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# Performance, Health Status, and Colostrum Yield of Twin-Bearing Afshari Ewes as Well as Growth and Survival of Their Offspring are not Affected by Increasing Dietary Metabolizable Protein in Late Pregnancy

Research Article

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## ABSTRACT

The aim of this study was to investigate the effects of two dietary metabolizable protein (MP) concentrations on the performance and health status of Afshari ewes and survival and growth of their lambs during late pregnancy. For 6 weeks prior to lambing, 32 Afshari ewes were randomly assigned to two dietary treatments, containing low (LMP) or high (HMP) MP concentrations. The ewes in LMP (n=16) and in the HMP (n=16) were individually fed with isoenergetic (2.39 Mcal ME/kg DM) diets that contained 99.4 and 116.5 g crude protein (CP) and 70.5 g and 84.6 g MP/kg dry matter (DM) respectively. The concentration of MP in the late pregnancy diet did not affect changes in body weight and body condition score of ewes as well as blood glucose, total protein, non-esterified free fatty acids (NEFA), β-hydroxy butyrate (βHBA), insulin concentrations, insulin sensitivity and total number of white blood cells (WBC), red blood cells (RBC) and other blood cells. Similarly, the amounts and composition of colostrum obtained during the first 24 h after lambing were not affected by MP level. It was concluded that increasing the MP content of the diet for 6 weeks prior to lambing above the standard requirements resulted in no benefit in terms of the productive performance and health indices of twin-bearing ewes and their offspring.

KEY WORDS gestation, lamb performance, metabolizable protein, nutrition, sheep.

## INTRODUCTION

The last third of pregnancy is the most important period regarding maternal metabolism and fetal growth (Symonds and Clarke, 1996), Eighty percent of fetal development occurs in the last 2 months of pregnancy leading to a significant increase in nutrient requirements of the ewe (Bell, 1995; Dawson *et al.* 1999; NRC, 2007). On the other hand, ketosis or pregnancy toxemia is a real problem for ewes bearing more than one fetus (Moallem *et al.* 2010) during the last 4-6 wk of gestation. A combination of 1) the increased ewe's net protein requirements for udder growth and colostrum production (Robinson, 1985). 2) the decreased voluntary feed intake (Orr and Treacher, 1984). 3) the elevated requirements for gluconeogenesis from amino acids to alleviate pregnancy toxemia (Amanlou *et al.* 2011) and 4) the higher growth rates of the fetus in the third part of gestation suggest that excess crude protein (CP) or meta-

bolizable protein (MP) should be fed. This is stated in the latest version of national research council (NRC, 2007) recommendations compared to the older versions.

The literature contains contradictory results regarding the increasing of CP or MP concentrations during the last one-third of pregnancy. Van Emon *et al*. (2014) showed that when ewes fed diets similar in total energy with increased MP (60, 80 and 100% of NRC recommendations) during late gestation, they gained body weight (BW) and body condition score (BCS), but the increased MP had minimal effects on lamb birth weights or weaning weights. Accordingly, our previous study (Amanlou *et al*. 2011) showed that increasing MP concentration to 14 and 24% higher than NRC (2007) recommendations resulted in greater colostrum production, but there was no difference in ewes BW changes and lamb birth BW as well as weaning performance. In contrast, Ocak *et al*. (2005) showed that increasing dietary CP during late pregnancy in singleton-bearing ewes resulted in lower colostrum yield, lambing difficulty and reducing lamb survival rate prior to weaning. On the contrary McNeill *et al*. (1997) and Dawson *et al*. (1999) did not observe any effects of higher concentration of MP on the performance of pregnant ewes with regard to colostrum yield and postnatal lamb survival. Part of these discrepancies could be attributed to the source of protein and the share of degradable *vs*. un-degradable protein (UDP). Nevertheless, little research has been conducted to evaluate the effects of low MP concentration on the immune system and blood metabolites related to insulin sensitivity in periparturient sheep. Houdijk *et al*. (2000) showed that high MP can enhance the expression of immunity of twin-bearing ewes. In this study, we hypothesized that the changes in body weight, body condition score and selected blood parameters of ewes in late pregnancy as well as the yield and composition of colostrum and survival and growth of lambs obtained from these ewes can be affected by increasing MP via high rumen degradable protein (RDP) sources (corn gluten meal and fish meal).

## MATERIALS AND METHODS

### Animals and feeding

The study was conducted at the experimental farm of the University of Zanjan, Zanjan, Iran. Multiparous Afshari ewes (n=32) were synchronized using controlled intravaginal drug release device (CIDR; Eazi-Breed CIDR; Pharmacia and Upjohn Pty Limited, Rydalmere, Australia). The CIDR devices were inserted on d-14 and on d 0 CIDRs were removed and lambs were injected by 400 units of pregnant mare's serum gonadotrophin (PMSG; Intervet Inc., Millsboro, DE). Forty-eight hours after removal, ewes were mated with Afsahri rams (5 ewes per each ram).

Breeding marks were observed 3 times per week to determine day of breeding. Expected lambing date was determined by breeding marks. All ewes were maintained on pasture and supplemented with alfalfa hay, corn silage and concentrate until day 85 of pregnancy. Thereafter, ewes were fed the Adaptation diet. Six weeks prior to expected parturition ewes were allocated to one of two treatment groups, namely low (LMP) and high (HMP) MP and housed in individual stalls (2 $m^2$). The ewes in LMP (n=16) and in the HMP (n=16) were individually fed with isoenergetic (2.39 Mcal ME/kg DM) diets that contained 99.4 and 116.5 g crude protein (CP) and 70.5 g and 84.6 g MP/kg dry matter (DM) respectively. The diets were formulated by the CNCPS software (Cannas *et al*. 2004). The composition of the experimental diets is shown in Table 1.

**Table 1** Composition of diets used to evaluate the effects of dietary metabolizable protein concentration during late pregnancy on ewe and lamb performance

| Ingredient composition (% DM) | LMP | HMP |
|---|---|---|
| Alfalfa hay | 25 | 25 |
| Corn silage | 31.89 | 32 |
| Barley straw | 11.45 | 11.57 |
| Barley grain | 30 | 24.46 |
| Soybean meal (440 g CP/kg, solvent) | 0.67 | 4.48 |
| Corn gluten meal | 0 | 1 |
| Fish meal | 0 | 0.5 |
| Vitamin mix[1] | 0.5 | 0.5 |
| Mineral mix[2] | 0.5 | 0.5 |
| Nutrient composition (g/kg DM) | | |
| Crude protein CP | 99.4 | 116.5 |
| Rumen degradable protein (% CP)[3] | 70.92 | 70.17 |
| Rumen undegradable protein (% CP)[3] | 29.08 | 29.83 |
| Metabolizable protein | 70.5 | 84.6 |
| Metabolizable energy (Mcal/kg DM) | 2.39 | 2.39 |
| Ether extract | 22.0 | 25.2 |
| Ca | 5.8 | 8.2 |
| P | 3.4 | 4.8 |

[1] Vitamin mix: vitamin A: 1500000 IU/kg; vitamin D: 400000 IU/kg and vitamin E: 6000 IU/kg as guaranteed by the supplier.
[2] Mineral mix: Ca: 200 g/kg; Mg: 90 g/kg; Co: 35 mg/kg; Cu: 3500 mg/kg; I: 151 mg/kg; Fe: 17500 mg/kg; Mn: 13500 mg/kg; Se: 90 mg/kg and Zn: 14300 mg/kg as guaranteed by the supplier.
[3] MAFF (1990), for a rumen outflow rate of 0.02 h-1.
LMP: low metabolizable protein and HMP: high metabolizable protein.
CP: crude protein and DM: dry matter.

The diets were individually offered, *ad libitum* as a total mixed ration (TMR) thrice a day at 0800, 1600 and 2400 h. Ewes had access to clean water. Groups were homogeneous in BW (~94.05 kg), BCS (~3.5), age (second lactation period) and milk yields and litter size of the previous lactation period.

### Measurements

The amount of TMR offered and orts were weighted daily. Samples of all TMR and diet ingredients were analyzed for DM (AOAC, 2006; ID 934.01), CP (AOAC, 2006; 984.13).

The BW and BCS (1-5 scale; Russell *et al*. 1969; Aliyari *et al*. 2012; Vatankhah *et al*. 2012) of ewes were recorded before morning feeding on a weekly basis pre-lambing and post-lambing, within 24 h of parturition and at weaning. The colostrum yield was determined within 24 h after birth by calculating the difference between pre- and post-sucking weights of lambs (Ocak *et al*. 2005) and milking method to inject oxytocin was measured (Purroy and Jaime, 1995). Immediately after lambing, the colostrum were sampled and analyzed for fat, protein, lactose and solids non fat (SNF) using a Milk-O-Scan minor (78110; Foss, Denmark), and placenta weight was measured using a standard protocol (Benirschke, 1961). Blood samples were obtained by jugular venipuncture 1 h pre-morning feeding weekly pre-lambing using heparinised syringes. Blood was centrifuged at 2000 × g for 15 min to harvest plasma, which was stored at -20 ˚C for later analysis. Plasma glucose, blood urea nitrogen (BUN), albumin, total protein, cholesterol, creatinine (Pars Azmun Laboratory, Tehran, Iran), β-hydroxybutyrate (βHBA; Abbott Diabetes Care Ltd. Rang Road. Witney, Oxin, OX29 OYL., UK) and non-esterified free fatty acids (NEFA) concentrations (NEFA-HR(2) assay kit, Wako Chemicals GmbH, Neuss, Germany) were determined enzymatically using commercially available kits. The plasma samples were analyzed by a BT 1500 automatic biochemistry analyzer (Biotechnica Instruments S.p.A, Rome, Italy) which simultaneously measures plasma samples for all the aforementioned diagnostic tests. Insulin concentrations were determined with an ELISA kit (Mercodia Ovine Insulin ELISA, Mercodia AB; Uppsala, Sweden). Aspartate amino transferase (AST) was analyzed using a kit (AST kit; Pars Azmun kits. Pars Azmun Laboratory, Tehran, Iran) based on the method of Persijn and Vander Slik (1976).

Red blood cells (RBC) and white blood cells (WBC) were counted with hemocytometers. Packed cell volume (PCV) was determined using the microhaematocrit method.

Haemoglobin (Hb) concentration was measured by the cyanmethaemoglobin method (Schalm *et al*. 1975).

To indirectly evaluate insulin homeostasis and sensitivity, a homeostasis model of insulin resistance (HOMA-IR) and an insulin sensitivity check index (RQUICKI) were calculated according to the equations suggested by Muniyappa *et al*. (2008):

HOMA-IR= [glucose (mmol/mL) + insulin (μU/mL)] / 22.5

QUICKI= 1 / [log (glucose in mg/dL) + log (insulin in μU/mL)]

RQUICKI= 1 / [log (glucose in mg/dL) + log (insulin in μU/mL) + log (NEFA in mmol/L)]

RQUICKIβHB= 1 / [log (glucose in mg/dL) + log (insulin in μU/mL) + log (NEFA in mmol/L) + log (βHBA in mmol/L)]

**Statistical analysis**

The BW, BCS changes, dry matter intake (DMI), colostrum yield and composition and selected blood parameters of ewes were analyzed using the MIXED procedure of statistical analysis system (SAS, 2003). Repeated measures analysis was performed for DMI, BW, and BCS data using autoregressive (1). Lamb sex was proven not to be significant for these dependent variables and was excluded from the model. Significance was declared at ($P<0.05$) and trends at ($P<0.10$).

## RESULTS AND DISCUSSION

The average daily DMI during the pre-partum period was unaffected by MP ($P>0.10$; Table 2).

**Table 2** The dry matter intake, body weight (BW), body condition score (BCS), gestation length, placenta weight and lamb performance, of ewes offered different concentrations of metabolizable protein during late pregnancy

| Items | LMP | HMP | SEM | P-value |
|---|---|---|---|---|
| Dry matter intake (kg/d) | 1.93 | 1.98 | 0.15 | NS |
| BW of ewes (kg) | | | | |
| At beginning of experiment | 94.00 | 94.10 | 5.43 | NS |
| At pre lambing | 98.56 | 99.76 | 5.49 | NS |
| At post lambing | 90.32 | 87.56 | 6.79 | NS |
| At 30 d after lambing | 86.15 | 82.21 | 1.41 | NS |
| At 50 d after lambing | 80.51 | 78.12 | 5.94 | NS |
| BW change of ewes (kg) | | | | |
| At the last 6 weeks of pregnancy | 4.56 | 5.66 | 1.96 | NS |
| Between 1 d pre-till 1 d post lambing | -8.26 | -12.20 | 4.86 | NS |
| BCS of ewes | | | | |
| At beginning of experiment | 3.47 | 3.53 | 1.41 | NS |
| At pre lambing | 3.73 | 3.67 | 0.38 | NS |
| BCS change from 105 days gestation to lambing | +0.26 | +0.14 | 0.08 | NS |
| Gestation length (d) | 149.83 | 149.57 | 0.5 | NS |
| Litter size | 1.67 | 1.81 | 0.34 | NS |
| Mortality rate (live births to weaning %) | 4.00 | 3.45 | 0.35 | NS |
| Placenta weight (g) | 732.17 | 806.86 | 70.82 | NS |
| BW of lambs (kg) | | | | |
| At birth weight (kg) | 4.88 | 4.84 | 0.17 | NS |
| At 1 week (kg) | 6.10 | 6.17 | 0.26 | NS |
| At 3 weeks (kg) | 10.37 | 10.44 | 0.42 | NS |
| Average daily gain (kg) | 0.295 | 0.261 | 0.02 | NS |

LMP: low metabolizable protein and HMP: high metabolizable protein.
SEM: standard error of the means.
NS: non significant.

There was no difference between BW change, at lambing, or at 30 d and 50 d after lambing ($P>0.10$, Table 2). The addition of protein pre-partum had no effect on the weights of the lambs at birth and the BW gains from birth to week 3 as well as on lamb survival ($P>0.10$; Table 2). Neither gestation length nor placenta weight was affected by the treatments (Table 2).

Results of milk yield and constituents for both treatments are presented in Table 3.

**Table 3** The yield and composition of colostrum produced by ewes offered different concentrations of metabolizable protein during late pregnancy

| Items | LMP | HMP | SEM | P-value |
|---|---|---|---|---|
| Colostrum yield (g/d) | 472.50 | 660.00 | 108.0 | NS |
| Colostrum yield (g/d) per kg lamb birth weight | 96.82 | 136.36 | 15.70 | NS |
| Composition (%) | | | | |
| Protein | 17.93 | 16.22 | 1.06 | NS |
| Fat | 13.23 | 12.77 | 0.77 | NS |
| Solids non fat | 21.64 | 20.26 | 1.06 | NS |
| Protein yield (g/d) | 84.72 | 107.05 | 13.75 | NS |
| Fat yield (g/d) | 62.51 | 84.28 | 10.14 | NS |
| Solids non fat yield (g/d) | 102.25 | 133.72 | 16.58 | NS |

LMP: low metabolizable protein and HMP: high metabolizable protein.
SEM: standard error of the means.
NS: non significant.

Colostrum yields in both groups showed no difference (P>0.1). A similar pattern was observed for colostrum composition (P>0.1). Results for blood parameters for both treatments are presented in Table 4.

**Table 4** The selected blood components of pre-lambing of ewes, offered different concentrations of metabolizable protein during late pregnancy

| Items | LMP | HMP | SEM | P-value |
|---|---|---|---|---|
| Glucose (mg/dL) | 60.75 | 68.75 | 4.90 | NS |
| BUN (mg/dL) | 24.24$^{b}$ | 31.69$^{a}$ | 1.14 | < 0.0001 |
| Colesterol (mg/dL) | 55.00$^{b}$ | 67.56$^{a}$ | 9.85 | 0.0161 |
| Albumin (g/dL) | 4.98 | 4.57 | 0.23 | NS |
| Total protein (g/dL) | 6.43 | 6.50 | 0.26 | NS |
| Globulin (g/dL) | 1.45 | 1.93 | 0.15 | 0.0659 |
| Cratinin (mg/dL) | 1.00 | 1.01 | 0.07 | NS |
| NEFA (mmol/L) | 0.25 | 0.37 | 0.07 | NS |
| βHBA (mmol/L) | 0.291 | 0.288 | 0.08 | NS |
| AST (U/L) | 55.00 | 56.25 | 3.62 | NS |
| Insulin (µIU/mL) | 4.14 | 1.91 | 1.11 | NS |
| HOMA-IR | 11.42 | 5.87 | 4.57 | NS |
| QUICKI | 0.45 | 0.55 | 0.09 | NS |
| RQUICKI | 0.62 | 0.73 | 0.12 | NS |
| RQUICKIβHB | 0.94 | 1.67 | 0.69 | NS |

LMP: low metabolizable protein and HMP: high metabolizable protein.
SEM: standard error of the means.
NS: non significant.
BUN: blood urea nitrogen; NEFA: non-esterified free fatty acids; βHBA: β-hydroxybutyrate; AST: aminotransferase and HOMA-IR: homeostasis model of insulin resistance.

As expected, the increment in dietary MP concentration pre-lambing increased blood urea nitrogen (BUN; P<0.05).

Also plasma cholesterol concentrations increased in high MP fed ewes (P<0.05). Other blood parameters including glucose, total protein, albumin, globulin, creatinine, NEFA and βHBA did not show any difference between treatments (P>0.10). In addition, AST concentration, as a health index of liver was not influenced by dietary changes (P>0.10). The supplementation with MP did not have any effects on insulin and insulin sensitivity (P>0.10).

Blood cell counts including WBC, RBC, hematocrit, neutrophil and monocytes were not affected by MP concentration (P>0.10; Table 5).

**Table 5** The selected blood components of pre-lambing of ewes, offered different concentrations of metabolizable protein during late pregnancy

| Items | LMP | HMP | SEM | P-value |
|---|---|---|---|---|
| White blood cells (/µL) | 7675.3 | 9025.1 | 1280.4 | NS |
| Red blood cells (106/µL) | 11.64 | 11.86 | 0.259 | NS |
| Neutrophil segmented (%) | 34.50 | 47.75 | 5.34 | NS |
| Lymphocyte (%) | 63.25 | 50.25 | 5.26 | NS |
| Monoocyte (%) | 1.00 | 0.50 | 1.23 | NS |
| Eosinophil (%) | 1.25 | 1.25 | 0.48 | NS |
| Hemoglobin (g/dL) | 11.17 | 12.32 | 0.53 | NS |
| Hematocrit (%) | 33.65 | 35.62 | 1.55 | NS |
| Platelets (105/µL) | 4.45 | 4.94 | 0.43 | NS |

LMP: low metabolizable protein and HMP: high metabolizable protein.
SEM: standard error of the means.
NS: non significant.

Although nutrient demands are greater during late gestation due to the rapid growth of the fetus, it appears that our results do not support our primary hypothesis. Daily DMI was similar between treatments which is in agreement with Houdijk *et al*. (2000) and Annett *et al*. (2008), which means that ewes on high MP diet received more protein. In contrast, Dawson *et al*. (1999) showed a decrease in DMI when adding digestible undegradable protein (DUP) to the diet, although ME intake probably had an effect on that observation because diets were not isoenergetic.

The current study showed that supplementation with MP to ewes during late pregnancy, neither affected their body weight nor lamb BW, which was in agreement with our previous reports in Afshari ewes (Amanlou *et al*. 2011) and in other breeds (Dawson *et al*. 1999).

Ocak *et al*. (2005) reported that feeding of pregnant ewes with 140% of the CP requirements during the last 6 weeks of pregnancy increased ewe live weight at lambing and lamb birth weight, which is in contrast with our observations in the present study. An explanation could be the high incidence of singleton-bearing ewes in their study *vs*. the twin-bearing frequency in the current one. Also ewes on the HMP group were given 120% of CP, much lower than the one given by the other authors. These discrepancies suggest that analysis should be obtained in ewes with equal litter size or data should be balanced for litter size effects. More recently and in contrast with the current study, Van Emon *et al*. (2014) in similar work with respect to litter size have shown that higher MP linearly increased BW and BCS at lambing, but the MP concentrations used in their experiment were 60, 80 and 100% of NRC recommendations so that their results would have been predictable. It should be considered that ewes in the present study were in a good body condition, so they were less likely to use dietary protein to replenish their own body reserves.

The present data suggest that for multiple-bearing ewes, supplementation with MP does not have any effects on lamb BW. The main parameters affecting this variable are litter size and sex.

In agreement with previous research (Dawson *et al.* 1999; Annett *et al.* 2008; Van Emon *et al.* 2014), in which differential MP concentrations were tested, the supplementation with DUP did not affect colostrum output linearly. However, Ocak *et al.* (2005) showed that offering high CP diet to ewes during late pregnancy decreased colostrum production. Our previous study (Amanlou *et al.* 2011) showed a tendency for higher colostrum production, probably because of higher concentration of DUP and CP compared to the current study. These discrepancies suggest that the type of protein [rumen undegradable protein (RUP) or rumen degradable protein (RDP) sources] used to increase CP in pre-lambing diets has differential effects on ewe and lamb characteristics.

The onset of colostrum production and the transition to milk secretion is controlled by a number of nutritionally sensitive hormones (De Louis *et al.* 1980) including progesterone, prolactin and glucocorticoids. Protein (soybean meal) supplementation during late pregnancy was shown by O'Doherty and Crosby (1996) to decrease serum progesterone on day 142 and 1 h post-lambing, leading to increased colostrum outputs. This is in agreement with the present data, but is of limited value because above that trial studied the effects of CP supplementation in ewes suffering from under-nutrition. Some studies have concluded that increasing pre-lambing dietary CP (Hatfield *et al.* 1995) or DUP when a constant CP concentration is maintained (Annett *et al.* 2005) did not affect CP content of colostrum. In agreement with Annett *et al.* (2008) and Dawson *et al.* (1999) neither colostrum composition nor colostrum components yield was affected by the current treatments. In agreement with our previous report (Amanlou *et al.* 2011) it appears that colostrum composition does not easily respond to dietary CP manipulation when diet CP is between 9 to 12%. In a review, Robinson *et al.* (1998) concluded that, in cows, additional amino acids (AA) increased milk protein yield (12 of 12 reviewed) and milk fat yield (9 of 12 reviewed), but there was no response at moderate concentrations which is in agreement with the current results.

Our results did not show a significant difference between treatment groups in lamb birth weight (Table 2). According to McNeill *et al.* (1997), increasing dietary CP concentration from 79 g CP/kg DM to 116 g CP/kg DM for twin bearing ewes resulted in higher lamb birth weight, while increasing CP to 157 g CP/kg DM did not affect lamb birth weight. Considering that AA transfer from the maternal circulation to fetal tissues is highly regulated and the low ability of the placenta to transfer the excess AA to the fetus (McNeill *et al.* 1997), it has been reported (Overton, 1999) that increasing dietary CP concentration above the requirements during the late pregnancy seems unlikely to have an important effect on the birth weights of calves and lambs.

On the other hand, Ocak *et al.* (2005) reported that high protein diet pre-partum increased lamb birth weight, but we did not report a similar effect (Amanlou *et al.* 2011). The lack of a similar response in the current study might be ascribed to the shorter experimental period, or it seems that diet LMP (9.94% CP) was adequate in covering protein needs of pregnant ewes. Indeed, the calculation of protein needs at the end of the trial suggested that LMP diet covered the requirements of ewes. The controversial reports on this issue are probably related to the concentration of CP in the basal diets, the concentration of CP increment, and especially the amount of RDP that is not transformed to MCP.

As expected, diet HMP had higher BUN (Table 4), probably due to lower efficacy of RDP transformation to MCP (Table 1). The concentration of BUN is known to be a function of dietary CP content, rumen degradability of CP and energy intake (Jordan *et al.* 1983). These results were in agreement with previous publications (McNeill *et al.* 1997; Amanlou *et al.* 2011) and in contrast to the results of Annett *et al.* (2008) and Dawson *et al.* (1999), probably because of isonitrogenous diets used in those experiments. McNeill *et al.* (1997) did not observe any differences in glucose concentration when dietary CP increased from 7.9 to 15.7% which is in accordance with our results. Lack of any responses in glucose concentration between groups was opposite to results of our previous report (Amanlou *et al.* 2011). The probable explanation would be the concentration of DUP and CP used in our previous study which was higher compared to the current study. Considering that glucose is the sole source of energy to the uterus and fetus, it is suggested that the energy cost for detoxification of excess ammonia destroyed any potential elevation in glucose concentration. Although high MP sources elevate blood glucose concentration (Milis *et al.* 2005), we should also consider the CP content of basal diets to make a better comparison.

The total protein, albumin and globulin were not influenced by treatments and this was in line with previous studies (Amanlou *et al.* 2011; Dawson *et al.* 1999; Annett *et al.* 2008). However, Houdijk *et al.* (2000) showed that high MP diets (130 *vs.* 85% of requirements) caused total protein and albumin but not globulin to increase during the last 3 wk of pregnancy. However, their basal diet contained 12.7% CP which is different from the current study. The albumin concentration might have a high priority to be maintained since albumin is involved in various maintenance functions such as regulating oncotic pressure and

carrying nutrients and enzymes (Ganong, 1975). Cholesterol concentration showed an interesting increase with the high MP diet; the reason is not clear, but high the MP diet might have increased the liver output of very-low-density lipoprotein (VLDL), in turn increasing the cholesterol concentration of plasma. This observation warrants more research to evaluate the effect of the MP on lipid deposition in the liver. Schlumbom *et al*. (1997) reported that an increase in cholesterol concentration during late pregnancy is related to insulin, which plays a direct role in adipose tissue metabolism during pregnancy and its responsiveness is significantly reduced in ewes during late pregnancy. However, because insulin concentration was not affected by treatments, this cannot be the case in the current study.

In accordance with McNeill *et al*. (1999) and Annett *et al*. (2008) blood parameters related to energy balance including NEFA and βHBA were not different between treatments, meaning energy balance was not altered by MP supply. Compared to the reported values of NEFA and βHBA by Annett *et al*. (2008), our values are lower, suggesting ewes in the current study were in lower energy balance.

This observation in combination with previous publications shows that MP concentration is not likely to be a great factor in determining energy status of twin-bearing ewes.
In combination with the similar insulin concentration between treatments, none of the insulin sensitivity indices showed an effect of MP. Except for HOMA-IR, a low index value indicates decreased insulin sensitivity. To our knowledge, nobody has evaluated the effect of MP during late pregnancy of sheep on insulin sensitivity. Schoenberg and Overton (2012) observed no effect of plane of nutrition on the insulin responsiveness evaluated by RQUICKI. Although Petterson *et al*. (1993) showed that insulin responsiveness decreased during late pregnancy, our results showed that the MP concentrations selected in the current study do not have any effects on insulin sensitivity. Results using RQUICKI as a measure of insulin resistance in ruminants have been mixed. It appears that RQUICKI may be an appropriate measure in some metabolic circumstances but may lack the ability to detect differences in others. In this case, RQUICKI was unable to detect treatment differences.
Blood cell responses to MP have not been extensively evaluated. Houdijk *et al*. (2000) showed that increasing dietary MP concentration (from 85 to 130% of requirements) during late pregnancy increased immunity against parasites. Nonnecke *et al*. (2003) did not observe any differences in total numbers of blood leukocytes, composition of mononuclear leukocyte populations, mitogen-induced DNA-synthesis or mitogen-induced IgM secretion in dairy calves reared with an intensified program (30% crude protein CP, 20% fat milk replacer fed at a rate of 2.4% of body weight) or an industry standard program (20% CP, 20% fat milk replacer fed at a rate of 1.4% body weight). Pisek *et al*. (2008) also did not observe any effect of supplemental selenium on the lymphocyte count of pregnant ewes; they concluded that supplementation of different forms of selenium did not markedly influence the dynamics of blood parameters in pregnant ewes if the intake of vitamins and other essential microelements was adequate. Therefore, lack of any differences to higher MP may imply that the NRC recommendation on MP is enough and higher MP concentrations will not cause blood parameters to dramatically change.

Liver function can be assessed through a variety of enzymes including gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST) and sorbitol dehydrogenase (SDH) and total bilirubin concentrations in the blood. To our knowledge, there is no specific recommendation for the safe range of AST in sheep, but it appears that our ewes were in the safe range, as has been recommended for dairy cattle (Cozzi *et al*. 2011).

## CONCLUSION

Our results show that supplementation with MP in isoenergetic diets during late pregnancy of ewes has no effect on colostrum yield and composition. The live weight of ewes and lambs at lambing were not affected by the protein level. Except for cholesterol and BUN which were elevated in the high MP group, none of the blood parameters showed any differences between groups. Neither insulin nor an insulin resistance index was influenced by the treatments. The MP concentration had no effect on hematology characteristics including white blood cells, red blood cells, lymphocytes, neutrophils, hemoglobin and hematocrit. Generally, our results show that supplementing twin-bearing ewes with an MP concentration higher than NRC recommendations has no beneficial effects on the performance and health status of ewes.

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