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Effect of UMMB Supplementation on Metabolic and Oxidative Parameters in Rambouillet Cross Sheep during Peripartum Period

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ABSTRACT

Twelve crossbred Rambouillet sheep in advanced pregnancy were divided into 2 groups of 6 animals each. Group A was kept as control while group B was supplemented with UMMB (@ 50 g/d/animal. Blood samples were collected at 4 weeks and 1 week before lambing followed by 1, 4 and 8 weeks after lambing. The body weight gain and feed intake was better in UMMB supplemented group. In treatment group, there was increase (P<0.05) in Hb level at +4 and +8 weeks post lambing. No significant change was observed in PCV values. Significant (P<0.05) improvement was observed in plasma calcium, phosphorus, magnesium, iron and zinc levels, however, plasma copper level was similar in two groups. The oxidative stress parameters in both treatment and control group showed significant changes in both groups. Hence, UMMB supplementation improved metabolic and oxidative status of sheep during peripartum period.

Key words: Minerals, Oxidative stress, Peripartum period, Sheep, UMMB

INTRODUCTION

The peripartum period, also called transition period, is a critical phase in the life of ruminants. In small ruminants, due to an increased demand of nutrients by the foetus the animal is subjected to sudden and intense changes in metabolic, endocrine and immune status (Stefanon *et al.,* 2005). In dairy cows and goats, the transition period is marked by variations in oxidative parameters like glutathione peroxidase, glutathione, superoxide dismutase and ceruloplasmin (Stefanon *et al.,* 2005; Celi *et al.,* 2010). The maternal nutrition during peri partum period also plays an important role in the survival of lambs (McDowell *et al.,* 1996). The sheep in the Kandi areas of subtropical and intermediate agro-climatic zones of Shivalik hills of Jammu region in India are dependent mainly on grazing poor quality feed resources which are characteristically low in fermentable nitrogen, mineral and readily available carbohydrate. Developing alternate feeding strategies for ruminant production based on agro-industrial wastes is, therefore, of prime importance.

The use of urea molasses mineral blocks (UMMB) for supplementing crop residues based diet of livestock has been well documented in ruminants (Sansoucy, 1995; Singh *et al.,* 2010a) and has the potential to increase the viability of livestock production (Leng *et al.,* 1991). The UMMB provide nitrogen over a longer period of time than any other urea source. Further, ruminants have the unique ability to convert NPN compounds in their diet to a microbial protein of high biological value. (Thu and Uden, 2000, 2001). A UMMB prepared from locally available agro-industrial by-products has been an adoptable feed supplement which improved nutritional status of animals (Singh *et al.,* 2010b). The aim of this study was to determine the effect of urea molasses multinutrient blocks in ameliorating metabolic and oxidative stress during peripartum period in sheep.

MATERIALS AND METHODS

The study was conducted on Rambouillet sheep reared at Government Sheep Breeding Farm, Panthal, Udhampur, Jammu. History including pregnancy status, previous disease condition, feeding and managemental practices was obtained from the farm records. All the sheep were dewormed before the start of trial using fenbendazole @ 7.5 mg/kg body weight. Twelve crossbred Rambouillet sheep in advanced pregnancy were divided into two groups of 6 animals each. Group A was kept as control while group B was supplemented with UMMB (@ 50 g/d/animal. The trial lasted for 67

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days and was conducted during January-March. The effect of UMMB supplementation was measured in terms of body weight, feed intake, hemato-biochemical parameters, oxidative and mineral status of animals. Blood samples were collected at 1 week before lambing followed by 1, 4 and 8 weeks after lambing. The UMMB were prepared using cold method by mixing molasses (35%), urea (10%), deoiled rice bran (10%), oiled rice bran (10%), groundnut meal (10%), cement (10%), area specific mineral mixture 14% (Singh, 2009) comprising of DCP-70%, $MgSO_4$-29%, $CuSO_4$-0.5%, $MnSO_4$-0.5%, Potassium iodate-0.09%, and common salt (1%).

For estimation of biochemical constituents and minerals, blood samples (~15 mL) were collected by jugular venipuncture into mineral free heparinised glass vials. The plasma was separated after centrifuging blood samples at 3000 rpm for 30 min. and stored at -10°C in deep freeze for subsequent analysis. For determining haemoglobin (Hb) and packed cell volume (PCV), 2 mL of blood sample was collected from each animal in sterile plastic tubes containing dipotassium salt of EDTA (Hi Media Mumbai, @ 2 mg/mL of blood) and analyzed (Jain *et al.,* 1986). Glucose was estimated immediately after collection of blood using gluco-chek strip. Standard kits (Span Diagnostics, Surat, India) were used for determination of total plasma protein, albumin, BUN, ALT and AST. Estimation of Ca, Inorganic fraction of phosphorus (Pi), Na and K was done using kits. Plasma samples (3 mL) were analysed for micromineral analysis by digesting in distilled concentrated nitric acid (15 mL). Digested samples were diluted to 10 mL with double glass distilled water. The concentrations of micro-elements *viz.*, Cu, Fe and Zn were measured by Polarized Zeeman Atomic Absorption Spectro-photometer (Z-2300, Hitachi).

Malondialdehyde (MDA; Shafiq-ur-Rehman, 1984), glutathione peroxidase (GPx; Hafeman *et al.,*1974), glutathione-s-transferase (GST; Habig *et al.,* 1974), superoxide dismutase (SOD; Marklund and Marklund, 1974) and catalase (CAT; Aebi; 1983) were estimated. The proximate analysis of feed stuff was carried out (AOAC, 1990). The statistical analysis of data was carried out using Turkey Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The CP, EE and CF contents of Dhaman leaves were 18.78, 2.80 and 3.65%, respectively. These values were in normal range (Singh *et al.,* 2010b). The minor variations found may be due to difference in cultivars, stage of agro-climatic conditions, season, stage of maturity, genetic makeup, soil fertility, harvesting methodology, post harvest storages and processing conditions like grinding and drying before analysis.

There was an average increase of 17.33% in BW of treated group compared with average decrease of -0.026% in control group (Table. 2). The findings are in agreement with Forsberg *et al.* (2002) and Singh *et al.* (2010a). UMMB has been suggested as a versatile supplement for sheep and goat (Golluscio *et al.,* 1998; Currier *et al.,* 2004; Singh *et al.,* 2010b). The overall DMI (kg/d) was higher ($P<0.05$) in treatment group as compared to control. These results clearly indicated that UMMB supplementation in peri-parturient sheep had a positive impact on weight gain and health status. UMMB supplementation enhances rumen microbial growth, feed intake of animals and increases feed digestion (FAO, 2007) allowing the animals to maintain and often improve productive performance (Bensalem and Nafzaoul, 2003). Sena *et al.* (2006) also reported that

Table 1. Proximate composition (% DM basis) of feedstuffs

Feed	Dry matter	Crude protein	Crude fibre	Ether extract	Total ash
Dhaman (*Grevia optiva*)	37.81	18.73	30.65	2.80	10.57
Oat grains	90.93	8.23	7.30	1.78	1.96
Pelleted feed	87.74	15.05	19.90	2.90	11.69
UMMB	86.35	21.75	1.10	6.60	28.21

Table 2. Effect of UMMB supplementation on body weight, haematology and biochemical parameters

Parameter	Week	Group	
		A	B
Body weight (kg)	-1 week	44.33±2.1	41.83±3.7
	+1 week	41.92±2.8	40.50±3.7
	+4 week	41.50±2.8	42.75±3.7
	+8 week	43.17±3.2	49.08±3.8
Overall DMI (kg/d)		1.87^{a}±2.03	1.98^{b}±2.11
Hb (g/dL)	-1 week	8.83^{a}±0.31	9.3^{a}±0.52
	+1 week	6.43^{b}±0.56	9.65^{a}±0.33
	+4 week	8.71^{a}±0.32	12.32^{b}±0.38
	+8 week	11.59^{b}±0.30	12.00^{b}±0.25
PCV (%)	-1 week	26.70^{a}±1.34	31.68^{a*}±2.25
	+1 week	23.58^{b}±1.67	32.79^{a*}±0.69
	+4 week	26.13±0.93	36.5^{a}±1.12
	+8 week	34.41^{c}±0.99	35.9^{a*}±0.76
Blood glucose (mg/dL)	-1 week	70.89^{a}±4.19	50.46^{a*}±5.20
	+1 week	82.56^{b}±5.64	60.07^{b}±3.31
	+4 week	61.33^{a}±3.87	65.77^{b*}±5.99
	+8 week	63.69^{a}±8.80	67.13^{b}±2.12
Total plasma protein (g/dL)	-1 week	7.75^{ab}±0.40	7.06^{a}±0.45
	+1 week	6.89^{c}±0.70	6.93^{a}±0.46
	+4 week	8.29^{a}±0.37	8.73^{a}±0.73
	+8 week	8.46^{b}±0.31	8.16^{a}±0.18
Albumin (g/dL)	-1 week	3.19^{a}±0.35	2.92^{ab}±0.14
	+1 week	2.06^{b}±0.13	2.44^{ab*}±0.13
	+4 week	2.41^{a}±0.13	2.55^{ab}±0.11
	+8 week	2.44^{a}±0.10	2.24^{c}±0.17
Globulin (g/dL)	-1 week	4.77^{a}±0.50	4.14^{a}±0.41
	+1 week	4.83^{a}±0.64	4.97^{ab}±0.18
	+4 week	5.87^{b}±0.37	6.18^{b}±0.78
	+8 week	6.02^{c}±0.32	5.93^{b}±0.09
BUN (mg/dL)	-1 week	21.87^{ab}±1.52	33.08^{a*}±2.03
	+1 week	19.22^{a}±1.98	30.58^{a*}±0.81
	+4 week	24.02^{b}±3.10	28.70^{b*}±1.15
	+8 week	20.97^{ab}±0.79	17.72^{c}±3.12
ALT activity (U/L)	-1 week	16.66^{ab}±1.72	18.51^{a*}±1.19
	+1 week	23.12^{c}±3.12	23.12^{a}±1.89
	+4 week	15.44^{a}±1.56	20.66^{a}±2.24
	+8 week	14.16^{a}±1.16	15.01±5.48^{a}
AST activity (U/L)	-1 week	80.93^{ab}±1.18	93.48^{a*}±2.32
	+1 week	101.38^{c}±1.67	96.47^{a}±2.78
	+4 week	95.42^{a}±2.13	94.92^{a}±2.63
	+8 week	92.72^{a}±1.62	93.21^{a}±2.14

^{a,b,c}Figures with different superscripts for a parameter in a column differ significantly (P<0.05); *Figures in a row differ significantly (P<0.05)

UMMB supplementation increased microbial activity by supplying nitrogen, minerals and energy in a balanced proportion.

The average values of Hb in groups A and B animals varied from 8.83 to 11.59 g/dL and 9.3 to 12.32 g/dL, respectively. In treatment group, there was increase (P<0.05) in Hb level at +4 and +8 weeks post lambing. Control group showed decrease (P<0.05) at +1 week and an increase (P<0.05) at +8 week compared with the level of Hb at 1 week before lambing (Table 2). The average values of PCV in groups A and B varied from 26.70 to 34.41% and from 31.68 to 36.5%, respectively. In control group, a decrease (P<0.05) in PCV level was observed at +1 week and an increase (P<0.05) was observed at +8 weeks as compared with the values observed at week 1. The elevated levels of hematological indices compared with control group could be attributed to higher DMI in the supplemented group. Singh *et al.* (2010a) also reported non-significant increase in Hb and PCV levels in animals supplemented with UMMB while Singh *et al.* (2017) reported rise (P<0.05) in PCV in beetal goats supplemented with UMMB on 30th day of trial.

The plasma glucose concentration in control and test group animals varied from 70.89 to 82.56 mg/dL and 50.46 to 67.13 mg/dL, respectively. An increase (P<0.05) in glucose level was observed at +1, +4 and +8 week in treatment group. In control group, significant increase (P<0.05) in glucose level was observed only at +1 week as compared to -1 week (Table. 3). The higher glucose level observed in supplemented group animals might be due to the fact that minerals in UMMB acted as cofactor and activator of many enzymatic systems associated with the

Table 3. Effect of UMMB supplementation on plasma minerals in sheep during peripartum period

Parameter	Week	Group	
		A	B
Ca (mg/dL)	-1 week	18.45^{b}±1.90	13.10^{ab*}±1.19
	+1 week	11.50^{a}±0.47	11.30^{a}±0.44
	+4 week	14.50^{b}±1.05	18.86^{c}±2.68
	+8 week	10.42^{a}±0.88	15.36^{b*}±0.97
Pi (mg/dL)	-1 week	8.02^{a}±0.83	5.70^{ab}±0.52
	+1 week	5.00^{b}±0.33	5.15^{a}±0.45
	+4 week	6.83^{c}±0.72	8.20^{c}±1.16
	+8 week	4.53^{b}±0.38	6.65^{d*}±0.43
Fe (µmol/L)	-1 week	385.53^{b}±94.67	234.38^{a}±78.60
	+1 week	106.66^{a}±23.11	131.48^{a}±21.22
	+4 week	58.87^{a}±19.70	122.25^{a*}±11.98
	+8 week	366.03^{b}±71.03	252.60^{a}±108.49
Zn (µmol/L)	-1 week	17.42^{b}±2.85	23.07^{ab}±3.24
	+1 week	21.52^{ab}±2.49	28.76^{ab*}±3.61
	+4 week	29.95^{a}±3.53	34.39^{c}±2.93
	+8 week	15.14^{b}±2.70	19.43^{b*}±1.45
Cu (µmol/L)	-1 week	20.42^{a}±4.66	12.36^{a}±3.12
	+1 week	23.39^{a}±6.91	19.99^{a}±4.77
	+4 week	11.19^{a}±2.59	23.48^{a}±7.56
	+8 week	19.06^{a}±2.52	8.05^{a}±1.43

^{a,b,c,d}Figures with different superscripts for a parameter in a column differ significantly (P<0.05); *Figures in a row differ significantly (P<0.05)

metabolism of nutrients (Mohapatra *et al.,* 2012).

The average values of total plasma protein (TPP) in control and test group animals varied from 6.89 to 8.46 g/dL and 6.93 to 8.73 g/dL respectively. In test group, there was no difference in TPP level at +1 week, +4 and +8 week whereas animals in control group showed decrease (P<0.05) at +1week as compared to -1 week. The average values of albumin among control and test group animals varied from 2.06 to 3.19 g/dL and 2.24 to 2.92 g/dL, respectively. In test group, an increase (P<0.05) in albumin level was observed at +1 week compared to control group. There was significant (P<0.05) decrease at +8 weeks as compared to -1 week. The average values of globulin in control and test group animals varied from 4.77 to 6.02 g/dL and from 4.14 to 6.18 g/dL, respectively. Significant (P<0.05) increase in globulin level was observed in treatment group at +4 and +8 weeks. Similar trend was observed in control group animals. Findings of the present study corroborated with those of Singh *et al.* (2010a) who reported non-significant effect of UMMB supplementation on total plasma protein and albumin level in anoestrus buffaloes after 4 weeks (Table 2). The role of mineral for improvement of serum protein level is mostly limited to the better availability of phosphorus in supplemented than un-supplemented group as phosphorus plays a significant role in amino acid and protein synthesis (Underwood and Suttle, 1999).

In treatment group, there was increase (P<0.05) in BUN level at +4 and +8 weeks as compared to -1 week. In control group, no significant (P<0.05) change in BUN level was observed. Qreshi *et al.* (2002)

Table 4. Effect of UMMB supplementation on oxidative stress indices of sheep during peripartum period

Parameter	Week	Group	
		A	B
MDA (µmol/mL)	-1 week	10.10^{b}±4.01	18.02^{b}±4.05
	+1 week	13.80^{b}±3.90	21.8^{ab}±3.9
	+4 week	20.34^{a}±4.29	28.34^{a}±4.29
	+8 week	24.57^{c}±1.21	32.57^{a}±1.21
SOD activity (U/mg of Hb)	-1 week	295.13^{a}±16.60	242.49^{bc}±14.81
	+1 week	252.94^{b}±8.85	219.06^{c*}±5.64
	+4 week	272.64^{ab}±9.02	289.14^{a}±14.32
	+8 week	282.45^{a}±15.82	289.14^{a}±14.32
Catalase activity (µmolH_2O_2 utilized/min/mg of Hb)	-1 week	112.75^{a}±4.44	105.74^{a*}±1.12
	+1 week	105.91^{a}±5.21	110.45^{a}±4.65
	+4 week	111.52^{a}±5.99	131.54^{b}±6.47
	+8 week	131.54^{b}±6.47	125.43^{a}±7.58
Glutathione S transferase activity (µmol of conjugate ofGSH-CDNB/min/ mg of Hb)	-1 week	0.03^{b}±0.00	0.02^{a}±0.00
	+1 week	0.01^{a}±0.00	0.02^{a}±0.00
	+4 week	0.02^{a}±0.00	0.03^{ab}±0.01
	+8 week	0.03^{b}±0.00	0.05^{b*}±0.00
GPx activity (U/mg of Hb)	-1 week	1.11^{a}±0.31	1.14^{a}±0.41
	+1 week	1.01^{a}±0.29	1.12^{a}±0.36
	+4 week	0.96^{a}±0.19	0.49^{b*}±0.14
	+8 week	1.34^{a}±0.20	1.15^{a}±0.20

^{a,b,c}Figures with different superscripts for a parameter in a column differ significantly (P<0.05); *Figures in a row differ significantly (P<0.05)

reported that excessive levels of crude protein in the diet elevated BUN levels. Hosamani *et al.* (1998) also reported increase (P<0.01) in blood serum urea following UMMB supplementation in Murrah buffaloes. No significant change was observed in ALT and AST levels in treatment groups, however, in control group an increase (P<0.05) in ALT and AST level was observed at +1 week as compared to -1 week (Table 2). During the periparturient period, liver is over burdened by the increased gluconeogenesis and lipid infiltration causing injury to liver cells and consequently increased liver specific enzymes (Pechova *et al.,* 1997; Lubojacka *et al.,* 2005). However, in the present study animals supplemented with UMMB had better protein and energy metabolism. Feeding of UMMB had no direct effect on liver function (Cenesiz *et al.,* 2006).

The average values of plasma Ca in groups A and B varied from 10.42 to 18.45 mg/dL and 11.30 to 18.86 mg/dL, respectively. In treatment group, there was increase (P<0.05) in Ca level at +4 weeks as compared to -1 week whereas control group animals showed decrease (P<0.05) at +1 and +8 weeks (Table. 3). The average values of Pi in groups A and B varied from 4.53 to 8.02 mg/dL and from 5.15 to 8.20 mg/dL, respectively. In treatment group, there was an increase (P<0.05) in Pi level at +4 and +8 weeks as compared to -1 week. However, in control group there was decrease (P<0.05) in Pi level at +1, +4 and +8 weeks as compared to -1 week values. The average values of Ca (9.0-11.6 mg/dL) and Mg (2.10-2.90 mg/dL) were within the normal range. Singh *et al.* (2017) reported rise (P<0.05) in the mean plasma inorganic P and Mg concentration on d 60th compared to 0 d values in beetal goats supplemented with UMMB.

No significant change in plasma Fe level was observed in control group. The animals supplemented with UMMB showed increase (P<0.05) in plasma iron level at +4 weeks compared with control group. Singh *et al.* (2017) reported decrease (P<0.05) in concentration of Fe levels of both UMMB supplemented and non-supplemented goats from day 30th of the trial. A significant (P<0.05) variation in plasma Zn level was observed among test and control groups. In animals supplemented with UMMB blocks, significant (P<0.05) increase in Zn levels was found at +4 and +8 weeks. However, in control group there was decrease (P<0.05) in plasma Zn level upto +4 weeks compared with -1 week level. No significant change in plasma Cu level was observed in treatment group (Table. 3). Singh *et al.* (2017) reported significant (P<0.05) increase in Zn concentration in UMMB supplemented beetal goats after 30 days of supplementation.

There was significant (P<0.05) increase in the MDA levels and SOD activity in control groups at +4 and +8 weeks in both control and treatment groups (Table 4). No significant change was observed in CAT level. However, in control group there was increase (P<0.05) in CAT level at +8 weeks as compared with -1 week and decrease (P<0.05) in CAT activity was observed at -1 week in test group compared with control group. In treatment group, there was increase (P<0.05) in glutathione S transferse activity at +8 weeks whereas there was decrease (P<0.05) in control group at +1 week as compared with -1 week. There was no change in GPx activity in control group whereas there was increase (P<0.05) in GPx activity at 4 weeks post treatment. There was not much change in the oxidative stress due to UMMB supplementation. The reason could be the low dose of UMMB supplement. Aantioxidant status of blood is reflection of their corresponding concentration in rest of body (Bouwstra *et al.,* 2008). The increased SOD and MDA activity in both supplemented and control animals might have been due to high ROS production. SOD catalyzes the dismutation of $\bullet O_2^-$ into oxygen and hydrogen peroxide (H_2O_2) which is removed by the coordinated activity of catalase and GPx. Therefore, the increased activity of catalase and GPx in UMMB supplemented groups could be atributed to high production of H_2O_2.

CONCLUSIONS

Supplementary feeding of urea molasses multi-nutrient blocks during peripartum period in sheep increased body weight along with increase in Hb post lambing. Plasma calcium, phosphorus, magnesium, iron and zinc levels improved significantly, however, copper

level was similar in 2 groups. The oxidative stress parameters showed significant changes in both treatment and control group. Therefore, UMMB being good source of protein, energy and minerals improved metabolic status of sheep during peripartum period.

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