# Effects of rumen protein availability on transition ewe's performance

Tayyebeh Ajam Rangraz and Asadollah Teimouri Yansari

Department of Animal Science, Animal Science and Aquaculture faculty, Agricultural and Natural Resource University, Sari, Mazandaran, Iran. astymori@yahoo.com

**Abstract:** Two experiments carried out to determine the effects of rumen protein availability on ewe's performance at transition period. The first trial were performed using the *in sacco* method with two fistulated Zel ewes to determine degradability of untreated (UCM) and formaldehyde treated canola meal (FTCM; 4 ml/ 100 g CM). In second experiment, sixteen 3-years old pregnant Zel ewes (133 ± 4day in pregnancy) were fed ad libitum two rations that consisted of UCM and FTCM with same composition that offered as a TMR twice daily at 0900 and 2100 h. Formaldehyde treatment decreased a, b, potential degradable fractions and effective degradability for dry matter (DM) and crud protein (CP), with no effect on neutral detergent fiber (NDF) degradation. The FTCM increased body weight before lambing and reduced body weight loss after parturition. There were no differences between DM intake (except on 2 weeks after lambing). Control treatment had greater digestibility for CP, NDF and ash than FTCM treatment. The body weight of lamb at birth, 7, 14 and 21 day after lambing and lamb's daily gain were higher in treatment than control. Milk production, fat (% and kg/day), protein (kg/day) and total solid (%) content of milk increased in sheep that fed ration contained the FTCM. However, using FTCM enhanced milk production (13.2 % based on control), therefore, can be beneficial to high-producing dairy ewes.
[Tayyebeh Ajam Rangraz and Asadollah Teimouri Yansari. Effects of rumen protein availability on transition ewe's performance. Journal of American Science 2011;7(10):276-283]. (ISSN: 1545-1003). http://www.americanscience.org.

**Key words:** transition ewe; formaldehyde treat; degradability; protein; milk yield

## 1. Introduction

The last six weeks of gestation is the most critical period in ewe because approximately 70 % of the fetal growth occurs at this time. Nutrient restrictions during this period may result in lighter lambs at birth, increased postnatal lamb losses, lower levels of milk production, and possibly pregnancy disease (ketosis). In addition, if protein is limited during late gestation, lower birth rates and lighter ewe fleece weights can be expected. The protein nutrition during late pregnancy influences fetal growth, postpartum health and lactation performance in ewes but the responses vary greatly according to the type and level of protein supplements. Substituting the ruminal undegradable protein sources in rations often has been used to increase microbial protein flow and dietary protein truly digested to the small intestine (Paul *et al*., 1998). Studies on the use of low degradable protein supplements, protected proteins or protected amino acids in milk production of sheep are very limited. However, most of the references were made using suckling ewes, altering the practical significance of data of milk composition in consequence. In addition, in some cases the results are not significant or contradictory. Milk composition was, however unchanged in most cases (Robinson *et al.* 1979; Hadjipanayiotou, 1992, 1995) and only significantly improved in the trials of Penning *et al.* (1988) and Purroy and Jaime (1995), when comparing soybean and fishmeal in suckling ewes. These last authors reported significant increased in milk protein but not in milk yield.

Canola meal (**CM**) is one of important protein source in ruminant's diet. Despite an excellent AA profile, CM is a poor source of metabolizable AA because it is extensively degraded in the rumen (Kendall et al., 1991; Cheng *et al.*, 1993), therefore various physical and chemical treatments have been used to decrease its extent of ruminal degradation (Khorasani *et al.*, 1993 and 1996). The FTCM supplement is one of the recommended procedures in practice. In this sense, comparison of the use of soybean, fishmeal and formaldehyde protected soybean in Chios dairy ewes was without significant effects on milk yield and milk composition (Hadjipanayiotou, 1992), even if milk fat and milk protein contents were slightly higher in ewes fed formaldehyde treated soybean. Hadjipanayiotou and Photiou (1995) found the use of formaldehyde protected soybean in Chios dairy ewes in negative energy balance did not affect milk yield and composition either. However, use of protected proteins for milk production in dairy sheep gave also interesting results, but in some cases was not significant or contradictory. Therefore, the objectives of this trial were to evaluate the effect of FTCM on rumen protein availability, to test whether transition ewe's performance could be improved by increasing dietary ruminal undegradable protein (RUP) via FTCM at the same protein level.

## 2. Material and methods

### 2.1. Experiments 1. *Ruminal degradability*

Two ruminally fistulated Zel ewes were used to estimate the ruminal degradability parameters of DM, CP and NDF of UCM and FTCM (4ml/100 g CM). Using the nylon bag (7cm×14cm; with pore size 40±10µm, and 26% porosity) three replications of 3 g DM equivalent samples were ruminally incubated for 0, 1, 3, 6, 12, 24, 36 and 48 h. All incubations started after the morning feeding. Bags were attached to a plastic tube (5- mm diameter) that was fixed to the outside of the fistula with a string. The bags and the tubes had free movement inside the rumen and reticulum. On removal, bags were washed using cold water until the effluent ran clear. The bags were dried in an oven at 55°C for 48 h, and weighed. Following the weighing, bags were opened, and residues from the three bags for each period were homogenized and placed in tightly capped plastic bottles. Samples were analyzed for Kjeldahl N and NDF (Van Soest et al., 1991). All analyses were made on combined residues of the three bags. The analyses were run available. Kinetics of DM, crud protein, and NDF disappearance *in situ* was estimated by the nonlinear regression procedure of SAS (1998). For each sample, the following model (Ørskov and McDonald, 1979) was fitted to the percentage of disappearance of DM, CP, and NDF: $Y = a + b\ (1 - exp^{(-c\ t)})$

Where, a, soluble fraction (%); b, slowly digestible fraction (%); c, fractional rate of disappearance (per hour); and t, = time of incubation (hours). The equation ED = [a + b × c/(c + *kp*)] was used to calculate effective degradability (ED). In this equation, *kp* represents the flow rate of particles out of the rumen that we theoretically consider equal to 0.02 (maintenance level), 0.04 and 0.06 %/h.

Table 1. Ingredients and chemical compositions of experimental diets

| Ingredient | Experimental diets that contained |
|---|---|
| Ingredients, % | |
| Barley | 33.08 |
| Canola meal | 10.15 |
| Wheat bran | 11.65 |
| Wheat straw | 44.36 |
| Mineral-vitamin | 0.76 |
| Chemical compositions, % of DM | |
| Dry matter | 89.04 |
| Crud protein | 12.03 |
| Neutral detergent of fiber | 45.6 |
| Non fiber carbohydrate | 33.47 |
| Ether extract | 2.42 |
| Ash | 6.48 |

### 2.2. Experiments *2.* Feeding trail

The study was carried out at the Ruminant Research Center of Sari Agricultural and Natural Resources University (SANRU), Sari, Iran. Sixteen Zel ewes of known mating date (BW=42.0 ± 2.7 kg) were selected for the study in five weeks before calving, weighed and divided into two different weight groups, which represent the replications of the experiment using a completely randomized design. The ewes were on average 3 years old, healthy and in good physical condition.

**Table 2.** Rumen degradation parameters for dry matter, crude protein and neutral detergent fiber of untreated (control) and formaldehyde-treated (Treatment) canola meal.

| Item | Degradability parameters[1] | | | | | Effective degradability at *Kp* | | |
|---|---|---|---|---|---|---|---|---|
| | *a* | *b* | *a* + *b* | UD | *c* | 0.02 | 0.04 | 0.06 |
| **Dry matter** | | | | | | | | |
| Control | 18.77$^{a}$ | 54.75$^{a}$ | 73.52$^{a}$ | 26.48$^{b}$ | 0.086 | 63.19$^{a}$ | 56.14$^{a}$ | 51.02$^{a}$ |
| Treatment | 16.40$^{b}$ | 46.03$^{b}$ | 62.43$^{b}$ | 37.57$^{a}$ | 0.073 | 52.53$^{b}$ | 46.136$^{b}$ | 41.66$^{b}$ |
| SEM | 0.672 | 1.043 | 1.051 | 1.500 | 0.010 | 0.422 | 0.436 | 0.479 |
| P-Value | 0.0081 | >0.0001 | 0.0273 | >0.0001 | 0.3046 | 0.0087 | 0.0107 | 0.0028 |
| ***Crude protein*** | | | | | | | | |
| Control | 25.50$^{a}$ | 52.59$^{a}$ | 78.09$^{a}$ | 21.91$^{b}$ | 0.087 | 68.26$^{a}$ | 61.53$^{a}$ | 56.62$^{a}$ |
| Treatment | 18.91$^{b}$ | 46.28$^{b}$ | 65.19$^{b}$ | 34.81$^{a}$ | 0.071 | 55.02$^{b}$ | 48.51$^{b}$ | 43.99$^{b}$ |
| SEM | 0.562 | 1.617 | 1.122 | 1.122 | 0.022 | 0.487 | 0.672 | 0.382 |
| P-Value | >0.0001 | >0.0001 | >0.0001 | >0.0001 | 0.9170 | 0.0076 | >0.0001 | >0.0001 |
| ***Neutral detergent fiber*** | | | | | | | | |
| Control | 25.1 | 51.42 | 65.51 | 34.48 | 0.069 | 64.96 | 57.65 | 52.60 |
| Treatment | 27.56 | 50.43 | 63.98 | 36.01 | 0.076 | 67.48 | 60.60 | 55.74 |
| SEM | 0.959 | 3.025 | 2.206 | 2.206 | 0.026 | 2.233 | 1.862 | 1.853 |
| P-Value | 0.1370 | 0.2411 | 0.8783 | 0.2783 | 0.1087 | 0.2884 | 0.2578 | 0.3221 |

[a,b]Means within rows with different letters differ ($P < 0.05$).[1] *a*, the water-soluble fraction; *b,* the slow degradation fraction, *a*+ *b*, the potentially degradable fraction; UD, Undegradable fraction; *c*, the rate of degradation.

Ewes were randomly allocated to one of two treatments by the order that they lambed. Dietary treatments were initiated approximately 5 weeks prior expected lambing dates (Two weeks for adaptation and 3 weeks as close up period) and continued through 3 weeks postpartum. All animals were allocated in two treatments to the experimental feeds at libitum to evaluate the effect of animals were fed diets consisting 44.36, 11.65, 10.15 and 0.76%, wheat straw, wheat bran, CM and mineral-vitamin mix supplement, respectively (Table 1), but untreated CM (UCM) in treatments 1 was substituted with formaldehyde treated CM (FTCM) in treatment 2. Both diets were formulated using the Sheep Cornell Net Carbohydrate and Protein System (Sheep CNCPS, 2007) to meet the requirements for dry and lactating ewes and offered as a total mixed ration. Ewes were housed in individual. Diets were fed individually twice a day at 0700 and 1900 hours at a level of 10% above ad libitum intake during the experiment. Refusals were collected and weighed every morning to obtain an estimate of intake. Daily record of feed intake was maintained throughout the experiment. Feces of all sheep collected on 14 d before lambing over 5 d. Samples of refusal and feces collected from individual animals every day were pooled over the entire experimental period and sub-sampled for analysis. The feeds were sampled regularly and analyzed for DM, crud protein, ether extract, ash at 605◦C (AOAC, 2002), NDF and ADF (Van Soest *et al.*, 1991). Non fibrous carbohydrate (NFC) was calculated by 100- (CP % + NDF % + Ash % + EE %)

(NRC, 2001) and digestibility was calculated for DM and all nutrients.

On the first day of the experiment and subsequently at weekly intervals before offering the morning feed on the same day of the week after withholding feed and water overnight ewes were weighed. The lambs were penned individually on slatted floors. All individually housed ewes lambed in their pens and remained there with their lambs until 24 h post partum. Then, lambs separated from the ewes and allowed 15 min access to their dams daily at 7, 12, 17 and 22 h. Lambs were weighed at birth and 3 weeks after birth. Lamb's daily gain (g/day) was calculated by the weight difference of the lamb between two consecutive times. The average daily body weight gain (ADWG) during the experimental period was calculated by regressing body weight of lamb on number of days of feeding.

Milk samples were manually collected daily at 6 and 16 h. Daily samples of milk were mixed and immediately frozen and maintained at -20◦C until analysis for fat, protein, lactose, total solid (TS), and solid not fat (SNF).

Lamb birth weights were recorded. The ewe's milk yields were measured during the 3 weeks of lactation using the lamb-suckling method. The lambs were separated from the ewes and then allowed 15 min access to their dams at 7, 12, 17 and 22 h. The lambs were weighed immediately before and after being suckled. The daily milk yields were calculated by the summated weight differences of the lambs. Milk fat and protein yield was calculated by multiplying milk yield by milk fat or protein percentage.

The experimental design consisted of a completely randomized design with repeated measurements on animals. Analysis of variance was conducted using the SAS General Linear Models procedure (SAS, 1998) by following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_{ij} + t_k + e_{ijk}$$

where $Y_{ijk}$ is the dependent variable, $\mu$ is the overall mean, $\alpha_i$ is the random effect of diets as treatments (i = 1 and 2), $\beta_{ij}$ is the random effect of animal j in treatment group i, $t_k$ is a fixed effect of time k, and $e_{ijk}$ is random error at time k on animal j in treatment i. Lamb sex was not a significant source of variation for any parameters and was omitted from the model. Means were separated using the Lsmeans procedure with an alpha level of 0.05.

## 3. Results

### 3.1. *Ruminal degradability*

Ruminal DM, CP and NDF degradability parameters of UCM and FTCM are presented in Table 2. Formaldehyde treatment significantly decreased *a, b,* potential degradable fractions and effective degradability at different $K_p$ for DM and CP, with no significant effect on NDF degradation parameters (Table 2).

### *3.2. Feeding trail*

The BW of ewes and DM intake during the experiment are shown in Table 3. Body weight in sheep that fed ration inclusion UCM and FTCM increased 1.89 and 2.56 kg over the last three weeks of gestation. Increased BW in sheep that fed FTCM was significantly greater than sheep that fed UCM in ration. Reduction of BW result in lambing was -7.86 and -7.28 kg in sheep that fed ration inclusion UCM and FTCM. There were no significant differences between DM intake (except on 2 weeks after lambing) for ewes those fed diets contained UCM and FTCM in prepartum and postpartum. The feeding FTCM to ewes during late gestation decreased the dry matter intake at 2 week lambing ($P = 0.0349$).

Apparent digestibility of DM, ether extract and NFC were the same across two treatments, but control treatment had significantly greater digestibility for CP, NDF and ash than FTCM treatment (Table 3). Although formaldehyde treatment reduced 25.8, 12.0 and 16.5 % for *a, b* and potential degradable fractions of protein, apparent digestibility of CP in control treatments was approximately 14 % lower than treatment that was result in higher digestion of FTCM in the lower compartment of gastrointestinal tract and formaldehyde treated provide most amount of dietary protein truly digested in the small intestine.

The blood serum glucose, cholesterol, triglyceride, HDL-cholesterol concentrations of ewes were fed UCM and FTCM diets are set out in Table 4. The plasma glucose, triglyceride and cholesterol concentrations of ewes fed UCM or FTCM diets were not significantly different ($P > 0.05$). However, serum HDL-cholesterol level were higher ($P = 0.0296$) in ewes were fed FTCM diet than in ewes were fed UCM diet.

Litter size and lambs daily gain are presented in Table 5. The body weight of lamb at birth, 7, 14 and 21 d after lambing were significantly higher in treatment than control. In addition, there was difference between control and treatment groups in lambs daily gain ($P = 0.0172$).

Milk yield and milk composition results are presented in Table 6. Milk production increased significantly in sheep that fed ration contained the formaldehyde treated CM than control. In addition, fat (percentage and yield), protein (yield) and total solid (percentage) content of milk were significantly higher for ewes that fed the FTCM diet postpartum versus ewes that fed the UCM diet postpartum. However, other milk compositions were not influenced by increasing the quantity of RUP available to the small intestine.

Table 3. Body weight, dry matter intake and nutrients digestibility in sheep that fed two ration inclusion untreated (control) and formaldehyde-treated (Treatment) canola meal

| Item | Experimental diets that contained | | SEM | P-values |
|---|---|---|---|---|
| | Control | Treatment | | |
| **Body weight, kg** | | | | |
| Three week before lambing | 43.67 | 43.57 | 0.363 | 0.1824 |
| One day before lambing | 45.56[b] | 46.13[a] | 0.421 | 0.0023 |
| After lambing | 37.70 | 38.85 | 0.569 | 0.2946 |
| Three week after lambing | 37.45 | 38.25 | 0.435 | 0.1774 |
| **Dry matter intake, kg** | | | | |
| Two week before lambing | 1.401 | 1.355 | 0.036 | 0.5142 |
| One week before lambing | 1.167 | 1.267 | 0.030 | 0.5861 |
| Day of lambing | 1.021 | 1.085 | 0.043 | 0.0623 |
| One week after lambing | 1.145 | 1.223 | 0.045 | 0.9365 |
| Two week after lambing | 1.407[a] | 1.306[b] | 0.015 | 0.0349 |
| **Apparent digestibility (%)** | | | | |
| Dry matter | 67.87 | 68.59 | 0.676 | 0.3452 |
| Ether extract | 68.60 | 68.87 | 0.853 | 0.4332 |
| Crud protein | 62.86[b] | 73.23[a] | 0.543 | <0.0001 |
| Neutral detergent of fiber | 55.40[b] | 52.30[b] | 0.535 | <0.0001 |
| Ash | 72.94[a] | 69.08[b] | 0.436 | <0.0001 |
| Non carbohydrate fiber | 90.88 | 91.72 | 0.930 | 0.5501 |

[a,b]Means within rows with different letters differ ($P < 0.05$).

Table 4. Blood metabolites of ewes

| Item | Experimental diets | | SEM | P-values |
|---|---|---|---|---|
| | Control | Treatment | | |
| Glucose, mg/dl | 49.16 | 48.12 | 1.015 | 0.6725 |
| Cholesterol, mg/dl | 49.64 | 57.59 | 2.231 | 0.1738 |
| HDL-cholesterol, mg/dl | 17.69[b] | 25.22[a] | 1.147 | 0.0296 |
| Triglyceride, mg/dl | 58.23 | 62.47 | 5.397 | 0.9624 |

[a, b] Means within rows with different letters differ ($P < 0.05$).

Table 5. Litter size and lambs daily gain of lamb for of ewes that fed two ration inclusion untreated (control) and formaldehyde-treated (Treatment) canola meal

| Item | Experimental diets | | SEM | P-values |
|---|---|---|---|---|
| | Control | Treatment | | |
| Lambs weight, g | | | | |
| Birth | 2557.50[b] | 3402.50[a] | 55.451 | 0.0359 |
| 7d | 3378.75[b] | 4605.00[a] | 69.644 | 0.0208 |
| 14d | 4261.25[b] | 6011.25[a] | 79.914 | 0.0418 |
| 21d | 5205.50 | 7237.50 | 87.362 | 0.0252 |
| Daily gain, g/d | 143.15[b] | 203.81[a] | 20.577 | 0.0172 |

[a,b] Means within rows with different letters differ ($P < 0.05$).

## 4. Discussion

### 4.1. *Ruminal degradability*

As the data in table 2 have shown, degradation parameters for UCM, the values for *a, b,* potential degradable fractions and effective degradability at different $K_p$ for DM and CP were in normal range those before reported by researchers. The soluble protein (*a*) content of CM ranges from 18.6 to 29.8% and the proportion of potentially degradable protein (*b*) ranges from 56.7 to 84.9% (Khorasani *et al*., 1993). The rate of degradation of the *b* fraction has been reported to vary from 2.48 to15.7%/hr (Cheng *et al.*, 1993). Ha and Kennelly (1984), Kendall *et al.* (1991), Cheng *et al.* (1993) and Piepenbrink and Schingoethe (1998) found that the effective degradability of CM protein was 65.8, 51.5, 62.5, and 53.1%, respectively. Mustafa *et al.* (1996) reported effective degradability of protein for regular, low and high fiber CM to be 74.9, 75.3 and 72.5%, respectively. There is a large variation in the kinetic CM parameters and in the measurement of effective degradability. Variability of effective degradability of protein is related to the diet, the processing conditions and the ruminal turnover rate (Khorasani *et al.*, 1993; Kendall *et al.*, 1991). Cheng *et al.* (1993) reported a potentially degradable protein fraction (*b*) for CM of 72.5% on hay based diets and 59.6% on concentrate based diets. Also the effective degradability of protein of CM was 62.5% with a concentrate diet and 74.9 and 72.3% on a hay and straw diet, respectively. However, in current experiment, diets were similar therefore ewe variations and samples type also may influence degradability.

Khorasani *et al.* (1996) indicate that treatment of protein with acetic acid, formic acid, and propionic acid decreases CP solubility and degradability, but intestinal digestibility of CP is not depressed. Although the mode of action of all treatments in reducing protein degradation in the rumen may be the Maillard reaction, it appears that only with HCI treatments are the bonds created resistant to postruminal enzymatic digestion (Khorasani *et al.,* 1996). Chemical treatment of CM reduced the soluble protein fraction determined *in situ* which might have contained AA and peptides that are essential for microbial protein synthesis. A further reduction in the availability of AA and peptides may have resulted from the formation of isopeptide bonds between lysine residues and the *β*- or y-carboxamide group of Asparagine and Glutamine residues formed in the Maillard reaction. Because AA and peptides stimulate bacterial growth, the net shift of soluble CP to the potentially degradable and indigestible pools may have inhibited bacterial growth (Khorasani *et al.*, 1993 and 1996).

### 5.2. Feeding trail

There were no significant differences between DM intake (except on 2 weeks after lambing) for ewes those fed diets contained UCM and FTCM in prepartum and postpartum. Body weight in sheep that fed ration inclusion UCM and FTCM increased 1.89 and 2.56 kg over the last three weeks of gestation. In addition, reduction of BW result in lambing was -7.86 and -7.28 kg in sheep that fed ration inclusion UCM and FTCM. The feeding FTCM to ewes during late gestation decreased the DM intake at 2 week lambing ($P = 0.0349$). The effect of FTCM supplementation on DM in this study is consistent with previous results. Koritnik *et al.* (1981) restricted (50% of control) ewe diets the last 50 to 60 d of gestation and found that ewes on the restricted diet lost 7.0 kg and ewes on the control diet gained 7.7 kg. They concluded that the

reduction in maternal BW of the restricted ewes was a result of nutritional priority being directed to the fetus. The weight differences in the ewes on this study were not as large and increased before lambing. Therefore, the protein supplementation increased BW before lambing and reduced BW loss after parturition. During three weeks before lambing reduction of DM intake for ewes those fed diets contained UCM and FTCM were 27.1 and 20.0 %, respectively. In contrary, during three weeks after lambing increasing of DM intake for ewes those fed diets contained UCM and FTCM were 35.1 and 20.4 %, respectively.

However, level of intake was low in both of treatment. One reason is high concentration of NDF in rations. The NDF consumed by lambs in this study was 1.31% (± .023) and 1.35% (± .034) of BW and did not differ among treatments. In addition, dry matter content of the heat-treated CM and the Lignosulfonate-treated CM supplements were slightly lower than for the UCM supplement (Wright, 2005). Approximately 70 % of fetal growth, most of the ewe's mammary growth occurs during the last 4 to 6 weeks of pregnancy when her rumen capacity is decreasing. The primary result is the need for increased feed and a more nutrient-dense diet. Extra nutrition is needed to support fetal growth, especially if there are multiple fetuses, to support mammary development and ensure a plentiful milk supply and to prevent the occurrence of pregnancy toxemia (ketosis). It will ensure the birth of strong, healthy lambs. During late gestation, energy is the nutrient most likely to be deficient. The level of nutrients required will depend upon the age and weight of the ewe and her expected level of production, i.e. singles, twins, or triplets. However, in current experiment, the ewe's feeding requirements for late pregnancy were fulfilled with balance ration using the Sheep Cornell Net Carbohydrate and Protein System (Sheep CNCPS, 2007). In addition, in this study pregnancy toxemia, small and weak lambs, lamb mortality did not observe. The objectives of feeding the pregnant ewe are: 1) to produce healthy lambs of sufficient body weight to ensure good subsequent growth, 2) to ensure adequate udder development for lactation, and 3) to maintain the health of the ewe. On the other hand, some weight loss can be tolerated during the first three weeks provided that the ewe is well fed during her last 3 weeks. The possibility is suggested that the protein status of the animal is a component of a chemoregulatory mechanism governing the intake of low nitrogen diets by sheep. Therefore, balancing rations for protein degradability may improve animal performance and reduce the environmental impact of livestock production. Although microbial protein is the primary protein source for ruminants, increasing dietary RUP can increase the flow of AA above microbial AA supply. Specific requirements are not reported for RUP in pregnant and lactating ewe diets, but the NRC (2007) indicates that increasing dietary RUP from 3.4 to 9.3% of DM decreases dietary CP requirements from 17 to 15.5% of DM, indicating increased efficiency of N utilization from RUP. Formaldehyde treatment reduced 25.8, 12.0 and 16.5 % for *a, b* and potential degradable fractions of protein. Therefore, treatment significantly increased dietary ruminal undegradable protein from 3.45 to 8.39 % of DM that may be decreased dietary protein requirements and increased efficiency of N utilization.

The mean blood-sugar values determined by the REÍD (1950) in non-pregnant ewes in Australia and in England were 34.8 ±3.06 and 39.1 ± 3.37 mg/dl, respectively. The observed range in both pregnant and non-pregnant ewes was 18-57 mg/dl, but 94% of values fell between 25 and 46 mg/dl. The level of blood sugar was affected neither by the plane of nutrition nor by the bodily condition of non-pregnant ewes. Gestation in ewes in good condition was observed not to affect the level, although evidence was obtained of lowered blood-sugar levels during the last two months of gestation in ewes in poor condition. The present study showed that the mean values of glucose for sheep were greater than ranges that reported by REÍD (1950). The effects of nutrition are too little on ruminant blood glucose level, because fermentation carries out in rumen. Blood glucose concentrations in ruminants are considerably lower than those of nonruminants; ruminants are relatively insensitive to insulin. Glucose metabolism is expected to increase with feeding, because propionate, the major precursor for gluconeogenesis, is produced in the rumen and absorbed after feeding. Protein treatment had no effect ($P>0.10$) on blood acetate, propionate, triglyceride or glucose (Jaquette, 1986). The type of CM supplement (CM treated with heat and Lignosulfonate and Untreated CM) did not influence (P 0.05) hematocrit or blood glucose levels (Wright, 2005). Research has shown that the quantity of dietary protein affects lipid metabolism in a number of mammalian and avian species. In studies with growing ruminant animals and lactating cows, a reciprocal relationship has been observed between the dietary protein level and plasma cholesterol concentration. This relationship suggests that the amount of dietary protein acts as a regulator of plasma cholesterol by exerting its influence upon rates of cholesterolgenesis. The influence of dietary protein on plasma cholesterol level may lead to changes in concentrations or metabolism of cholesterol in other tissues and vital organs. When HDL-cholesterol was expressed as percentage total cholesterol, a significant increase in the type of lipoprotein bound cholesterol was observed for the high dietary protein group. This finding, along with the increase in LCAT enzymatic

activity for the high dietary protein treatment over the low protein treatment, suggests that HDL-cholesterol and structural apolipoprotein moieties might have been the main substrates as well as activators for LCAT. Only lipids of HDL may serve as direct substrates for LCAT, probably because the reaction is activated by the apolipoprotein AI moiety, the main protein of HDL. In ruminants, although the HDL-LCAT system has been recognized for its existence we know too little about the relative importance and the underlying mechanisms of this system, especially in the synthesis of plasma cholesteryl esters (Park, 1985). The underlying mechanisms whereby dietary protein level acts as a regulator of plasma cholesterol remain unknown. However, three possible explanations from our results and those found in the literature are: 1) dietary protein may influence the turnover rate of the mevalonic acid pool, possibly through regulation of the activity of β-hydroxy- β -methylglutaryl Coenzyme A reductase, 2) an imbalance of dietary amino acids, due to either protein deficiency or the protein: energy ratio of the diet, may alter plasma cholesterol level, and 3) dietary protein may affect the intricate metabolism of lipoprotein-LCAT complex, which controls cholesterol-lipid distribution in the liver and possibly the mammary gland (Park, 1985).

Table 6. Milk yield and composition of ewes that fed two ration inclusion untreated (control) and formaldehyde-treated (Treatment) canola meal

| Item | Experimental diets | | SEM | P-values |
|---|---|---|---|---|
| | Control | Treatment | | |
| Milk, g/d | 890.83[b] | 1008.72[a] | 42.011 | 0.0050 |
| Milk composition | | | | |
| Fat (%) | 4.70[b] | 5.50[a] | 0.150 | 0.0011 |
| Fat (g/d) | 43.93[b] | 56.32[a] | 2.650 | 0.0192 |
| Protein (%) | 6.20 | 6.50 | 0.272 | 0.4091 |
| Protein (g/d) | 57.30[b] | 65.48[a] | 2.142 | 0.0039 |
| Lactose (%) | 5.36 | 5.17 | 0.101 | 0.1802 |
| Lactose (g/d) | 50.11 | 52.32 | 5.802 | 0.7735 |
| Total solid (%) | 16.26[b] | 17.17[a] | 0.203 | 0.0121 |
| Total solid (g/d) | 151.34 | 174.13 | 17.177 | 0.3272 |
| Solid non fat (%) | 11.56 | 11.67 | 0.279 | 0.7882 |
| Solid non fat (g/d) | 107.51 | 117.80 | 10.835 | 0.4810 |

[a,b] Means within rows with different letters differ ($P < 0.05$).

Although the late gestation nutrition has been shown to have an effect on the subsequent lamb growth rate, there is still a lack of information on the effect of CP supplementation to ewes during production is, therefore, extremely important and underfeeding ewe's energy and protein in late pregnancy reduced the total yield of colostrums produced during the first 18h after birth by decreasing the prenatal accumulation of colostrums and its subsequent rates of secretion. The improving ewe protein nutrition pre-lambing increased the lambs efficiency to absorb colostral IgG during the first 24 h of life (Dawson, 1999).

Effects of dietary protein level on milk production of early lactating ewes are mainly attributed to energy savings as a consequence of an increase in body fat mobilization and utilization (Robinson et al., 1979). Studies on the use of low degradable protein supplements, protected proteins or protected amino acids in milk production of sheep are very limited and most of the references were obtained from suckling ewes, altering the practical significance of data of milk composition. In addition, in some cases the results are not significant or contradictory. In regard to low degradability protein supplements Robinson et al. (1979), Hadjipanayiotou (1992) and Purroy and Jaime (1995) showed increases in milk yield during early lactation when included or substituted a degradable protein by RUP for example fishmeal as in lactating ewes. Purroy and Jaime (1995) found significant increases in milk protein (+2.9 g/l, +6.2%) but not in milk yield, probably as a consequence of the reduction of undernutrition (70-80% of energy requirements) applied in the experiment. Robinson et al. (1979) also found a slight increase ($P<0.10$) in milk protein in ewes fed fishmeal, when compared with those fed soybean or peanuts protein supplements. Effects of RUP are attributed to an increase in the amount and profile of amino acids absorbed in the small intestine and that are available for milk synthesis.

Use of protected proteins also gave interesting results, but in some cases they are not significant or contradictory. Treatment of protein supplements with formaldehyde must be done at optimum doses. In this sense, compared the use of soybean, fishmeal and formaldehyde protected soybean in Chios dairy ewes were without significant effects on milk yield and milk composition (Hadjipanayiotou, 1992), even if milk fat and milk protein contents were slightly higher in ewes fed formaldehyde treated soybean. The use of formaldehyde protected soybean in Chios dairy ewes in negative energy balance also did not affect milk yield and composition (Hadjipanayiotou and Photiou, 1995).

Sloan *et al*. (1988) reported that increased dietary RUP reduced milk fat percentage. Milk production responses to RUP supplementation from FTCM have been inconsistent (Olmos, 2006). Unlike the results of current experiment, Khorasani *et al*., (1996) reported that an increase in available RUP had not response in milk protein content. The milk production of sheep is an important economic activity in many countries. Increased milk production and composition can result of increased microbial protein synthesis, propionate concentration (glucogenic precursor), postruminal digestion of starch, and bypass protein (Dann *et al*, 1999). Most dairy animal in early lactation and rapidly growing ruminant animals are unable to meet their requirements for absorbable protein from rumen microbial protein alone, making it

essential that the diet contain slowly degraded protein with a high potential for rumen escape. Feeding strategies for late pregnancy that recognize the need for diets to supply increasing amounts of RUP are now accepted in practice (Dawson, 1999). Some researchers have investigated whether the quantity and quality of CP in the diet can affect milk production and composition. However, indicated that treated CM increases RUP supplied from CM that improved production and N utilization. Overall, most studies have shown relatively little effect of different true protein sources on milk yield (Brito and Broderick, 2007). The NRC (2001) model predicts that the dietary CP late gestation on postnatal lamb growth performance (Oack, 2005). Lambs are born hypoimmunocompetent with a small store of energy, and rely on colostrums to supply maternal immunoglobulin and energy. Colostrums concentration required for a specific level of milk production decreases when RUP is supplemented in the diet. If this approach were successful, efficiency of conversion of dietary N into milk protein would be improved and economic advantage would be gained due to reduced feed costs rather than increased production. As speculated above formaldehyde treatment reduced 25.8, 12.0 and 16.5 % for *a, b* and potential degradable fractions of protein, but apparent digestibility of crud protein was 14 % higher for FTUC in the lower compartment of gastrointestinal tract. Therefore, RUP is increased in the diet and milk production improved because the with an increased amount of RUP has been shown to increase the flow of essential AA to the small intestine as required for milk synthesis in high-producing dairy animal. Studies using soybean meal reported no improvement in milk yield of animal fed diets supplemented with soybean treated to reduce ruminal degradability. However, these results were not unexpected considering that the diets containing untreated soybean meal were likely not limiting in protein. The discrepancy in response to increased RUP between CM and soybean meal may also be attributable to the more suitable balance of AA associated with CM (Khorasani *et al*., 1996). However, in current study, increased RUP significantly enhanced milk production (13.2 % based on control). In trials with stored feeds, RUP supplementation increased milk yield by 13 to 27% in nondairy ewes (Robinson et al., 1979). In dairy ewes, supplementing RUP from expeller soybean meal increased milk yield by 14% in low- and high-milk-yielding ewes (Mikolayunas-Sandrock et al., 2009). Few trials have evaluated the effect of RUP supplementation to lactating ewes on pasture. Penning et al. (1988) reported a 23% increase in milk yield and a 15% increase in lamb growth when dams were supplemented with fish meal compared with supplementation with barley or no supplement. Therefore, RUP supplementation may be beneficial to high-producing dairy ewes.

**Corresponding Author:**
***Dr. Asadollah Teimouri Yansari***
Department of Animal Science, Animal Science and Aquaculture faculty, Agricultural and Natural Resource University, Sari, Mazandaran, Iran
E-mail: astymori@yahoo.com

**References**

1. Association of Official Analytical Chemists, 2002. Official Methods of Analysis. Vol. 1. 17th ed. AOAC, Arlington, VA.
2. Brito AF, Broderick GA. Effects of Different Protein Supplements on Milk Production and Nutrient Utilization in Lactating Dairy Cows. Journal Dairy Science. 2007: 90(6):1816–1827.
3. Cheng KJ, McAllister TA, Rode LM. Use of acidulated fatty acids to increase the rumen undegradable protein value of canola meal. Tenth project report. Research on Canola Seed, Oil and Meal. Canola Council of Canada. Winnipeg. Canada. 1993.
4. Dann HM, Varga GA, Putnam DE. Improving Energy Supply to Late Gestation and Early Postpartum Dairy Cows. Journal Dairy Science. 1999: 82(7): 1765–1778.
5. Dawson LER, Carson A F, Kilpatrick DJ. The effect of the digestible undegradable protein concentration of concentrates and protein source offered to ewes in late pregnancy on colostrums production and lamb performance. Animal Feed Science and Technology. 1999: 82(1): 21-36.
6. Ha JK, Kennelly JJ. In situ dry matter and protein degradation of various protein sources in dairy cattle. Canadian Journal of Animal Science. 1984: 64(3): 443-452.
7. Hadjipanayiotou M. Effect of protein source and formaldehyde treatment on lactation performance of Chios ewes and Damascus goats. Small. Ruminant Research. 1992: 8(3): 185-197.
8. Hadjipanayiotou M, Photiou A. Effects of level of inclusion and formaldehyde treatment of soybean meal on the performance of lactating Chios ewes in negative energy balance. Livestock Production Science. 1995: 41(2): 207-215.
9. Jaquette RD, Rakes AH, Croom WJ. Effects of Dietary protein on milk, rumen, and blood parameters in dairy cattle fed low fiber diets. Journal Dairy Science. 1986: 69(5):1026-1034.
10. Kendall EM, Ingalls JR. Boila RJ. Variability in the rumen degradability and post ruminal digestion of the dry matter, nitrogen and amino acids of canola meal. Canadian Journal of Animal Science. 1991: 71(2):739-754.

11. Khorasani GR, de Boer G, Kennelly J J. Response of early lactation cows to ruminally undegradable protein in the diet. Journal Dairy Science. 1996:79 (2): 446–453.
12. Khorasani GR, Robinson PH, Kennelly JJ. Effect of canola meal treated with acetic acid on rumen degradation and intestinal digestibility in lactating dairy cows. Journal Dairy Science. 1993: 76(7): 1607-1616.
13. Koritnik DR, Humphrey W D, Kaltenbach CC, Dunn TG. Effects of maternal under nutrition on the development of the ovine fetus and the associated changes in growth hormone and prolactin. Biology Reproduction. 1981: 24 (1) :125-137.
14. Mikolayunas-Sandrock C, Armentano LE, Thomas DL, Berger YM. Effect of protein degradability on milk production of dairy ewes. Journal Dairy Science. 2009. 92 (11): 4507–4513.
15. Mustafa AF, Christensen DA, McKinnon JJ. Chemical characterization and nutrient availability of high and low fiber canola meal. Canadian Journal of Animal Science. 1996: 76(4): 579–586.
16. National Research Council. Nutrient Requirements of Dairy Cows. 7th rev. ed. National Academic Science. Washington, DC. 2001.
17. National Research Council. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. National Academic Science. Washington, DC. 2007.
18. Oack N, Cam MA, Kuran M. The effect of high dietary protein levels during late gestation on colostrums yield and lamb survival rate in singleton-bearing ewes. Small Ruminant Research. 2005:.56(1):89-94.
19. Olmos Colmenero JJ, Broderick GA. Effect of Amount and Ruminal Degradability of Soybean Meal Protein on Performance of Lactating Dairy Cows. Journal Dairy Science. 2006: 89(7): 1635–1643.
20. Ørskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal Agricultural Science (Cambridge). 1979: 92 (3): 499-509.
21. Park CS. Metabolites of Growing calves Influence of Dietary Protein on Blood Cholesterol and Related. Journal Animal Science. 1985: 61(4): 924-930.
22. Paul JW, Dinn NE, Kannangara T, Fisher LJ. Protein content in dairy cattle diets affects ammonia losses and fertilizer nitrogen value. Journal Environment Quality. 1998: 27(3): 528–534.
23. Penning PD, Orr RJ, Treache TT. Responses of lactating ewes offered fresh herbage indoors and when grazing, to supplements containing differing protein concentrations. Animal Production. 1988: 46(2): 403-415.
24. Piepenbrink MS, Schingoethe DJ. Ruminal degradation, amino acid composition and estimated intestinal digestibilities of four protein supplements. Journal Dairy Science. 1998: 81(2):454-461.
25. Purroy A, Jaime C. The response of lactating and dry ewes to energy intake and proteinsource in the diet. Small Ruminant Research. 1995. 17 (1): 17-24.
26. REÍD R L. Studies on the carbohydrate metabolism of sheep. I. The range of blood sugar values under several conditions. Australian Journal of Agricultural Economics. 1950:1(1):182-199
27. Robinson JJ, McHattie I, Calderon-Cortés JF, Thompson JL. Further studies on the response of lactating ewes to dietary protein. Animal Production. 1979: 29(2): 257-269.
28. SAS User's Guide: Statistics, version 8.2 Edition. SAS Inst. Inc., Cary, NC. 1998.
29. Sloan BK, Rowlinson P, Armstrong DG. The influence of a formulated excess of rumen degradable protein or undegradable protein on milk production in dairy cows in early lactation. Animal Production. 1988: 46(1): 13–22.
30. Van Soest P J, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharide in relation to animal nutrition. Journal Dairy Science. 1991:74 (11): 3583– 3597.
31. Wright CF, Von Keyserlingk MAG, Swift ML, Fisher LJ, Shelford JA, Dinn NE. Heat- and Lignosulfonate-Treated canola Meal as a Source of Ruminal Undegradable Protein for Lactating Dairy Cows. Journal Dairy Science. 2005:88(2):238–243.

6/5/2011