP, respectively. Level of ration's crude protein was considered equal by them and its amount was 15%. These conclusions were in conformity with the results of present experiment and show deduction of ration's RDP doesn't affect density of N-NH_3 of rumen. It can be

considered that upper density of N-NH_3 in rations of SBM+Urea and MM+Urea was due to urea degradation in rumen by urease. Davidson *et al.* (2003) compared the degree of crude protein and RUP and stated important differences among treatments in regarded to density of N-NH_3. The highest density belonged to dose fed treatment which including 19.4% CP. Reynal and Broderick (2005) reportec that when degradability of treatments decreased 13.2-10.6% RDP; therefore, density of N-NH_3 will go down linearly ($p<0.001$). Bohnert *et al.* (1999) explained when MBM gradually increased from 25 and 75%, amount of N-NH_3 in rumen will fall linearly ($p<001$).

This experiment contains six treatment MBM 0 and urea 100%, MBM 25 and urea 75%, MBM 50 and urea 50%, MBM 75 and urea 25%, MBM 100% and soybean 100%. It reported that with incresing RUP, density of N-NH_3 in rumen will decreased. These results illustrate reducing of digestible protein is as a result of reduction of RDP or increase RUP of allowance reduce of DIP cause protein to drop for digestion and microbial degradation and it has reversed influence to drop for digestion and microbial decomposition and it has reversed influence on ammonia's density in rumen. The lowest density of N-NH_3 in rumen is linked to treatment of SBM+MM. Sufficiently this density of N-NH_3 is upper than certain level for maximum of microbial growth *in vivo* (2.94 MM; Satter and Slyter, 1974) *in vitro* and (1.18-2.94 MM; Slyter *et al*., 1971).

There for rumen available nitrogen didn't has to affect amount of microbial growth in this experiment. Reed *et al.* (2007) increased the degree of UIP from 8-40.6% but they did not observed significant differences among treatments in regard to pH. Similarly, Lu *et al.* (1990) didn't report important diversity among SBM, MBM and Urea+MBM. Reynal and Broderick (2005) noticed that treatment's didn't change pH in the rumen. This results were confirm wih data presented with other researchers (Koster *et al*., 1996; Heldt *et al*., 1999; Mathis *et al*., 2000). Changes in pH is depended changes in ruminal fermentation and in reference to that point treatments take amount of crude protein and similarly nitrogen in rumen and ruminal fermentation and microbial growth were available, It isn't clear why percentage RUP increase so amount of pH will reduce.

Blood metabolites: There was no significant differences among glucose, Blood Urea Nitrogen (BUN) and total protein between treatments ($p>0.05$) (Table 4). However, the highest and lowest amount of BUN was attributed to treatments SBM+Urea and MBM+Urea and SBM+Urea respectively. Cattle fed diets containing MM+Urea contains the highest amount of protein in plasma compare to group whom received SBM which had lowest protein in plasma.

Table 4: Influence of supplemental protein sources with different degradability on blood metabolites

	Treatments					
Item	SBM+ Urea	SBM	SBM+ MBM	MBM+ Urea	SEM	p-value
Glucose (mg dL^{-1})	60.10	58.10	57.10	59.80	3.60	0.310
BUN (mgL^{-1})	20.25	19.37	18.00	18.00	0.94	0.690
Total protein (mgL^{-1})	7.96	7.70	7.92	8.08	0.13	0.290

Means in the same row without a common superscript differ ($p<0.05$)

Reed *et al.* (2007) increased amount of UIP in ration from 8-40.6%, they in regarded to density of glucose finded out significant differences among treatment of SBM, MBM+Urea and MBM. They decreased amount of DIP from 43.1-36.2% and in regard to density of plasma glucose and wasn't observed differences. Knaus *et al.* (1998) raised UIP (from 0-7.8%) and noticed that there weren't significant differences among treatments. These results falls on findings of present experiment and confirm them. Density of blood urea nitrogen is dependent on amount of crude protein within ration. Considering that Reed *et al.* (2007) increased amount of ration's UIP and crude protein and observed density of BUN increased linearly (from 6.53-14.42%). Many researchers have reported similar result (Wiley *et al*., 1991). Davidson *et al.* (2003) observed significant differences among treatments most density of BUN is related to treatment which including the highest level of CP. When content of crude protein decreased, Reynal and Broderick (2005) noticed linear decrease in density of BUN. Knaus *et al.* (1998) stated that with increasing ration, density of BUN had an upward trend. On the basis of these results and in regard to this point which amount of CP of treatments of present experiment is considered similarly. It is expected to have such conclusion on the other hand, it isn't likely to not observe significant differences among treatments. These results confirmed higher density of BUM in treatment of SBM+Urea is more than higher density observed N-NH_3 in rumen (Lu *et al*., 1990). Respecting total protein, no significant differences weren't observed among treatments (Table 4) ($p>0.05$). Lu *et al.* (1990) reduced RIP from 43-36% and didn't observed significant differences among treatments of SBM, MBM+Urea and MBM.

Milk production and its compositions: Total amount of milk production, protein, fat, lactose and total solid and SNF had no significant differences among treatments ($p>0.05$) (Table 5). Animal which received SBM+MBM had highest milk production, protein, lactose and SNF and animal fed SBM+Urea had highest total solid and fat in their milk. As it is shown in Table 5, the lowest level of milk production, fat, protein, lactose and SNF was observed in treatment of MBM+Urea. Period had significant effect on milk composition factors. Effect of period on fat, protein and lactose was significant ($p<0.001$). Fat shows an upward trend from first period to forth period. Amount of lactose and SNF imply a

Table 5: Influence of supplemental protein sources with different degradability on milk production and milk composition

	Treatments					
Item	SBM+ Urea	SBM	SBM+ MBM	MBM+ Urea	SEM	p-value
Milk production	28.96	28.98	29.18	28.85	0.58	0.51
Fat	3.73	3.45	3.42	3.31	0.14	0.25
Protein	2.61	2.67	2.70	2.61	0.02	0.19
Lactose	3.73	3.74	3.76	3.61	0.04	0.12
TS	11.47	11.18	11.18	11.09	0.16	0.41
SNF	7.35	7.42	7.50	7.32	0.05	0.17

Means in the same row without a common superscript differ ($p<0.05$)

downward trend. Both amount of total solid materials protein don't follow certain trend. Lu *et al.* (1990) stated that by decreasing RIP in ration, milk production, FCM, protein, fat, lactose, total solid and SNF don't change. In this experiment usage of MBM cause to in crease milk production normally but there weren't significant differences among treatments. Davidson *et al.* (2003) reported there is no difference between treatments of course with regard to milk production, fat, FCM, TS and SNF. These researchers came to conclusion treatments with low level of crude protein doesn't decrease amount of milk production, fat and protein. Similarly, Reynal and Broderick (2005) reported when they reduced amount of ration's RDP (13.2-0.6%), this reduction had no effect on milk production, fat, protein, TS and SNF. Grummer and Luck (1994) stated with increasing ration's RUP (from 32.2-36.2% CP), there weren't significant differences among treatments.

In the present study among three treatments (SBM, SBM+MM and roasted SBM) milk production in treatment of SBM+MM was higher than other treatments numerically. These researchers declared that treatments didn't has any significant effect on protein, fat, TS and SNF. Mansfield *et al.* (1990) and Sandrucci *et al.* (1992) reported similar conclusions and admit supplementing MBM doesn't affect milk production and its composition. As it observed in this experiment, the highest milk production is related to treatment of SBM+MM and this point is similar to Grummer and Luck (1994) and Lu *et al.* (1990) results.

They regulated rations according to amount of crude intake protein and crude intake protein was equal in all treatments. In the other hand, ration was isonitrogenous, when content of RUP go up, So amount of content RDP decreased among four treatments, we had the highest production level in treatment of SBM+MM and was related to providing amino acid for absorbing or related to better utilization of nitrogen or reducing requirements of energy for detoxification ammonia yet, It isn't clear.

Protozoal count: Protozoal count in cows fed SBM+Urea diet was higher ($p<0.001$) than those fed SBM, SBM+MBM and MBM+Urea diets at 3 h post feeding (Table 6). However, there was no difference between

Table 6: Influence of supplemental protein sources with different degradability on protozoal count

	Treatments					
Item	SBM+ Urea	SBM	SBM+ MBM	MBM+ Urea	SEM	p-value
Protozoal count	1206.25^{a}	705.50^{b}	696.50^{b}	689.13^{b}	0.2	0.0003

Means in the same row without a common superscript differ ($p<0.05$)

SBM, SBM+MBM and MBM+Urea diets. The protozoal count in cows fed SBM+Urea diet remained highest across all time period whereas it was lowest in cows fed MBM+Urea diet. A linear decrease ($p<0.001$) in protozoal count was observed when dietary RUP was increased from 25.5-36.3% at 3 h post feeding. The increased protozoal count in cows fed SBM+Urea diet was due to higher level of dietary RDP than those fed MBM+urea. Hoover and Stokes (1991) reported that increased microbial growth with increased dietary RDP level in *in vitro*. Meng *et al.* (2000) reported that protozoal count was lower (0.4×10^{3} cell mL^{-1}) when total RDP was supplied from urea compared with when urea base RDP was replaced with 30 or 70% soybean meal (3.0×10^{3} or 4.8×10^{3} cell mL^{-1}) in a continuous culture fermenters. Lower protozoal count in cows fed MBM+Urea diet than those fed SBM+MBM, SBM and SBM+Urea diets was due to lower level of dietary RDP in MBM+Urea diet.

CONCLUSION

The current experiment show that milk production and it composition was not varies with different RDP in rumen. In all treatments blood metabolites were not affected by treatments. However, ruminal protozoa was attend with RDP in rumen.

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