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REGULAR ARTICLES

Effect of physiological status and parity on metabolic and trace element profile of crossbred Rambouillet sheep of Himalayan region

R. Singh[1] · **A. Singh**[1] · **S. A. Beigh**[2] · **N. Sharma**[1] · **V. Singh**[1]

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Abstract

The study was designed to evaluate the effect of physiological status and parity on metabolic profile in crossbred Rambouillet ewes of the Himalayan region. The study was conducted on 20 ewes divided into two groups, primiparous (PP) and multiparous (MP), with 10 ewes in each group. Blood samples were collected on 4- and 1-week pre-lambing and 1- and 4-week post-lambing to measure metabolic parameters and minerals. The glucose ($p < 0.01$), total plasma protein (TPP) ($p < 0.05$), albumin ($p < 0.05$), blood urea nitrogen (BUN) ($p < 0.05$), cholesterol ($p < 0.05$), triglyceride ($p < 0.01$), high-density lipoprotein cholesterol (HDL-C) ($p < 0.05$), calcium (Ca) ($p < 0.01$), phosphorus (Pi) ($p < 0.05$), magnesium (Mg) ($p < 0.01$), copper (Cu) ($p < 0.05$), and zinc (Zn) ($p < 0.01$) levels revealed significant change along the time with the concentration decreasing from 3-week pre-lambing to immediate post-lambing; thereafter, levels increased steadily. Significant increase ($p < 0.01$) was observed in non-esterified fatty acid (NEFA), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), iron (Fe) ($p < 0.05$), and bilirubin ($p < 0.05$) concentrations along the sampling time. No group difference was observed in any of the parameters; however, parity and time interaction was observed in glucose, NEFA, GGT, Ca, and Pi. While NEFA levels were significantly high in pre-lambing in PP ewes compared to MP ewes, the post-lambing levels were significantly high in MP ewes. Pre-lambing levels of GGT were at par between the two groups; however, post-lambing levels were significantly high in MP ewes. Glucose, Ca, and Pi were low during pre-lambing in PP ewes and post-lambing in MP ewes. The result showed that ewes show a significant change in metabolic profile and trace minerals during late gestation and immediate postpartum; however, these changes were more pronounced during late gestation in primiparous and post-lambing in multiparous.

Keywords Himalayan · Metabolic · Mineral · Multiparous · Primiparous · Rambouillet

Introduction

Nutritional requirement of an animal depends on the physiological stage, and periparturient period is a very critical physiological state during which there is large increase in nutritional requirement of an animal. A substantial cost to the animal is imposed by pregnancy as nutrients are required to the extent of 75% towards the end of pregnancy compared to non-pregnant animals, and for successful outcome of pregnancy, major changes in dams' physiology and metabolism are needed (Castagnino et al. 2015). Lactation, on the other hand, especially the first half of lactation, is also a very stressful period for an animal as its nutritional needs are increased, and nutritional requirements increase substantially because of milk production. In late gestation and early lactation, energy intake is lower if compared to animals' needs, indicating negative energy balance (NEB), which mobilizes body reserves (Castagnino et al. 2015).

Consequently, significant changes may occur in the ewes in late gestation and early lactation periods, leading to metabolic disorders. Mobilization of body reserves causes a change in serum NEFA and beta-hydroxybutyric acid (β-HBA) concentrations (Van Knegsel et al. 2007) and some other blood metabolites like insulin; glucose; protein; and cholesterol triglyceride, BUN, and creatinine (Piccione

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✉ R. Singh
rajivrajiv101@gmail.com

[1] Division of Veterinary Medicine, Faculty of Veterinary Sciences and Animal Husbandry, Sher-E-Kashmir University of Agricultural Sciences and Technology of Jammu, R. S. Pura, Jammu and Kashmir 181 102, India

[2] Division of Clinical Veterinary Medicine & J, Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Srinagar, Jammu and Kashmir, India

et al. 2009). Moreover, substantial losses of body minerals occur during pregnancy and lactation. Therefore, estimating the concentration of macro- and micro-minerals in the serum during different physiological statuses becomes imperative (Elnageeb and Adelatif 2010).

The identification of metabolic changes during various physiological phases, the assessment of abnormal metabolic states, and prediction of some metabolic disorders in advance could provide some benefits to the farmers (Balikci et al. 2007). The blood metabolic profile provides an important diagnostic tool to assess the nutritional status and general health of an animal (Herdth 2000) and gives us an idea about the overall well-being of an animal much earlier than apparent changes becoming visible in the animal (Doaa et al. 2014; Antunović et al. 2017).

Parity has been seen to affect the pattern of metabolic changes in cows (Wathes et al. 2007); however, to our best knowledge, no such report is available for ewes. Understanding the basis for such metabolic responses may assist us in determining how they affect the health status of ewes. Furthermore, very less information is available on crossbred sheep of north western Himalayan region of India, particularly about crossbred Rambouillet sheep which are generally high-producing dual-purpose sheep (wool and mutton) and need considerable attention in their feed and nutrition. Keeping in view these facts, the present study was aimed to understand the effect of physiological status and parity on metabolic and trace element profile of crossbred Rambouillet sheep of the Himalayan region.

Materials and methods

Animals, husbandry, and nutrition

The study was carried out on 20 healthy crossbred Rambouillet sheep, at an organized Government Sheep Breeding Farm, Panthal, Udhampur, Jammu and Kashmir, India, between December and February, when the climate is suitable and there is no risk of heat or cold stress (Chauhan et al. 2014). The animals were divided into two groups according to their parity, 10 sheep in each group. The first group comprises primiparous (PP) ewes and the second group comprises ewes that have lambed multiple times (MP). Three ewes lambed for the second time, five ewes lambed for the third time, and two lambed for the fourth time. All ewes were lambed without assistance. All the animals were offered fresh water ad libitum and daily diet comprising oat hay (3.5% of live weight) and concentrate containing maize (35% DM), mustard oil cake (30% DM), wheat bran (28% DM), molasses (5% DM), salt (1% DM), and mineral mixture (1%; Mineral Mx, Mx Pharmaceuticals) at a rate of 0.3 kg/sheep twice daily (Table 1). The animals were kept in open shaded areas with daily access to sunny exercise areas.

Body condition scoring of all animals was done at the beginning of the experiment using a standard technique of 1–5 scale described by Russel et al. (1969). The sheep were homogenous for BCS (PP ewes: 2.85 ± 0.21 and MP ewes: 2.586 ± 0.15) with no statistical significant difference between the two groups.

Collection of samples

Blood samples were collected through jugular venipuncture from all sheep between 8:00 and 10:00 a.m., 4 weeks and 1 week before lambing followed by 1 and 4 weeks after lambing. For the estimation of biochemical constituents and minerals, blood samples (~ 15 ml) were collected into mineral-free heparinized glass vials (dipped overnight in 2 N HCl). The blood samples were transported in an ice box to prevent hemolysis. Blood samples were centrifuged at $500 \times g$ for 10 min to separate plasma immediately after collection to prevent hemolysis. Plasma samples were stored at – 10 °C in deep freeze for subsequent analysis.

Laboratory analysis

Biochemical analysis of plasma samples was carried in triplicate using commercial kits (Transasia, ERBA, or DiaSys commercial kits) following the manufacturer's instructions. The estimation of non-esterified fatty acids (NEFA; DiaSys

Table 1 Proximate chemical analysis of concentrate mixture and composition of mineral mixture supplementation fed to ewes

Composition of feed	
Composition	**Percentage**
Dry matter	86.94
Crude protein (% DM)	15.36
Crude fiber (% DM)	7.12
Ether extract (% DM)	3.8
Ash (% DM)	4.66
Mineral mixture composition (amount in 1 kg)	
Mineral	Gram (g)
Calcium	20
Phosphorus	12
Magnesium	5
Sulfur	1.8–3 g
Copper	0.10
Zinc	0.80
Manganese	0.125
Cobalt	0.012
Iodine	0.02
Iron	0.04

kit), glucose (Transasia, ERBA), total plasma protein (TPP; Transasia, ERBA), albumin (Transasia, ERBA), blood urea nitrogen (BUN; Transasia, ERBA), creatinine (Transasia, ERBA), aspartate transaminase (AST; Transasia, ERBA), gamma-glutamyl transferase (GGT; Transasia, ERBA), cholesterol (Transasia, ERBA), triglyceride (Transasia, ERBA), and high-density lipoprotein cholesterol (HDL-C; Transasia, ERBA) was done.

Mineral analysis

Estimation of calcium (Ca) and inorganic fraction of phosphorus (Pi) by Transasia (ERBA), and sodium (Na) and potassium (K) by DiaSys kit was carried out. Trace mineral estimation was done by Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI) as per the method described by Kolmer et al. (1951) with little modification. Briefly, 3 ml of plasma sample was digested in 15 ml distilled concentrated nitric acid. Approximately, 1 ml of leftover digestate was diluted to make a final volume of 10 ml with double distilled water and the concentrations of micro-minerals, viz. copper (Cu), iron (Fe), and zinc (Zn), were measured.

Statistical analysis

Overall descriptive statistics (mean and standard error) for each blood constituent were calculated. The data were tested for normality by applying the Shapiro–Wilk normality test and homogeneous variance by Levene's test. Data was subjected to repeated measure test and multiple comparisons, considering parity (G), sampling time (T), and their interactions ($T \times G$) as fixed effects was done by Bonferroni's adjustment. The model used was

$$Y_{ij} = \mu + G_i + T_j + (T \times G)_{ij} + e_{ij}$$

In which Y_{ij} is the observed value of the dependent variable, μ is the overall mean, G_i is the fixed effect of the ith parity, T_j is the fixed effect of the jth sampling, $(T \times G)_{ij}$ is the interaction between group and sampling time, and e_{ij} is the residual error. Only significant ($p < 0.05$) group or group and time interaction was kept and represented in figures.

Results

The glucose and NEFA concentration showed a significant ($p < 0.01$) change along the time (Tables 2 and 3), with glucose concentration being lowest at 1-week pre-lambing in PP and 1-week post-lambing in MP ewes (Fig. 1). The NEFA concentrations were highest at 1-week post-lambing in both the groups (Fig. 2). No significant group differences in glucose and NEFA levels were observed; however, time × group interaction revealed significantly low glucose ($p < 0.05$) and high NEFA ($p < 0.01$) 1-week pre-lambing in PP compared to MP and significantly decreased glucose ($p < 0.01$) and increased NEFA ($p < 0.05$) in MP at 1-week post-lambing (Figs. 1 and 2).

Table 2 Results (p values) of repeated measures with ewes as random effect group, and time, group, and group × time interaction as fixed effect for the dependant variables in blood (biochemical parameters)

Parameters	Time (weeks)	Group	Group × time
Glucose (mg/dl)	0.003	0.059	0.01
NEFA (mmol/l)	0.007	0.068	0.022
Total plasma protein (g/dl)	0.025	0.482	0.052
Plasma albumin (g/dl)	0.017	0.341	0.031
BUN (mg/dl)	0.031	0.201	0.098
Creatinine (mg/dl)	0.048	0.736	0.218
AST (IU/l)	0.005	0.570	0.117
GGT (IU/l)	0.016	0.117	0.317
Bilirubin (mg/l)	0.032	0.418	0.569
Cholesterol (mg/dl)	0.004	0.091	0.171
Triglyceride (mg/dl)	0.002	0.114	0.068
HDL-C (mg/dl)	0.040	0.234	0.528
Sodium (m eq/l)	0.172	0.221	0.241
Potassium(m eq/l)	0.038	0.079	0.192
Calcium (mg/dl)	0.013	0.082	0.037
Phosphorus (mg/dl)	0.020	0.187	0.024
Iron (μmol/l)	0.018	0.421	0.216
Zinc (μmol/l)	0.027	0.103	0.780
Copper (μmol/l)	0.031	0.328	0.129

NEFA, non-esterified fatty acid; *BUN*, blood urea nitrogen; *AST*, aspartate aminotransferase; *GGT*, gamma-glutamyl transferase; *HDL-C*, high-density lipoprotein cholesterol

Total plasma protein (TPP) in both the groups showed a significant ($p < 0.05$) change with time, with significant decrease in concentration from 4-week pre-lambing to 1-week post-lambing when concentrations reached to their lowest value, and thereafter, a steady increase was observed (Tables 2 and 3). A significant change in albumin ($p < 0.05$), BUN ($p < 0.05$), and creatinine ($p < 0.05$) was also observed with albumin and BUN concentrations being lowest at 1-week post-lambing while creatinine concentration reached to peak value at the same time (Tables 2 and 3). No significant group difference was observed between the groups (Figs. 3 and 4).

Significant change was observed in AST ($p < 0.01$), GGT ($p < 0.01$), and bilirubin ($p < 0.05$) with the activities of enzymes reaching to their peak at 1-week post-lambing and thereafter decreasing steadily (Table 3). While there was no significant group difference in any parameter, the time × group revealed significantly high GGT activities in

Table 3 Biochemical and mineral profile of Rambouillet ewes at different physiological stages

Parameters	– 4 weeks	– 1 week	1 week	4 weeks
Glucose (mg/dl)	53.01 ± 2.16^{a}	40.89 ± 2.28^{b}	42.56 ± 2.64^{b}	61.35 ± 3.87^{a}
NEFA (mmol/l)	0.337 ± 0.01^{b}	0.401 ± 0.02^{ab}	0.465 ± 0.03^{a}	0.447 ± 0.05^{a}
Total plasma protein (g/dl)	8.31 ± 1.06^{a}	7.71 ± 1.0^{ab}	6.83 ± 0.70^{b}	7.29 ± 1.07^{ab}
Plasma albumin (g/dl)	3.23 ± 0.07^{a}	3.13 ± 0.18^{ab}	2.97 ± 0.13^{b}	3.07 ± 0.23^{ab}
BUN (mg/dl)	28.95 ± 1.62^{a}	21.87 ± 1.52^{ab}	19.22 ± 1.98^{b}	24.02 ± 3.10^{ab}
Creatinine (mg/dl)	1.28 ± 0.11^{b}	1.33 ± 0.14^{b}	1.89 ± 0.11^{a}	1.35 ± 0.09^{b}
AST (IU/l)	80.93 ± 5.18^{a}	76.61 ± 6.76^{b}	101.38 ± 7.67^{a}	95.42 ± 4.13^{a}
GGT (IU/l)	41.82 ± 3.31^{b}	51.86 ± 3.25^{a}	53.04 ± 4.47^{a}	46.45 ± 3.17^{b}
Bilirubin (mg/l)	0.23 ± 0.01^{b}	0.29 ± 0.12^{a}	0.40 ± 0.12^{a}	0.17 ± 0.05^{c}
Cholesterol (mg/dl)	57.89 ± 8.28^{a}	41.75 ± 3.48^{b}	31.00 ± 3.28^{c}	60.67 ± 3.91^{a}
Triglyceride (mg/dl)	64.78 ± 3.88^{ab}	56.12 ± 3.22^{b}	45.62 ± 1.72^{c}	67.59 ± 2.53^{a}
HDL-C (mg/dl)	22.79 ± 3.48^{a}	17.41 ± 3.22^{ab}	10.48 ± 2.44^{b}	25.59 ± 2.53^{a}
Sodium (m eq/l)	149.25 ± 10.09^{a}	145.08 ± 6.70^{a}	147.64 ± 5.32^{a}	153.61 ± 8.05^{a}
Potassium (m eq/l)	4.96 ± 0.31^{ab}	4.16 ± 0.13^{b}	5.14 ± 0.22^{a}	5.21 ± 0.39^{a}
Calcium (mg/dl)	12.25 ± 1.52^{a}	11.05 ± 1.81^{a}	8.7 ± 0.91^{b}	10.71 ± 1.47^{a}
Phosphorus (mg/dl)	5.33 ± 0.83^{a}	4.92 ± 0.83^{ab}	4.27 ± 0.64^{b}	5.00 ± 0.33^{a}
Iron (μmol/l)	240.52 ± 50.42^{a}	185.53 ± 44.67^{a}	106.66 ± 23.11^{b}	58.87 ± 19.70^{b}
Zinc (μmol/l)	13.76 ± 1.19^{b}	17.42 ± 2.01^{b}	16.52 ± 2.2^{b}	30.18 ± 3.53^{a}
Copper (μmol/l)	19.06 ± 2.52^{a}	20.42 ± 4.66^{a}	23.39 ± 6.91^{a}	11.19 ± 2.59^{b}

Values with at least one similar superscript did not differ significantly

NEFA, non-esterified fatty acid; *BUN*, blood urea nitrogen; *AST*, aspartate aminotransferase; *GGT*, gamma-glutamyl transferase; *HDL-C*, high-density lipoprotein cholesterol

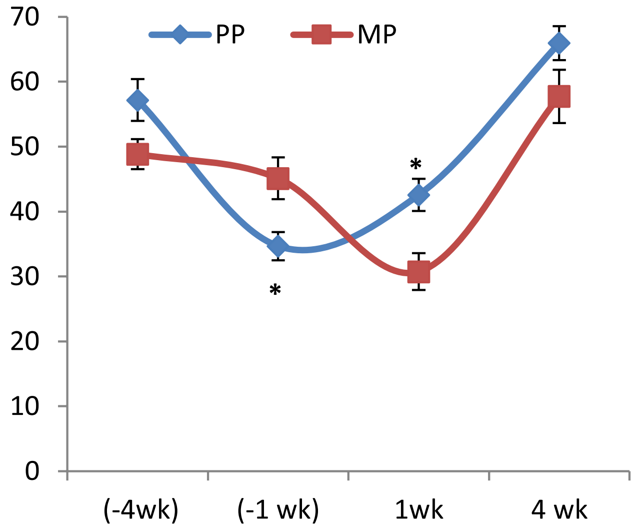

Fig. 1 Effect of parity and physiological stage on glucose (mg/dl) concentration in ewes. *Significant difference between (PP) primiparous and (MP) multiparous

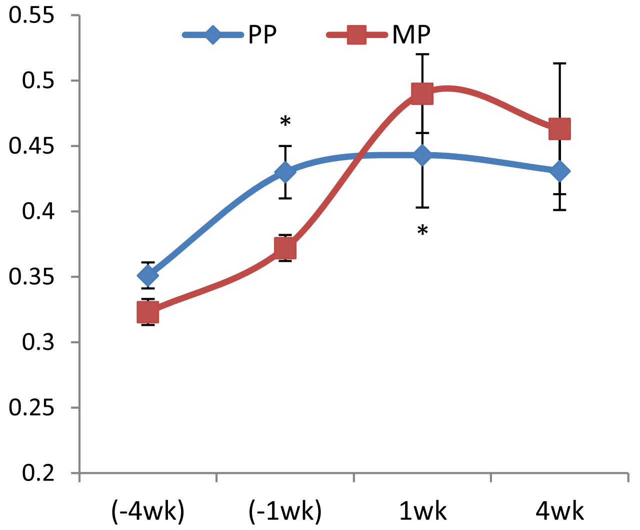

Fig. 2 Effect of parity and physiological stage on non-esterified fatty acid (mmol/l) concentration in ewes. *Significant between (PP) primiparous and (MP) multiparous

MP animals at 1-week and 4-week post-lambing (Table 2 and Fig. 5).

Significant change was observed in cholesterol ($p < 0.05$), triglyceride ($p < 0.01$), and HDL-C ($p < 0.05$) in both the groups (Tables 2 and 3). The concentration of cholesterol, TG, and HDL-C decreased along the time, reaching to their lowest concentration at 1-week post-lambing, and thereafter, a steady increase in concentration was observed (Table 3). No significant group and time × group interaction was observed between the groups (Table 2).

Among the plasma minerals, significant change was observed in Ca ($p < 0.01$), Pi ($p < 0.05$), and K ($p < 0.01$)

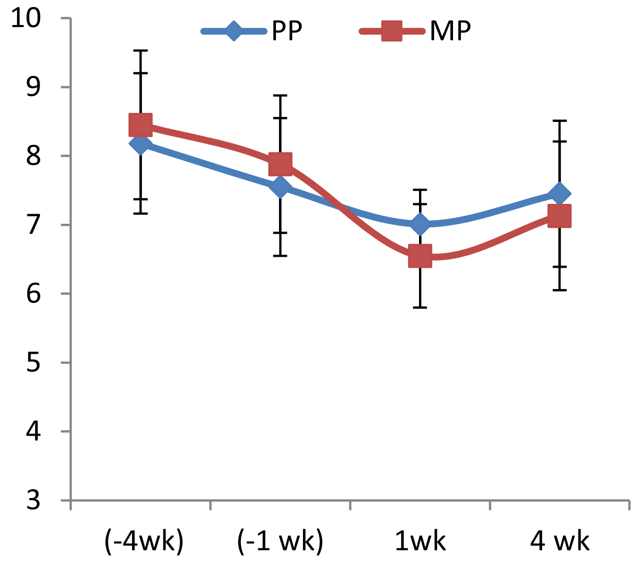

Fig. 3 Effect of parity and physiological stage on total protein (mg/dl) concentration in ewes. PP, primiparous; MP, multiparous

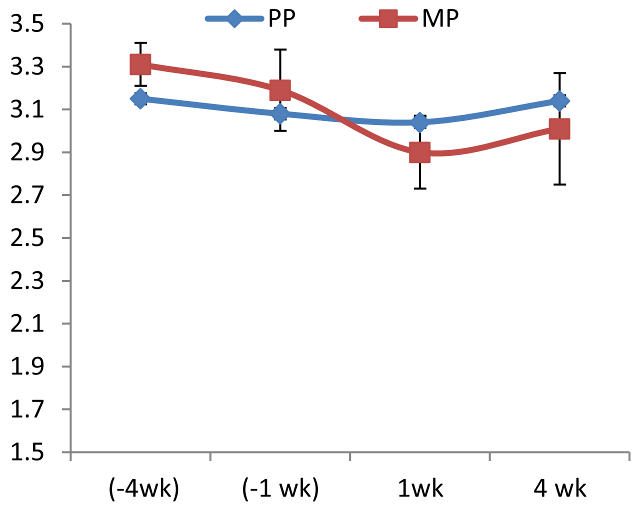

Fig. 4 Effect of parity and physiological stage on albumin (mg/dl) concentration in ewes. PP, primiparous; MP, multiparous

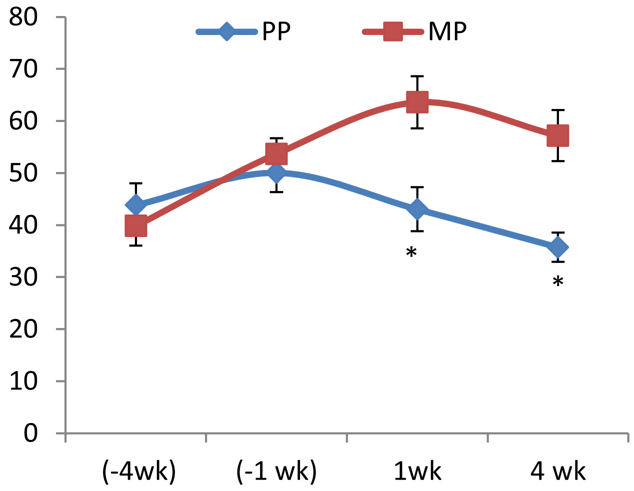

Fig. 5 Effect of parity and physiological stage on gamma-glutamyl transferase (IU/l) concentration in ewes. *Significant difference between (PP) primiparous and (MP) multiparous

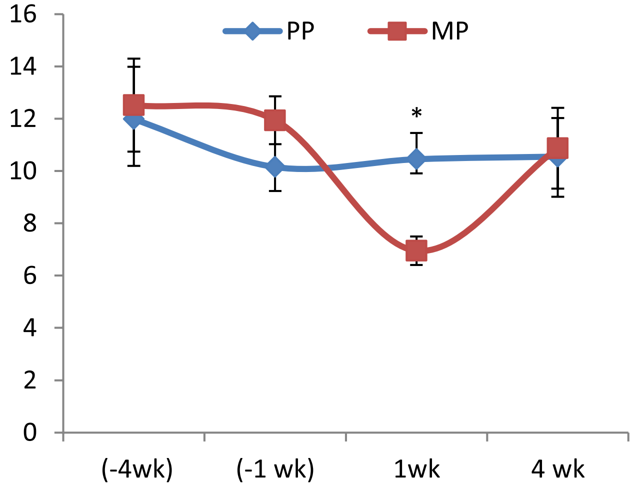

Fig. 6 Effect of parity and physiological stage on calcium (mg/dl) concentration in ewes. *Significant difference between (PP) primiparous and (MP) multiparous

in both the groups. While calcium levels in PP were lowest at 1-week pre-lambing, in MP, the lowest concentration was observed at 1-week post-lambing (Fig. 6); Pi levels were lowest at 1-week post-lambing in both the groups, while K concentrations were less during pre-lambing with the lowest concentration at 1-week pre-lambing (Table 3). While there was no significant group difference, a significant interaction was observed between the two groups with significantly low Ca levels in MP ewes at 1-week post-lambing and significantly low Pi in PP ewes at 1-week pre-lambing (Table 2; Figs. 6 and 7). No significant change was observed in Na concentration.

Plasma trace minerals revealed a significant change along the time in Fe ($p < 0.01$), Cu ($p < 0.05$), and Zn ($p < 0.01$; Table 2). While Fe levels revealed a decreasing trend at all sampling periods, plasma copper levels were significantly higher from 4-week pre-lambing to 1-week post-lambing and plasma Zn levels were lower during the same period compared to the levels at 4-week post-lambing (Table 3). No significant group and time × group interaction was observed between the groups (Table 2).

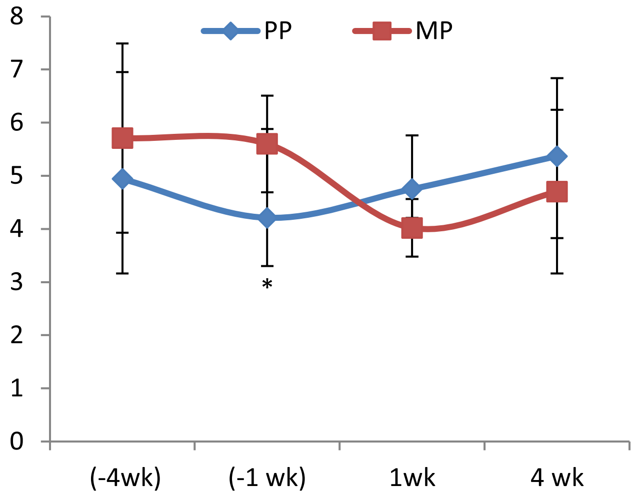

Fig. 7 Effect of parity and physiological stage on phosphorus (mg/dl) concentration in ewes. *Significant difference between (PP) primiparous and (MP) multiparous

Discussion

The nutrition partition during the periparturient period put considerable strain on animal health. This strain is considerably increased by intensive farming methods adopted for higher quality products increasing the susceptibility to peripartum disorders. In the present study, glucose concentrations were higher in lactation than pregnancy and similar findings were reported by other researchers (Balikici et al. 2007; Moghaddam and Hassanpour 2008; Taghipour et al. 2011). Increased glucose levels after lambing indicate that ewe feed intake has recovered and her energy status has improved. Glucose is the main source of energy for the developing fetus as well as for placenta, uterine tissue, and supporting membrane which together put heavy demand on maternal glucose supply during late pregnancy (Khan and Ludri 2002; Magistrelli and Rosi 2014). The last 6 weeks of gestation accounts for more than 50% of fetal growth (Mohammadi et al. 2016), and during this period, fetal glucose metabolism accounts for 40 to 70% total glucose metabolized in sheep, resulting in low systemic glucose concentration as observed in late part of gestation in our study.

After lambing, plasma glucose levels were higher in PP ewes compared to MP. The largest part of circulating glucose is used by the lactating mammary gland for lactose production, and due to incomplete development of the mammary gland in primiparous animals, the mammary glucose uptake is low resulting into greater systemic glucose level (Magistrelli and Rosi, 2014).

Despite the fact that glucose is the principal metabolic fuel and is required for crucial organ function, particularly fetal growth and milk production, it remains an insensitive indicator of energy status due to its tight homeostatic regulation (Rayan et al. 2019). Monitoring the energy status of pregnant sheep by measuring serum NEFA concentration is an alternative and useful technique.

As the glucose utilization by the developing fetus and extra uterine tissue increases, the required increase in glucose production may be inadequate to meet their demands which lead to mobilization of lipid reservoir and increasing free fatty acid concentration in blood. The significant increase in plasma NEFA concentration during the last month of gestation and early lactation indicates that the animals were in NEB. To overcome this NEB, the body mobilizes the fat to compensate for the shortage in energy needed, resulting in an increase of NEFA concentration in the blood (Caldeira et al. 2007). The increase in NEFA in the late pregnancy and early lactation coincided with the decline in glucose concentration, and this type of adjustment is necessary to meet the energy demand of growing fetus and mammary gland for lactogenesis and increased milk secretion. The rise in NEFA levels coincided with a drop in glucose levels in late pregnancy and early lactation, and this adjustment in metabolism is required to fulfill the energy demands of the growing fetus and mammary gland for lactogenesis (Samira et al., 2016).

Comparing the two groups, NEFA was significantly high in 1-week pre-lambing in PP while MP ewes had significantly high NEFA at 1-week post-lambing. Animals in early parties are still in the growing stage and require nutrients both for the growth of the fetus and the animals themselves (Wathes et al. 2007), which leads to a significant decrease in glucose and increase in NEFA in the last stage of pregnancy as observed in PP ewes in the present study. Thus, the increased need of energy in early parities causes increased mobilization of body fats leading to the increased NEFA concentration. This is in contrast to natural belief that primiparous ewes are less prone to NEB and metabolic disorders, like pregnancy toxemia. The present study confirms that primiparous ewes are equally susceptible to NEB and hence to the subsequent metabolic disorders. With increasing parities, udder development naturally increases, resulting in steadily increased milk production, and around the fourth or fifth lactation, the maximal milk yield is reached (Pavlicek et al. 2006; León et al. 2012; Magistrelli and Rosi 2014; Abraham et al. 2017). Thus, the significantly high NEFA concentration and corresponding low glucose concentration observed in MP in post-lambing could be attributed to the more energy demand for milk production in MP ewes and hence more mobilization of body reserves for synthesis and maintenance of milk during early lactation.

A significant change was observed in the TPP and albumin levels, with concentration reaching its lowest value 1-week post-lambing. El-Sherif and Assad (2001) reported decreased plasma protein and albumin in late gestation and early lactation; however, Baumgartner and Pernthaner

(1994) did not find significant effect of the physiological stage on the serum protein concentration in Karakul sheep. Since ruminants' hepatic gluconeogenesis is predominantly accomplished using gluconeogenic amino acids, the reduction in TPP and albumin levels with the progression of pregnancy may be attributed to the greater protein and energy requirements for gestation (Balikci et al. 2007). During the last stages of gestation, proteins are the primary nutrients for uterine tissue, and the fetus synthesizes all its protein from the amino acid derived from the dam (Antunovic et al. 2002; Schmitt et al. 2018), and during this period, the fetus tissues particularly muscle grow exponentially resulting in a corresponding decrease in maternal protein levels. The immediate decrease in TPP after lambing could be attributed to the removal of γ-globulin from the blood for milk secretion after parturition (Cepeda-Palacios et al. 2018). Celi et al. (2008) reported that TPPs are significantly low after parturition and contributed to the removal of γ-globulin from the maternal circulation.

Urea and creatinine are constituents of nitrogen metabolism (Cepeda-Palacios et al. 2018), and their increased levels are associated with kidney damage; however, their decreased concentration is related protein and energy levels in diet (Samira et al. 2016). BUN level is a significant indicator of dietary protein intake, synthesis, and degradation in both sheep and goats (Schroder et al. 2003). In the present study, significant decrease in BUN concentration was observed from 4-week pre-lambing to 1-week post-lambing when BUN levels were lowest; however, no significant change in creatinine was observed except at 1-week post-lambing when levels were highest. The decrease in BUN could be due to increased urea recycling into the digestive tract (Gurgoze et al. 2009) or the use of urea for protein synthesis on the rumino-hepatic route, as reported by Yokus et al. (2006) in sheep, to compensate for inadequate protein uptake during late gestation. Similar findings were reported by Mohammadi et al. (2016) in Makouei breed of sheep and contributed this increase to the decrease in feed intake due to stress and hormonal changes. The amount of creatinine secreted daily remains unaffected by diet, age, sex, or exercise but is a function of the muscle mass (Njidda et al. 2013). High need for energy by ewe during lactation leads to an increase in protein catabolism which increases blood creatinine level to an extent above the ability of kidneys to eliminate (El-Sherif and Assad 2001), and thus, the observed increase in creatinine 1-week post-lambing might have been because of high protein catabolism during this stage as corresponding protein levels were lowest 1-week post-lambing. Our finding corroborates with the findings of El-Sherif and Assad (2001) and Piccione et al. (2009) who reported increased creatinine levels because of increased protein mobilization for growing fetus and for initiation of lactation.

During the periparturient period, the liver and kidneys are in the state of hyperfunction (El-Sherif and Assad 2001), resulting in the corresponding biochemical changes in the blood. In the present study, there was a significant change in AST and GGT level with the activity increased steadily towards the end of gestation and reached the highest activity in the immediate post-lambing period. The activity of AST provides an estimate of liver function (Donia et al. 2014) and is best associated with impaired hepatic function in fatty liver disease and has been used in herd monitoring programs to detect fatty liver disease. Change in activities of enzyme may be related to reduced dry matter intake and subsequent increase in fat mobilization around parturition leading to hepatic lipidosis and thus altering the normal function of the liver (Greenfield et al. 2000). To provide the energy and protein requirements for the onset and maintenance of milk synthesis, there is an intense burden on the liver (Roubies et al. 2006) in lactating ewes, resulting in increased liver enzymes as observed in the present study. Antunovic et al. (2011) reported the highest AST activity in ewes in the first 3 weeks of lactation when milk production was highest, while El-Sherif and Assad (2001) reported more increase in AST during late pregnancy than lactation because of impairment in some muscle and liver cells due to rapid gluconeogenesis associated with pregnancy.

GGT is a membrane-bound enzyme found in cells with higher rates of absorptive or secretory capacity. Although GGT activity is seen in many organs, it is predominantly used as a serum marker in animals to diagnose liver illness (Milinković-Tur et al. 2005). Antunovic et al. (2011) reported increased GGT in ewes during lactation due to intense liver function which corroborates with our findings. The mammary gland's GGT activity is also significant, and during milk synthesis initiation and maintenance, GGT is released from the alveolar cell membrane into colostrum or milk, varying its activity in serum (Ramos et al. 1994). A small part can reach the blood, which will contribute to the increase in serum level; however, the major part comes from the liver because of its overactivity during the periparturient period. Since initiation and maintenance of milk production are directly related to GGT levels, the probable significant increase in MP at 1- and 4-week post-lambing could be because of the increase in milk production in MP ewes.

Besides liver-specific enzymes, plasma bilirubin is also an indicator of liver injury (Lubojacka et al. 2005). The present study revealed a significant increase in bilirubin concentration in late gestation and early lactation. A similar finding was reported by Bertoni and Trevisi (2013), who observed a significant increase in bilirubin concentration during the periparturient period in dairy cows. Bilirubin is not a protein, but its clearance is due to some liver-specific enzymes. Its increase is probably because of the lower synthesis of enzymes responsible for its clearance, which mainly occurs during the liver insult (Bertoni and Trevisi 2013). Our findings suggest that liver and kidney functions of crossbred Rambouillet sheep were experiencing a state of hyperfunction during late gestation and early lactation which can result in subsequent metabolic disorders.

During pregnancy, serum cholesterol and triglyceride levels gradually declined and reached their lowest levels after lambing. Antunović et al. (2002) reported higher cholesterol in late pregnancy compared to lactating ewes while El-Bassiony et al. (2018) reported low cholesterol and triglycerides in ewes during late gestation and early lactation. Cholesterol is synthesized in the small intestine epithelium for the transportation of dietary lipids; therefore, lower plasma levels may be expected because of lower dry matter intake around the periparturient period (Douglas et al. 2006). Also, cholesterol is the precursor of various steroid hormones whose concentration increases in late gestation (McDonald et al. 2002). Cholesterol is also an important component of milk, and during lactation, an increase in norepinephrine and epinephrine production stimulates free fatty acid mobilization, whereas lipogenesis and esterification are inhibited, resulting in a drop in cholesterol levels in the immediate post-lambing period (Nazifi et al. 2002; Tanvi et al. 2016). HDL constitutes about 60% cholesterol (Sevinc et al. 2003), so the observed HDL change in the present study could be due to a corresponding decrease in cholesterol levels.

A significant decrease in serum triglycerides was observed in 1-week pre- and post-lambing, and this drop could be explained as the effect of increased lipolysis, which is regulated by hormones, and not an indication of energy insufficiency. Karapehlivan et al. (2007) reported decreased plasma triglyceride levels on the first day of lactation due to NEB, and Antunović et al. (2011) reported increased triglyceride as the lactation advanced because of decreased milk yield and less NEB. The NEFA extracted from the liver are oxidized or esterified into triglycerides, and either exported in very low-density lipoproteins (VLDL) or accumulated in liver tissue, and ruminants have lesser capability to synthesize and secrete VLDL from the liver, but a similar capacity to reconvert NEFA back to triglyceride (Graulet et al. 1998). Thus, the imbalance of the liver's ability to uptake fatty acid due to NEB and its capacity to secrete lipoproteins synthesized from triglycerides (Pysera and Opalka 2000) decreases plasma triglyceride levels. Moreover, the circulating triglycerides also contribute considerably to synthesis of milk fat (Nazifi et al. 2002; Tanvi et al. 2016), and thus, the observed decrease 1-week pre- and post-lambing could also be due to the mobilization of triglycerides for initiation and maintenance of milk synthesis during early lactation.

Plasma Ca and Pi levels significantly decreased in the last month of gestation and continued to decrease up to 1-week post-lambing. Antunović et al. (2011) reported significant drop in plasma Ca and increase in plasma Pi in Tsigai ewes due to more drain of Ca in milk and Doaa et al. (2014) reported decreased Ca levels in late pregnancy and early lactation; however, no change was observed in Pi. Calcium levels required for pregnancy and lactation are much higher than those for maintenance; therefore, to meet the increased requirements at tissue level, Ca and Pi absorption from the gastrointestinal tract and resorption from bones should increase (Donia et al. 2014). However, during high demand of pregnancy and lactation, this process is unable to balance the loss of ions from blood, and hence, concentrations of these ions decrease (Elnageeb and Adelatif 2010). Thus, the increased requirement of Ca for fetal skeleton mineralization during late gestation and increased secretion of Ca in milk during early lactation (Liesegang et al. 2007; Antunović et al. 2017) coupled with less dry matter intake results in decreased Ca concentration. The decreased Pi has been attributed to a decrease in dry matter intake and increased utilization to enhance carbohydrate metabolism of pregnancy. Moreover, it has been reported that with the increase in milk production, more Pi from the ingested amount is transferred to milk and less is secreted with feces, causing more drop in blood Pi levels (Valk et al. 2002) and this might have resulted in low Pi concentration in immediate post-lambing in the present study. Parity was found to affect mineral levels; PP ewes had low Ca and Pi pre-lambing than MP, while MP had less mineral post-lambing. The Ca and Pi requirement is more in young ones for skeletal growth and since the PP animals besides having increased demand of minerals for mineralization of fetal skeleton are themselves in their active growing stage resulting in more drain of mineral in them (Wathes et al. 2007).

Though there was no significant change in Na levels with the time, K levels decreased significantly in the last month of gestation. Elnageeb and Adelatif (2010) reported that K levels decreased significantly during late gestation and attributed these changes to decreased plasma progesterone and increased aldosterone levels, resulting in more K excretion, hence decreased levels in the blood.

Plasma Fe levels decreased during late pregnancy and continued to fall 1- and 4-week post-lambing. The drop in plasma Fe levels observed during late pregnancy and early lactation may be due to the fetus's high need for Fe. Similar findings were reported by Yokus and Cakir (2006) and Tanritanir et al. (2009). In blood, Fe is mainly bound with proteins called transferrin and ferritin, and the amount of ferritin in maternal blood has been considered to indicate the amount of Fe stored in the body, and its concentration falls as pregnancy advances. During pregnancy, substantial quantity of ferritin is deposited on placental villous tissue and gets integrated into the placenta via pinocytosis in the trophoplast, thereby decreasing its blood levels (Swenson and Reece 1993).

Physiological status affects the trace mineral levels and Zn concentration has been found to decrease along the gestation and lactation periods (Elnageeb and Adelatif 2010). Doaa et al. (2014) and Elnageeb and Adelatif (2010) reported decreased Zn and Cu levels in late pre-partum and early postpartum Saudi ewes and Desert ewes, respectively, which corroborates with the finding of the present study. Developing fetus accumulates almost 1 to 2 mg of Zn per day. The demand for zinc in later gestation increased many folds when fetus is growing exponentially (Donia et al. 2014; Elnageeb and Adelatif 2010), resulting in the decreased concentration of Zn in maternal blood. Zn is primarily bound to albumin and the change in albumin concentration may have a significant effect on Zn levels. In the present study, the albumin 1-week post-lambing was lowest resulting in a corresponding decrease in the Zn concentration (Elnageeb and Adelatif 2010). Moreover, there is also a heavy loss of Zn in colostrum and milk (Pavlata et al. 2004) which might have led to a further decrease in Zn concentration in post-lambing.

Similar to Zn, blood Cu status also fluctuates during the periparturient period. The increase in Cu concentration during late pregnancy could be related to high progesterone levels or the fetal demands and mobilization of stored maternal Cu for the development of the nervous system (Elnageeb and Adelatif 2010). The immediate postpartum period is often stressful, and animals undergoing stressful periods usually have high concentration of ceruloplasmin, a Cu transport protein (Ward and Spears 1999). Ceruloplasmin is an acute-phase protein, and its levels rise in response to injury, infection, and inflammation. This could explain why the blood level of Cu was higher in post-lambing, as lambing and immediate lambing are a stressful period with tissue damage, such as in the uterus (Meglia et al. 2001).

Conclusion

This study evaluated the pattern of effect of parity and physiological stage on biochemical and mineral profile in crossbred Rambouillet sheep of the Himalayan region. The present study suggests that parity plays an important role in the biochemical and mineral alternation seen in ewes at different physiological stages. As a result of this finding, we propose that primiparous ewes suffer more pronounced changes than multiparous ewes in the immediate pre-lambing to maintain the nutrient supply for their continued body growth and the growth of fetus. In immediate post-lambing, primiparous ewes are better equipped than multiparous ewes to cope up with the metabolic stress because of underdeveloped udder and hence low milk production. These findings indicate that primiparous ewes suffer from NEB during pre-lambing and are thus equally susceptible to the metabolic disorders resulting from NEB, like pregnancy toxemia, and hence need proper management as well as simultaneous monitoring of blood parameters that would reduce the occurrence of such disorders. However, there is one limitation in this study: a number of subject in the experiment were less, and to this end, additional large-scale studies are needed. Nevertheless, these findings could be taken into consideration in the development of better diets and management plans for late-gestating and early-lactating sheep of various parities.

Author contribution R Singh and V Singh planned and designed the research. A Singh followed the clinical process. A Singh and V Singh made laboratory measurements. S A Beigh analyzed statistical data. R Singh and N Sharma discussed the results and contributed to the final manuscript.

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Data availability On request.

Declarations

Ethics approval The Institutional Animal Ethics Committee (IAEC) of SKUAST-Jammu, India, has authorized all of the techniques utilized in this study.

Conflict of interest The authors declare no competing interests.

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