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Milk Yield and Blood Metabolite Profile in Late Pregnancy in Lori Ewes Receiving Diets Containing Undegradable Protein Sources

Research Article

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ABSTRACT

An experiment was conducted to investigate the effects of supplementing Lori ewes with rumen-undegradable protein (RUP) in late pregnancy on their performance and that of their lambs. Milk yield and blood metabolite profile were measured. Forty pregnant ewes were used in a completely randomized design trial with four treatments: 1) a basal diet (control); 2) with a supplement of 5% fishmeal; 3) with a supplement of 5% extruded soybean and 4) with a supplement combining both sources. Supplementation started 30-days before the estimated parturition date. Dietary inclusion of fish meal significantly improved body weight at lambing compared to the other groups (P<0.05). Lambs of ewes fed diets supplemented with rumen undegradable protein (RUP) were heavier at birth and weaning compared to other groups (P<0.05). Soybeans, of the combined supplement improved the colostrum concentration of IgG in postpartum ewes (P<0.05). Diets containing extruded soybeans resulted in milk with higher milk urea nitrogen content than the control diet (P<0.05). Serum concentration of glucose was significantly decreased in ewes fed diets containing RUP pre-partum (P<0.05), while post-partum glucose concentration was only decreased in ewes fed diets containing fish meal without or with extruded soybeans (P<0.05). Plasma concentration of beta-hydroxybutyric acid (BHBA) and non-esterified fatty acid (NEFA) were significantly decreased in the treated groups compared to the control group in post-partum ewes (P<0.05).

KEY WORDS colostrum, extruded soybean, fish meal, late pregnancy, Lori ewes, reproductive performance.

INTRODUCTION

Animal husbandry faces major challenges in management and improvement of reproductive performance and milk production during late pregnancy. Dietary protein is known as one of the most important nutrients for ruminants in late pregnancy. And, it has become widely accepted as a standard breeding tool due to the savings in costs associated in animal husbandry.

Nutritional requirements of female ruminants increase with advancing parturition and onset of lactation, but a gradual decrease in dry matter intake (DMI) occurs over the last three weeks before lambing, thus dairy cows cannot received enough energy for their needs, resulting in negative energy balance (NEB) (Rodney *et al.* 2015), resulting in body reserve mobilization and increased levels of NEFA and BHBA in serum, and the incidence of metabolic disorders.

This can lead to decreased postpartum reproductive performance as reported in dairy cows (Ospina *et al.* 2010). In ruminants, dietary protein is usually found as either rumen undegradable protein (RUP) and rumen degradable protein (RDP).

The former not only bypasses rumen degradation, but it also has the potential to be digested in the small intestine providing indispensable amino acids to the animal (Akhtar *et al.* 2016).

Fish meal and some protein supplements of plant origins are the main sources of RUP (McDonald *et al.* 2002). Studies have reported an increase in nitrogen balance and decreased nitrogen excretion in ruminants fed diets rich in RUP (Pattanaik *et al.* 2003; Paengkoum *et al.* 2004). It has been shown that protein requirement of ewes significantly increases in the late pregnancy due to a higher net protein requirement for udder development and colostrum production (Abd-Allah, 2013). Ramsey *et al.* (2000) reported a higher lamb survival rate in ewes fed 150 g/d of a pelleted concentrate containing 26% crude protein during the final stages of pregnancy.

Newborn ruminants are born as a gamma-globulinaemic, because no antibodies are transferred to the fetus via the placenta by them other. Thus, they are completely dependent on gaining passive immunity from their mothers after birth by consuming colostrum.

The colostrum immunoglobulins, particularly IgG, along with the ability of the neonatal gut to help unrestricted passage of the large immunoglobulin molecules, provide the newborns with passive immunity (Boucher, 2014). Thus, it is essential to provide enough dietary protein in the late pregnancy to support colostrum production of high quality.

In the dairy ewe industry of Iran a few studies have also evaluated the effect of RUP sources on postpartum performance. Thus, this study was conducted to investigate the effects of RUP sources during late pregnancy on performance, milk yield and blood metabolite profile of postpartum Lori ewes.

MATERIALS AND METHODS

Chemical and ethics
All procedures used were approved by the Standard Ethical Committee of Arak University (Arak-Iran: No. T5235) for care and treatment of animals. Unless otherwise indicated, all reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Animals
In present studyforty pregnant Lori ewes were used. Estrus synchronization was performed in the breeding season by using intra vaginal progesterone sponges (Hipra Co., Spain) on the first day, with administration of 400 IU of PMSG (Hipra Co., Spain) on day 12. Following the administration of PMSG, ewes were introduced to rams for mating. Ewes had free access to pasture from mating until the fourth month of pregnancy, and were then individually housed in crates, measuring 1.0×1.5 m, until parturition. There was free access to water. They were fed a total mixed ration twice daily at 09:00 and 17:00 h, in amounts to allow for 5% to 10% refusals. The diets were formulated according to NRC (2007) (Table 1).

Animals were used in a completely randomized design trial of four dietary treatments: 1) basal diet (control); 2) containing 5% fish meal; 3) containing 5% extruded soybean; and 4) 2.5% fishmeal and 2.5% extruded soybean. These diets were fed from 30 days before the estimated parturition date. Extruded full-fat soybean used in this study was prepared by wet extruding in an Anderson expander-extruder cooker at 120 ˚C. The formulated diets were isocaloric and isonitrogenous (Table 1).

Colostrum sampling
In order to record colostrum production, both udders of the ewes were covered with cotton clothes. Oxytocin (5 IU) was administered intramuscularly to each ewe and then both udders were milked. Lambs were bottle-fed by for the first 6 h after birth.

Colostrum samples were collected 6 and 12 h after lambing, and stored at -20 ˚C until they were analyzed for IgG by ELISA method, as described by Daniels *et al.* (2000). Colostrum and milk concentrations of fat, lactose and protein were determined using Milk-O-Scan 133B analyzer (FOSS Electric A/S, Hillerød, Denmark).

Blood chemical analyses
Blood samples from each ewe were taken10 days before the expected lambing date, and at parturition. They were obtained via coccygeal venipuncture into vacuum tubes without any anticoagulant, and, then centrifuged immediately at 3000 rpm for 15 minute at room temperature. Serum was harvested and stored at -20 ˚C. Glucose, total protein, blood urea nitrogen (BUN), non-esterified fatty acid (NEFA), and beta-hydroxybutyric acid (BHBA) concentrations in the serum were analyzed with enzymatic colorimetry using an auto-analyzer (ALYCON 300, USA), with special enzymatic kits.

Body weight and body condition score
Body condition score (BCS) and body weight (BW) of each ewe was recorded weekly throughout the experiment. The BCS was assessed based on a 5-point scale (Russel, 1984).

Table 1 Feed and chemical composition of experimental diets

Item (%)	Control	Extruded soybeans	Fish meal	Combination form
Alfalfa hay	15.00	15.00	15.00	15.00
Corn silage	5.00	5.00	5.00	5.00
Wheat straw	25.00	25.00	25.00	25.00
Barley grain	10.00	10.00	10.00	10.00
Beet pulp	10.00	10.00	10.00	10.00
Soybean meal	17.00	13.00	9.50	7.00
Extruded full-fat soybean	0.00	5.00	0.00	9.00
Fish meal	0.00	0.00	5.00	2.50
Wheat bran	16.50	15.20	19.50	15.20
Mineral and vitamins[1]	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Carbonate	-	0.30	0.00	0.20
DCP	0.50	0.50	0.00	0.10
Chemical composition				
Dry matter (DM, %)	78.40	78.30	78.20	78.30
Metabolizable energy (Mcal/kg)	2.28	2.34	2.32	2.34
Crude protein (% DM)	16.00	16.00	16.00	16.00
Rumen degradable protein (% DM)	12.00	8.50	8.10	8.30
Rumen undegradable protein (% DM)	4.00	7.50	7.90	7.70
Neutral detergent fiber (% DM)	46.10	46.10	47.00	46.50
Acid detergent fiber (% DM)	27.60	27.70	27.60	27.70
Calcium (% DM)	0.67	0.68	0.68	0.68
Phosphorous (% DM)	0.43	0.43	0.47	0.43

[1] Composition per kg contained: vitamin A: 2000000 IU; vitamin D_3: 40000 IU; vitamin E: 400 mg; Mn: 12.80 g; Zn: 9.00 g; I: 1.56 g; Fe: 6.42 g; Cu: 1.60 g; Co: 50 mg and Se: 32 mg.

Statistical analysis

The following mixed model (model 1) was used for the analysis of repeated measurement of blood chemical analysis (Littell *et al.* 1998):

$$y_{ijk} = \mu + G_i + a_{ij} + T_k + GT_{ik} + e_{ijk} \text{ (model 1)}$$

Where:

y_{ijk}: repeated measurment recorded in relation to j^{th} animal in i^{th} treatment and at k^{th} time.

μ: overall mean.

G_i: fixed effect of ith treatment.

a_{ij}: random error with mean 0 and variance σ^2_a which is the covariance between two sequential records of each animal.

GT_{ik}: interaction effect between treatments i and time k.

e_{ijk}: random error with the mean 0 and variance σ^2_e which is equal to the variance between measurements within the animals.

To analyze the other traits, we used the following linear model:

$$y_{ij} = \mu + G_i + b (x_{ij} + X) + e_{ij} \text{ (model 2)}$$

Where:

y_{ij}: j^{th} observations of the trait in i^{th} treatment.

b: regression of the dependent trait (milk production, udder volume, lamb birth weight) on ewe body weight

x_{ij}: body weight as a covariate.

In the model 2, lamb birth weight was considered as a covariate to analyze the weaning weight. Data for milk chemical component were analyzed using model 2 without including the covariate. All data were analyzed using SAS software v. 9.2 (SAS, 2003). Duncan's multiple range tests were used for comparisons of means, and significance was declared at $P \leq 0.05$. Lambing and survivability rates (percentages) were calculated as follows (Olivier, 2014):

Lambing rate= (lambs born/ewes inseminated) × 100
Survivability= (lambs weaned/lambs born alive) × 100

RESULTS AND DISCUSSION

Effects of the experimental treatments on lambing and survivability are shown in Table 2. The diet supplemented with RUP sources had no significant effect on reproductive performance.

Dry matter intake (DMI), neutral detergent fiber intake (NDFI), metabolism energy intake (MEI) and crude protein intake (CPI) were also not affected by the addition of RUP to the diet (P>0.05) (Table 3).

Table 2 Effects of dietary rumen-undegradable protein sourceson lambing and weaning percentages (%) in late gestation of Lori ewes

Groups	Survivability	Lambing
Control	100.00	100.00
Fish meal	100.00	100.00
Extruded soybeans	100.00	100.00
Fish meal + extruded soybeans	100.00	100.00
P-value	NS	NS
SEM	0.2	0.5

SEM: standard error of the means.
NS: non significant.

Table 3 Effects of dietary rumen-undegradable protein sources on feed intake in late gestation of Lori ewes

Groups	DM (kg/day)	NDF (g/day)	CP (g/day)	ME (MJ/day)
Pre-parturition				
Control	1.58	14.90	252.80	16.40
Fish meal	1.54	14.70	246.40	16.60
Extruded soybeans	1.53	15.30	244.80	16.58
Fish meal + extruded soybeans	1.62	16.60	259.20	17.20
P-value	NS	NS	NS	NS
SEM	0.15	1.14	3.58	0.84

DM: dry matter; NDF: neutral detergent fiber; CP: crude protein and ME: metabolizable energy.
SEM: standard error of the means.
NS: non significant.

Ewes fed diets containing fish meal in late pregnancy were heavier at lambing than control ewes and ewes fed diets supplemented with a mixture of fish meal and extruded soybeans ($P<0.05$), but similar to ewes fed diets containing extruded soybeans (Table 4). Lambs of ewes fed diets supplemented with fishmeal and extruded soybeans were heavier at birth and weaning than those born from control and fishmeal plus extruded soybeans supplemented ewes ($P<0.05$). However, there were no difference observed for BCS between treatment groups ($P>0.05$).

The fat tail length in the control, extruded soybean and combined form of fish meal and extruded soybean groups was the same, but in the treatment fish meal group was it was longer ($P<0.05$) (Table 5).

The effects of treatment on the quality of colostrum are presented in Table 6. Dietary supplementation of ewes with fishmeal alone or in combination with extruded soybeans in late pregnancy improved colostrum concentration of crude protein (CP) ($P<0.05$), but had no significant effect on fat and lactose contents. The highest colostrum yield was observed in ewes supplemented with fishmeal alone or in combination with extruded soybeans treatments. Colostrum concentration of IgG was greatest in the ewes supplemented with fishmeal ($P<0.05$).

Effects of dietary inclusion of RUP sources on milk yield and milk compositions are shown in Table 7. The treatments had no significant effects on milk concentrations such as fat, CP, lactose and dry matter percentage ($P>0.05$), but milk urea nitrogen (MUN) was significantly higher in ewes supplemented with extruded soybeans compared to control ewes and those supplemented with a combination of fishmeal and extruded soybeans ($P<0.05$).

Serum metabolites of ewes pre-partum and post-partum are shown in Table 8. Serum concentration of glucose was significantly decreased in ewes fed diets containing RUP before lambing ($P<0.05$), but after lambing glucose concentration was only decreased when ewes were fed diets containing fishmeal, either without or with extruded soybeans ($P<0.05$). Serum concentration of NEFA was significantly decreased in ewes fed diets supplemented with a combination offish meal and extruded soybeans pre-partum ($P<0.05$).

However, it was significantly lower in control and extruded soybeans-fed ewes compared to those receiving fishmeal post-partum. Serum concentration of BHBA was significantly decreased in the treated groups in comparison to the control group post-partum ($P<0.05$). Serum concentration of protein was not significantly influenced by the experimental treatments pre- and post-partum ($P>0.05$). Also, blood urea nitrogen (BUN) levels post-partum and IgG concentration during pre-partum were not affected by the treatments. The greatest value for pre-partum BUN was observed in fish meal and extruded soybeans fed ewes followed by control ewes, fish meal-fed and extruded soybeans-fed ewes ($P>0.05$). Post-partum serum concentration of IgG was significantly greater in ewes supplemented with fishmeal than the other ewes, with the lowest value for control ewes ($P>0.05$).

During pregnancy and early lactation dairy ewes in Iran are often underfed, resulting in an energy deficient intake. This leads to an increase in metabolic disease such as such as ketosis and fatty liver, both of which decrease lactation performance reproductive efficiency as reported in dairy cows (Ospina *et al.* 2010).

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Table 4 Effects of dietary rumen-undegradable protein sources on production performance of ewes and their lamb

Groups	WAL (kg)	BW (kg)	WW (kg)	ADG (kg)	BCSB (score)	BCSA (score)
Control	52.44[b]	3.69[b]	31.40[b]	0.308	3.36	3.49
Fish meal	58.59[a]	4.44[a]	37.70[a]	0.325	3.17	3.06
Extruded soybeans	55.07[ab]	4.46[a]	34.40[a]	0.325	3.31	3.14
Fish meal + extruded soybeans	51.92[b]	3.92[b]	32.50[b]	0.318	3.34	3.28
P-value	*	*	*	NS	NS	NS
SEM	4.58	0.36	1.11	0.022	0.24	0.22

WAL: weight after lambing; BW: birth weight of lambs; WW: weaning weight of lambs; ADG: average daily gain of lambs; BCSB: body condition score before parturition and BCSA: body condition score after parturition.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* (P<0.05).
NS: non significant.

Table 5 Body measurements in Lori ewes fed rumen-undegradable protein sources in late gestation

Groups	WH (cm)	SR (cm)	HR (cm)	TL (cm)
Control	41.20	38.70	26.60	10.80[b]
Fish meal	40.90	40.30	26.40	13.40[a]
Extruded soybeans	39.81	37.10	26.10	11.80[b]
Fish meal + extruded soybeans	40.00	38.40	26.90	10.90[b]
P-value	NS	NS	NS	*
SEM	2.30	4.80	0.80	1.30

WH: withers height; SR: shoulder round; HR: head round; TL: fat tail length; LRT: lamb rectum temperature and ERT: ewe rectum temperature.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* (P<0.05).
NS: non significant.

Table 6 Effects of rumen-undegradable protein sources on colostrums content in late gestation of Lori ewes

Groups	DM (%)	CP (%)	Fat (%)	Lactose (%)	Volume (mL)	IgG (mg/dL)
Control	25.60	8.40[b]	11.20	5.20	228.70[c]	4212.60[c]
Fish meal	24.40	9.30[a]	11.20	5.50	256.70[b]	5260.80[a]
Extruded soybeans	25.40	7.60[b]	11.60	5.60	257.80[b]	4480.40[bc]
Fish meal + extruded soybeans	25.80	9.20[a]	12.40	5.90	332.00[a]	4771.30[b]
P-value	NS	*	NS	NS	**	**
SEM	0.60	0.60	1.60	0.10	5.02	150.00

DM: dry matter and CP: crude protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* (P<0.05) and ** (P<0.01).
NS: non significant.

To counteract these responses the present trial was undertaken, making use of the growing knowledge regarding the role of RUP and its effect on digestibility. In the current experiment the difference in reproductive performance, milk yield and milk composition between control (basal diet) with different sources of RUP in late pregnancy is probably due to differences in DMI. In the present study, lambing rate and lambs' survivability were not affected by the supplementation of RUP sources in late pregnancy. In line with the current results, Hoaglund *et al.* (1992) showed that dietary inclusion of different sources of RUP had no significant effect on lamb's survivability. However, Encinias *et al.* (2004) reported that lambs born from ewes fed a high fat diet during late pregnancy had a greater survivability in compared to lambs born from the ewes fed a low fat pre-partum diet. Annett *et al.* (2008) reported that dietary inclusion of RUP improved lamb survivability.

In another study, supplementation of ewes in late pregnancy with 454 g/d of a pellet containing 20% CP improved lambs' survival (Burfening and Kott, 1993). The conflicts in previous studies may be explained by breed, amount and type of basal diet and different types of grain in the diet.

With RUP supplementation for 30 d in late pregnancy, feed intake variables efficiency was similar across all treatments.

Everts (1990) reported that nutrient requirements of ewes drastically increase during late pregnancy due to the increased embryo growth. In such a situation, ewes may benefit from supplementary protein with low rumen degradability. However, in line with our results, Annett *et al.* (2008) also reported that increasing the level of RUP in the diet had no effect on DMI, NDF and intake of protein (what does this mean, is this a substitution or a supplement- clarify).

Table 7 Effects of rumen-undegradable protein sources in late gestation on milk composition of Lori ewes

Groups	DM (%)	Fat (%)	CP (%)	Lactose (%)	MUN (mg/dL)
Control	10.10	6.92	4.80	5.12	8.14[b]
Fish meal	10.20	5.85	4.98	5.08	9.21[ab]
Extruded soybeans	11.00	5.83	4.79	5.02	10.05[a]
Fish meal + extruded soybeans	11.80	5.05	4.93	5.21	9.46[ab]
P-value	NS	NS	NS	NS	*
SEM	0.96	0.08	0.60	0.12	0.46

DM: dry matter; CP: crude protein and MUN: milk urea nitrogen.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* (P<0.05).
NS: non significant.

Table 8 Effects of rumen-undegradable protein sources on blood metabolite profiles in late gestation of Lori ewes

Groups	Glucose (mg/dL)	NEFA (mmmo/dL)	BHBA (mmol/dL)	Protein (g/dL)	BUN (mg/dL)	IgG (mg/mL)
Pre-partum						
Control	152.67[a]	0.171[a]	0.47	3.23	16.11[a]	22.50
Fish meal	115.67[b]	0.116[b]	0.52	3.73	14.45[a]	23.60
Extruded soybeans	87.05[b]	0.065[c]	0.57	3.81	10.22[b]	22.80
Fish meal + extruded soybeans	91.93[b]	0.113[b]	0.47	3.63	16.19[a]	21.90
P-value	**	*	NS	NS	**	NS
SEM	2.54	0.11	0.14	0.59	2.83	1.70
Post-partum						
Control	185.16[a]	0.206[b]	0.55[a]	3.53	18.79	37.60[c]
Fish meal	173.68[a]	0.450[a]	0.30[c]	3.94	18.63	55.30[a]
Extruded soybeans	110.58[b]	0.141[b]	0.44[b]	3.60	22.11	43.70[bc]
Fish meal + extruded soybeans	151.54[a]	0.450[a]	0.33[c]	3.79	22.48	47.50[b]
P-value	**	*	**	NS	NS	*
SEM	28.4	0.19	0.06	0.76	6.25	4.20

NEFA: non-esterified fatty acids; BHBA: beta-hydroxy butyrate and BUN: blood urea nitrogen.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* (P<0.05) and ** (P<0.01).
NS: non significant.

Hence, understanding the rate of feed intake through the dairy ewes at a late pregnancy period could offer the potential to help develop improved nutritional management programs for dairy farming.

Feeding Lori ewes in late pregnancy with diets supplemented with fishmeal and extruded soybeans improved ewes' body weight at lambing and resulted in heavier lambs at birth and weaning (Table 4). It has been demonstrated that inadequate intake and unbalanced feed during late pregnancy decreases birth weight of the lambs (Mellor and Murray, 1985). Hatfield *et al.* (1995) have also shown that birth weights of lambs were significantly higher in ewes fed higher levels of protein in comparison to those fed lower levels in late pregnancy. In contrast to our finding, Annett *et al.* (2008) reported that feeding ewes with large amounts of RDP during late pregnancy had no effect on ewes' live weight, but dietary inclusion of fish oil led ewes to lose 3.8 kg more live weight than those receiving no fish oil.

Current findings showed that supplemented ewes with fishmeal alone or in combination with extruded soybeans resulted in colostrum with a raised CP content.

The colostrums of ruminants is a complex of lacteal secretions and compounds from blood serum, which consist of immune-globulins and other serum proteins (Foley and Otterby, 1978). Under-nutrition of ewes can decrease the contents of lactose, lipids and protein in colostrum (Hashemi *et al.* 2008).

Therefore, supplying sufficient dietary protein is essential in late pregnancy to produce high quality colostrum (Ocak *et al.* 2005). O'Doherty *et al.* (1998) showed that supplementing ewes with a protein source such as soybean meal in late pregnancy increased colostrum production 1-18 h after birth. In the present study, supplementing ewes with a combination of fishmeal and extruded soybean improved colostrum concentration of IgG, which could be attributed to the improved amino acids profile of the diet. IgG is the most predominant (Thompson *et al.* 2013) immune-globulin in colostrum (Mech *et al.* 2011), and is responsible for pathogen clearance (Mech *et al.* 2011), which protects the neonate from pathogens.

Milk production and milk compositions are of great economic significance for dairy ewe farmers.

Milk urea nitrogen concentration can be used as a management tool to monitor protein metabolism and protein intake in lactating ewes (Cannas *et al.* 1998). Feeding ewes with diets containing extruded soybeans in the late pregnancy resulted in milk with higher MUN content than ewes on the control diet. This may indicate that they may have received more rumen degraded protein than the control ewes.

During a period of NEB blood concentration of glucose, insulin and Insulin-like growth factor-1 decrease (Grummer *et al.* 1995), raising negative factors for successful reproductive performance. Alleviation of this condition (NEB) can be achieved by increasing dietary energy density, by increasing the concentrates to forage ratio or the use of an RUP supplement in the diet.

Glucose is considered as the main source of energy for the fetus. Increased glucose concentration in pregnant ewes could be due to metabolic capacity of the ewes under severe stress, because increased size of fetus raises the stress on maternal carbohydrate metabolism (Sigurdsson, 1988) (clarify, point not clear). Sporleder (1998) also reported that insulin responsiveness was significantly decreased in sheep in late pregnancy with reduced glucose turnover and intake through muscle and fat tissues. In the present research, pre-partum serum glucose concentration was significantly higher (at what stage before lambing) in the control group compared to treated groups (Table 8). It seems that supplementing ewes with RUP sources decreased the tissue breakdown of proteins to supply glucogenic amino acids for providing glucose in late pregnancy.

Protein catabolism and increased requirement for energy in ewes during late pregnancy may increase urea levels to an extent above the ability of the kidneys to excrete the excessive urea from plasma. Blood urea N can also be used to monitor nitrogen metabolism in ruminants, and a higher BUN could be an indication of a higher rumen degradation of protein (Broderick and Clayton, 1997). The lower pre-partum BUN in ewes fed extruded soybeans compared to the other treatments indicated that there was less protein degraded in the rumen. However, in the present study, dietary treatments had no effect on BUN concentration. Lane *et al.* (2000) showed that plasma BHBA is an indication of ketosis. Several researchers have been shown that inclusion of forage or diet type had little effect on BHBA concentration (Terré *et al.* 2013; Laarman *et al.* 2012). Although plasma concentrations of BHBA were in the normal range reported for sheep (Kaneko, 1989), late pregnancy supplementation of ewes with fishmeal, extruded soybeans or their combination reduced plasma BHBA concentration compared with control ewes. Ewes fed diets supplemented with sources of RUP in late pregnancy also had lower plasma concentrations of NEFA pre-partum. Together with the lower post-partum plasma BHBA, the lowered pre-partum NEFA concentration indicated that ewes supplemented with extruded fishmeal and extruded soybeans may be less prone to ketosis than control ewes.

CONCLUSION

The results suggested that the addition of RUP grains can be advised for improving ewes' body weight at lambing and colostrum concentration of IgG, and reduce the presence of ketosis disorder during late pregnancy. Thus, RUP supplementation could be recommended under current feeding situations of dairy ewes.

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