Effect of Physiological Status And Parity On Metabolic And Trace Elements Profile of Crossbred Rambouillet Sheep of Himalayan Region

RAJIV SINGH (rajivrajiv101@gmail.com)
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu https://orcid.org/0000-0003-2854-1530

A SINGH
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

S A BEIGH
Sher-E-Kashmir University of Agricultural Sciences and Technology Kashmir

N SHARMA
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

V SINGH
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

Research Article

Keywords: Himalyan, Rambouillet, Metabolic, Mineral, Multiparous, Primiparous

Posted Date: September 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-838542/v1

Version of Record: A version of this preprint was published at Tropical Animal Health and Production on January 17th, 2022. See the published version at https://doi.org/10.1007/s11250-022-03068-z.

Abstract

The study was designed to evaluate the effect of physiological status and parity on metabolic profile in crossbred Rambouillet ewes of Himalyan 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-weeks post-lambing to measure metabolic parameters and minerals. The glucose($p<0.01$), Total plasma protein (TPP) ($p<0.05$), albumin ($p<0.05$), BUN ($p<0.05$), cholesterol ($p<0.05$), triglyceride ($p<0.01$), HDL-C ($P<0.05$), Calcium ($p<0.01$), Phosphours ($p<0.05$), Magnesium ($p<0.01$), Cu ($p<0.05$), and Zn ($p<0.01$) levels revealed significant change along the time with the concentration decreasing 3 weeks pre lambing to immediate post lambing thereafter levels increased steadily. Significant increase ($P<0.01$) was observed in NEFA, AST, GGT, Iron($P<0.05$), 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, calcium, and phosphorus. 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 shows a significant change in metabolic profile and trace minerals during late gestation and immediate post-partum, however these changes were more pronounced during late gestation in primiparous and post lambing in multiparous.

Introduction

Pregnancy and lactation are very critical physiological states in small ruminants during which nutritional requirements are increased manifold. 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. 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.

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 BHBA concentrations (Van Knegsel et al. 2007) and some other blood metabolites like insulin, glucose, protein, and cholesterol tryglyceride, BUN, and creatinine (Piccione 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 wellbeing of an animal much earlier than apparent changes become visible in animal (Doaa et al. 2014; Antunović et al. 2017).

Parity has been seen to effect 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. The present study aimed to understand the metabolic changes in ewes during periparturient periods and to compare the relationship in primiparous and multiparous during peri-parturient periods in crossbred Rambouillet sheep of Himalyan region.

Materials And Methods

Animals, husbandry and nutrition

The study was carried out on 20 healthy Rambouillet crossbred sheep, at an organized Government Sheep Breeding Farm, Panthal, Udhampur, Jammu and Kashmir, India between Decembers until February, when the climate is suitable and there is no risk of heat 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 ewes (PP) and the second group comprises ewes that have lambed multiple times (MP). All ewes lambed without assistance. All the animals were provided a daily diet comprising of oats hay (3.5% of live weight) in addition to concentrate with 16% crude protein @ 0.3 kg/ sheep twice daily. Freshwater was offered ad libitum and mineral supplementation was done on a daily basis, which included sodium (Na), chloride (Cl), potassium (K), phosphorous (P), magnesium (Mg), calcium (Ca), iodine (I) selenium (Se) and iron (Fe). 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 sheep: 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 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 heparinised glass vials (dipped overnight in 2N HCl). The blood samples were transported in ice box to prevent hemolysis.

Blood samples were centrifuged for 30min at 3000 rpm to separate plasma immediately after collection to prevent haemolysis. 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 manufacturer's instructions. The estimation of Non-esterified fatty acids (NEFA) (DiaSys 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-glutamyltransferase (GGT) (Transasia, ERBA), cholesterol (Transasia. ERBA), triglyceride (TG) (Transasia, ERBA), 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), Sodium (Na) and Potassium (K) by DiaSys kit were carried. For trace mineral analysis, 3ml of plasma sample was digesting in 15ml distilled concentrated nitric acid. Approximately, 1ml of left over digestate was then diluted with double distilled water and the concentration of micro-minerals viz. copper (Cu), iron (Fe), zinc (Zn), were then measured by Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI).

Statistical analysis

Overall descriptive statistics (mean and standard error) for each blood constituent were calculated. The data were tested for normality by applying Shapiro-Wilk normality test and homogeneous variance by Levene test. Data was subjected to repeated measure test and multiple comparisons, considering parity (G), sampling time(T), and their interactions (T×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 the fixed effect of the i^{th} parity, T_j is fixed effect of the j^{th} sampling, $(T\times G)_{ij}$ is interaction between group and sampling time, and e_{ij} is the residual error. Only significant group or group and time interaction was kept and represented in figures.

Results

The glucose and NEFA concentration revealed a significant ($p < 0.01$) change along the time (Tables 1 and 2), with glucose concentration being lowest at one week pre-lambing in PP and one week post lambing in MP ewes (Fig. 1). The NEFA concentrations were highest at one 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 ($p < 0.01$) one 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 1
Results (P values) of repeated measures with ewes as random effect group, time, group and group* time interaction as fixed effect for the dependant variables in blood (biochemical parameters)

Parameters	Time (Weeks)	Group	Group* Time
NEFA	0.003	0.059	0.01
Glucose (mg/dl)	0.007	0.068	0.022
Total 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
Triglyceride (mg/dl)	0.005	0.570	0.117
Cholesterol (mg/dl)	0.016	0.117	0.317
HDL-C (mg/dl)	0.032	0.418	0.569
AST (IU/L)	0.004	0.091	0.171
GGT (IU/L)	0.002	0.114	0.068
Bilirubin (mg/ l)	0.040	0.234	0.528
Na (m eq/l)	0.172	0.221	0.241
K (m eq/l)	0.038	0.079	0.192
Ca (mg/dl)	0.013	0.082	0.037
Pi (mg/dl)	0.020	0.187	0.024
Fe (µmol/ l)	0.018	0.421	0.216
Zn (µmol/ l)	0.027	0.103	0.780
Cu (µmol/ l)	0.031	0.328	0.129

Table : 2: Biochemical and mineral profile of Rambouliet 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}
Na (m eq/l)	149.25 ± 10.09^{a}	145.08 ± 6.70^{a}	147.64 ± 5.32^{a}	153.61 ± 8.05^{a}
K (m eq/l)	4.96 ± 0.31^{ab}	4.16 ± 0.13^{b}	5.14 ± 0.22^{a}	5.21 ± 0.39^{a}
Ca (mg/dl)	12.25 ± 1.52^{a}	11.05 ± 1.81^{a}	8.7 ± 0.91^{b}	10.71 ± 1.47^{a}
Pi (mg/dl)	5.33 ± 0.83^{a}	4.92 ± 0.83^{ab}	4.27.±0.64^{b}	5.00 ± 0.33^{a}
Fe (µmol/ l)	240.52 ± 50.42^{a}	185.53 ± 44.67^{a}	106.66 ± 23.11^{b}	58.87 ± 19.70^{b}
Zn (µmol/ l)	13.76 ± 1.19^{b}	17.42 ± 2.01^{b}	16.52 ± 2.2^{b}	30.18 ± 3.53^{a}

Values with at least one similar superscripts didn’t differ significantly

Parameters	-4 Weeks	-1 Week	1 Week	4 Weeks
Cu (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 superscripts didn't differ significantly				

Total plasma protein (TPP) in both the groups showed a significant ($p < 0.05$) change with time, with significant decrease in concentration from 4 weeks pre lambing to one week post-lambing when concentration reached to their lowest value and thereafter a steady increase was observed (Tables 1 and 2). 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 as creatinine concentration reached to peak value at the same time (Tables 1 and 2). No significant group difference was observed between the groups, however, pre-lambing protein and albumin concentrations were numerically low in PP and post lambing concentrations were numerically low in MP, albeit (Table 1; 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 one week post-lambing and there-after decreasing steadily (Table 2). While there was no significant group difference in any parameter, the time*group revealed significantly high GGT activities in MP animals at 1 week and 4 week post-lambing (Table 1; Fig. 5)

Significant change was observed in cholesterol ($p < 0.05$), TG ($p < 0.01$) and HDL-C ($P < 0.05$) in both the groups (Tables 1 and 2). 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 2). No significant group and time*group interaction was observed between the groups (Table 1).

Among the plasma minerals, significant change was observed in Ca ($p < 0.01$), Pi ($p < 0.05$), and K ($p < 0.01$) in both the group. While as in PP calcium levels were lowest at one week pre-lambing, in MP lowest concentration was observed at one-week post-lambing (Fig. 6), Pi levels were lowest at one-week post-lambing in both the groups, while as K concentrations were less during pre-lambing with lowest concentration at 1 weeks pre-lambing (Table 2). 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 one week post lambing and significantly low phosphors in PP ewes at one week pre-lambing (Table 1; 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 1). While as Fe levels revealed a decreasing trend at all sampling period, plasma copper levels were significantly higher from 4 week pre-lambing to one week post lambing and plasma zinc levels were lower during the same period compared to the levels at 4 week post-lambing (Table 2). No significant group and time*group interaction was observed between the groups (Table 1).

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 ewes 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 Lundri, 2002; Magistrelli and Rosi, 2014). The last six weeks of gestation accounts for mora than 50% of fetal growth (Mohammadi et al. 2016), and during this period fetal glucose metabolism account for 40–70% total glucose metabolized in sheep, resulting in low systemic glucose concentration as observed in late part of gestation.

After lambing plasma glucose levels were higher in PP ewes compared to MP. The lactating mammary gland uses the major part of circulating glucose for lactose production. Due to incomplete mammary gland development in primiparous animals, the mammary glucose uptake is low, resulting in 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 foetal 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.

During the later pregnancy as the required increase in glucose production to fulfill the demands of developing fetus and extrauterine tissue may be insufficient, mobilization of the lipid reservoir and increase free fatty acid content in the blood occurs to meet the energy demands. The significant increase in plasma NEFA concentration during last month of gestation and early lactation indicates that the animals were in negative energy balance (NEB). To overcome this NEB the body mobilize the fat to compensate for the shortage in energy needed, resulting in an increase of NEFA concentration in 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 (Samira et al. 2016). 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 fulfil the energy demands of the growing foetus and mammary gland for lactogenesis (Samira et al., 2016).

Comparing the two groups, NEFA was significantly high one 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 fetus and the animals itself (Wathes et al. 2007), which leads to a significant decrease in glucose and increase in NEFA in 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. 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 one-week post-lambing. Brozostowski et al. (1995) also reported decreasing trend of TPP and albumin levels during late pregnancy and gradual increases towards the end of lactation. 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). Protein is the primary nutrient for uterine tissue during the last stages of pregnancy (Schmitt et al.2018), and protein synthesized by fetus are made from amino acids obtained from dam (Antunovic et al.2002; Schmitt et al.2018) and during this period, the foetus 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 total proteins are significantly low after parturition and contributed to the removal of γ-globulin from the maternal circulation.

During late stage of gestation, numerically low TTP and albumin levels were observed in PP ewes had, while as reverse was observed in MP which had numerically low TPP and albumin during lactation and this could be due to the fact that PP ewes need nutrients for their growth as well as the growth of fetus resulting into more drain of available proteins in blood (Wathes et al. 2007). Morever, albumin is mostly directed toward foetal development tissue, whereas globulin is utilized in the production of milk (Balıkcı et al. 2007; Karapehlivan et al. 2007; Obidike et al. 2009), resulting into more decrease of albumin pre-lambing in PP compared to MP.

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). Plasma urea 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 urea concentration was observed from 4 weeks prelambing to 1 week post-lambing when urea levels were lowest, however no significant change in creatinine was observed except at one week post-lambing when level were highest. The decrease in BUN could be due to increased urea recycling into the digestive tract or better nutrition management (Gürgöze et al. 2009) or the use of urea for protein synthesis on the rumino-hepatic route, as reported by Yokus and Cakir 2006 in cattle, to compensate for inadequate protein uptake during late gestation. 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 one-week post-lambing might have been because of high protein catabolism during this stage as corresponding protein levels were lowest one-week post-lambing.

During the periparturient period, liver and kidneys are in hyper-function (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. 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 programmes to detect fatty liver disease. Reduced dry matter intake and consequent increase in fat mobilisation during the peripartum period, leading to hepatic lipidosis and hence affecting liver function, could be the cause of altered enzyme activity(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.

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). 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 over activity during the peri-parturient period. Since initiation and maintenance of milk production are directly related to GGT levels, the probable significant increase in MP at1 and 4 weeks 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 peri-parturient 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 occur during the liver insult.

During pregnancy, serum cholesterol and triglyceride levels gradually declined and reached their lowest levels after lambing. 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). During late gestation,

utilization of cholesterol by fetus (Guédon et al. 1999) increases, resulting in less plasma cholesterol levels. Cholesterol is also an important component of milk, and during lactation, an increase in nor-epinephrine and epinephrine production stimulates free fatty acid mobilisation, 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 one week pre- and post-lambing. This drop could be interpreted as the result of increased lipolysis, which is regulated by hormones, and not an indication of energy insufficiency. The NEFA extracted by 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 TGs (Graulet et al. 1998). Thus, the imbalance of the liver's ability to uptake fatty acid and its capacity to secrete lipoproteins synthesized from triglycerides (Pysera and Opalka, 2000) decreases triglyceride levels. Moreover, the circulating triglycerides also contribute considerably to synthesis of milk fat (Nazifi et al. 2002; Tanvi et al. 2016). Thus, the observed decrease one-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 P levels were significantly decreased in the last month of gestation and continued to decrease up to one week post lambing. 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 calcium 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 calcium concentration. The decreased phosphorus 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 phosphorus from the ingested amount is transferred to milk and less is secreted with faeces, causing more drop in blood phosphorus levels (Valk et al. 2002) and this might have resulted in low phosphorus concentration in immediate post-lambing in the present study. Parity was found to affect minerals 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 during in them (Wathes et al. 2007).

Though there was no significant change in sodium levels with the time, potassium levels decreased significantly in the last month of gestation. Elnageeb and Adelatif (2010) reported that potassium levels decreased significantly during late gestation and attributed these changes to decreased plasma

progesterone and increased aldosterone levels, resulting in more potassium excretion hence decreased levels in the blood.

Plasma Fe levels decreased during late pregnancy and continued to fall 1 and 4 weeks 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 iron. Similar findings were reported by Yokus and Cakir (2006) and Tanritanir et al. (2009). In blood, iron 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 lowering its maternal blood levels (Swenson and Reece(1993).

Physiological status affects the zinc levels, with decreased zinc along the gestation and lactation periods (Elnageeb et al. 2010). Developing feotus accumulates almost 1 to 2 mg of Zn/ day. The demand for zinc in later gestation increased many fold when feotus is growing exponentially (Donia et al. 2014; Elnageeb et al. 2010), resulting in the decreased concentration of zinc in maternal blood. Zinc 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 one-week post-lambing was lowest resulting in a corresponding decrease in the Zn concentration (Elnageeb et al. 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 zinc, blood Cu status also fluctuates during the periparturient period. The increase in copper concentration during late pregnancy could be related to high progesterone levels or the fetal demands and mobilization of stored maternal copper for the development of the nervous system (Elnageeb et al. 2010). The immediate post-partum period is often stressful, and stressed animals' blood levels of Cu and ceruloplasmin, a Cu transport protein, are frequently high (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 is a stressful period with tissue damage, such as in the uterus. (Meglia et al. 2010).

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 suggest 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 under-developed udder and hence low milk production. These findings may aid in the development of better diets and management plans for late gestating and early lactation sheep of various parities.

Declarations

Conflict of interest

There are no conflicts of interest for the authors to disclose.

Funding

The University Research Grant, SKUAST-Jammu funded the research.

Ethics approval

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

Availability of data

On request

Author's 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.

References

1. Abraham, H., Gizaw, S., & Urge, M., 2017. Milk production performance of Begait goat under semi-intensive and extensive management in Western Tigray, North Ethiopia. Livestock Research for Rural Development, 29, 12.
2. Antunović, Z., Novoselec, J., Šperanda, M., Steiner, Z., Ćavar, S., Pavlović, N., Lendić, K.V., Mioč, B., Paćinovski, N. and Klir. Z., 2017. Blood metabolic profile and milk quality of ewes, Mljekarstvo, 67(4), 243–252. doi:10.15567/mljekarstvo.2017.0401.
3. Antunović, Z., Senčić, Đ., Šperanda, M. and Liker, B., 2002. Influence of the season and the reproductive status of ewes on blood parameters. Small Ruminant Research, 45(1), 39–44. doi: 10.1016/S0921-4488(02)00109-8.
4. Balıkcı, E., Yıldız, A. and Gürdoğan, F., 2007. Blood metabolite concentrations during pregnancy and post-partum in Akkaraman ewes. Small Ruminant Research, 67 (2–3), 247–251.
5. Bertoni, G. and Trevisi, E., 2013. Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. Veterinary Clinics of North America: Food Animal Practice, 29(2), 413–431.
6. Brozostowski, H., Milewski, S., Wasilewska, A. and Tanski, Z., 1995. The influence of the reproductive cycle on levels of some metabolism indices in ewes. Arch. Vet. Pol., 35, 53–62.

7. Caldeira, R. M., Belo, A. T., Santos, C. C., Vazquez, M. I. and Portuuga, A.V., 2007. The effect of long-term feed restriction and over-nutrition on body condition score, blood metabolites and hormonal profiles in ewes. Small Rum. Res., 68, 242–255. http://dx.doi.org/10.1016/j.smallrumres.2005.08.026.
8. Celi, P., Di Trana, A. and Claps, S., 2008. Effects of perinatal nutrition on lactational performance, metabolic and hormonal profiles of dairy goats and respective kids. Small Ruminant Research, 79(2–3), 129–136.
9. Cepeda-Palacios, R., Fuente-Gómez, M.G., Ramírez-Orduña, J.M., García-Álvarez, A., Llinas-Cervantes, X., Angulo, C., 2018. Effects of pregnancy and post-kidding stages on haematochemical parameters in cross-bred goats. Journal of Applied Animal Research, 46(1), 269–273.
10. Chauhan, S. S., Celi, P., Fahri, F. T., Leury, B. J. and Dunshea, F.R., 2014. Dietary antioxidants at supranutritional doses modulate skeletal muscle heat shock protein and inflammatory gene expression in sheep exposed to heat stress. J. Anim. Sci., 92, 4897–4908. doi:10.2527/jas.2014-8047.
11. Doaa, F. T., Nashwa, A. H. A., Hanan, A., Tag El-Din., Safaa, M., Soud, A. E. and Hassan, O. H., 2014. Study on levels of some blood hormonal and biochemical constituents during different reproductive status in saudi ewes. Egypt. J. Sheep Goat Sci., 9 (3), 105–113.
12. Donia, G. R., Ibrahim, N.H., Shaker, Y.M., Younis, F.M. and Amer, Z.A., 2014. Liver and kidney functions and blood minerals of Shami goats fed salt tolerant plants under the arid conditions of southern Sinai, Egypt. J Am Sci., 10 (3), 49–59.
13. Douglas, G.N., Overton, T.R., Bateman, H.G., Dann, H.M. and Drackley, J.K., 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in holstein cows. Journal of Dairy Science, 89(6), 2141–2157.
14. Elnageeb, M. E. and Adelatif, A. M., 2010. The minerals profile in Desert Ewes (Ovis aries): Effects of pregnancy, lactation and dietary supplementation. Am. Eurasian J. Agric. Environ. Sci., 7 (1), 18–30. http://www.idosi.org/.../4.pdf
15. El-Sherif, M.M.A. and Assad, F., 2001. Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi-arid conditions. Small Ruminant Research, 40(3), 269–277.
16. Graulet, B., Gruffat, D., Durand, D., Bauchart, D., 1998. Fatty acid metabolism and very low density lipoprotein secretion in liver slices from rats and preruminant calves. Journal of Biochemistry, 124(6), 1212–1219.
17. Greenfield, R.B., Cecava, M.J., Johnson, T.R.and Donkin, S.S., 2000. Impact of dietary protein amount and rumen undegradability on intake, peripartum liver triglyceride, plasma metabolites, and milk production in transition dairy cattle. Journal of Dairy Science, 83(4),703–710.
18. Guédon, L., Saumande, J., Dupron, F., Couquet, C. and Desbals, B., 1999. Serum cholesterol and triglycerides in post-partum beef cows and their relationship to the resumption of ovulation. Theriogenology, 51,1405–1415. DOI:10.1016/S0093-691X(99)00083-7.

19. Gürgöze, S. Y., Zonturlu, A.K., Özyurtlu, N. and İÇen, H., 2009. İvesi koyunlarında gebelik sürecinde ve postpartum döneminde bazı biyokimyasal parametreler ile mineral maddelerin araştırılması. Kafkas Univ Vet Fak Derg. Published online. DOI:10.9775/kvfd.2009.858.
20. Herdth, T. H., 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Vet. Clin. North Am. Food Anim. Pract., 16, 215–230. DOI:10.1016/s0749-0720(15)30102-x.
21. Karapehlivan, M., Atakisi, E., Atakisi, O., Yucayurt, R. and Pancarci, S.M., 2007. Blood biochemical parameters during the lactation and dry period in Tuj ewes. Small Ruminant Research, 73(1–3), 267–271.
22. Khan, J. R. and Lundri, R. S., 2002. Hormonal profile during periparturient period in single and twin fetus bearing, goats. Asian Australas J Anim. Sci., 15, 346–351. Doi.org/10.5713/ajas.2002.346
23. León, J.M., Macciotta, N.P.P., Gama, L.T., Barba, C.and Delgado, J.V., 2012. haracterization of the lactation curve in Murciano-Granadina dairy goats. Small Rum. Res., 107, 76–84. DOI:10.1016/j.smallrumres.2012.05.012
24. Liesegang, A., Risteli, J. and Wanner, M., 2007. Bone metabolism of milk goats and sheep during 2nd pregnancy and lactation in comparison to first lactation. J. Anim. Physiol. Anim. Nutr., 91, 217–55. DOI: 10.1111/j.1439-0396.2007.00695.x
25. Lubojacká, V., Pechová, A., Dvořák, R., Drastich, P., Kummer, V.and Poul, J., 2005. Liver steatosis following supplementation with fat in dairy cow diets. Acta Vet Brno., 74(2), 217–224.
26. Magistrelli, D. and Rosi, F., 2014. Trend analysis of plasma insulin level around parturition in relation to parity in Saanen goats1. Journal of Animal Science, 92(6), 2440–2446.
27. McDonald, P., Edwards, R. A., Greenhalgh, J.F.D. and Morgan, C.A., 2002. Animal Nutrition. 6th Ed. Pearson Education Limited, Essex.
28. Milinković-tur, S., Perić, V., Stojević, Z., Zdelar-tuk, M. and Piršljin, J., 2005. Concentrations of total proteins and albumins, and AST, ALT and GGT activities in the blood plasma of mares during pregnancy and early lactation. Vet. Arhiv, 75, 195–202.
29. Moghaddam, G. and Hassanpour, A., 2008. Comparison of blood serum glucose, beta hydroxybutyric acid, blood urea nitrogen and calcium concentrations in pregnant and lambed ewes. J. Anim. Vet. Adv. 7, 308–311. http://medwelljournals.com/abstract/?dio=javaa.2008.308.311.
30. Mohammadi, V., Anassori, E. and Jafari, S., 2016. Measure of energy related biochemical metabolites changes during peri-partum period in Makouei breed sheep. Vet. Res. Forum, 7 (1), 35–39.
31. Nazifi, S., Saeb, M. and Ghavami, S.M., 2002. Serum lipid profile in iranian fat-tailed sheep in late pregnancy, at parturition and during the post-parturition period. J Vet Med Series A, 49(1), 9–12.
32. Njidda, A. A., Hassan, I. T. and Olatunji, E. A., 2013. Haematological and biochemical parameters of goats of semi-arid environment fed on natural grazing rangeland of northern Nigeria. IOSR J Agri. Vet Sci., 3(2), 2319–2372. DOI:10.9790/2380-0320108.
33. Obidike, I.R., Aka, L.O. and Okafor, C.I., 2009. Time-dependant peri-partum haem., biochemical and rectal temperature changes in West African dwarf ewes. Small Ruminant Research, 82(1), 53–57.

Doi.org/10.1016/j.smallrumres.2009.01.012

34. Pavlata L., Pechova, A. and Dvorak, R., 2004. Microelements in colostrum and blood of cows and their calves during colostral nutrition. Acta Vet. Brno., 73, 421–429. DOI: 10.2754/avb200473040421.
35. Pavlicek, J., Antunović, Z., Senčić, Č.and Šperanda, M., 2006. Production and goat milk contents depending on number and stage of lactation. Poljoprivreda, 12, 52–57.
36. Piccione, G., Caola, G., Giannetto, C., Grasso, F., Runzo, S.C., Zumbo, A. and Pennisi, P., 2009. **Selected biochemical serum parameters in ewes during pregnancy, post-parturition, lactation and dry period**. Animal Science Papers and Reports, **27** (**4**), **321–330**.
37. Pysera, B. and Opałka, A., 2000. The effect of gestation and lactation of dairy cows on lipid and lipoprotein patterns and composition in serum during winter and summer feeding. J Anim Feed Sci., 9(3), 411–424. Doi.org/10.22358/jafs/68061/2000
38. Ramos, J.J., Verde, M.T., Marca, M.C. and Fernández, A., 1994. Clinical chemical values and variations in Rasa Aragonesa ewes and lambs. Small Ruminant Research, 13(2), 133–139. Doi.org/10.1016/0921-4488(94)90088-4.
39. Rayan, A.O., El-abedeen, A.E.Z., Abd Ellah, M.R. 2019. Some Metabolic Parameters During Transition Period in Dairy Cows with and without Retained Fetal Membranes. J. Adv. Vet. Res. 9, 45–48.
40. Roubies, N., Panousis, N., Fytianou, A., Katsoulos, P.D., Giadinis, N., and Karatzias, H., 2006. Effects of age and reproductive stage on certain serum biochemical parameters of chios sheep under greek rearing conditions. J Vet Med Series A., 53(6), 277–281. DOI:10.1111/j.1439-0442.2006.00832.x
41. Russel, A. J. F., Doney, J. M. and Gunn, R. G., 1969. Subjective assessment of body fat in live sheep. J. Agr. Sci., 72, 451–454. DOI: 10.1017/S0021859600024874
42. Samira, A.M., Mohammed, A.R., Anaam, E.O., Sheeba, A. and Waleed, M.A.G., 2016. Biochemical and hematological profile of different breeds of goat maintained under intensive production system. Afr J Biotechnol, 15(24),1253–1257.
43. Schmitt, E., Maffi, A.S., Raimondo, R.F.S., Lima, M.E., Hoffmann, D.A.C., Farofa, T.S., Montagner, P., Rincón, J.A.A., Del Pino, F.A.B. and Corrêa, M.N., 2018. Energetic metabolic profile of ewes presenting low body condition score induced to subclinical hypocalcemia in early post-partum, Austral J. Vet. Sci., 50(1), 15–20.
44. Schroder, B., Schoneberger, M., Rodehutscord, M., Pfeffer, E. and Breves, G., 2003. Dietary protein reduction in sheep and goats: different effects on L-alanine and L-leucine transport across the brush-border membrane of jejunal enterocytes. J. Comp. Physiol., 173, 511–518. DOI:10.1007/s00360-003-0359-3
45. Sevinc, M., Basuglu, A. and Guzelbektas, H., 2003. Lipid and lipoprotein levels in dairy cows with fatty liver. Turk Vet Anim Sci., 27, 295–299.
46. Swenson, M.J. and Reece, W.O., 1993. Water balance and excretion in Dukes' physiology of Domestic Animals, 11th edition, Cornell University Press, Ithaca, NY, 573–604.

47. Taghipour, B., Seifi, H.A., Mohri, M., Farzaneh, N. and Naserian, A., 2011. Variations of energy related biochemical metabolites during periparturition period in fat-tailed baloochi breed sheep. Iran. J. Vet. Sci. Tech., 2, 85–92. Doi.org/10.22067/veterinary.v2i2.8368.
48. Tanritanir, P., Dede, S. and Ceylan, E., 2009. Changes in some macro minerals and biochemical parameters in female healthy Siirt hair goats before and after parturation. J. Anim.Vet. Adv., 8 (3), 530–533.
49. Tanvi, D., Chaudhary, S.S., Singh, V.K, Patel, S.B. and Puri, G., 2016. Hematobiochemical profile in Surti goats during post-partum period. Vet. World, 9(1), 19–24. Doi: 10.14202/vetworld.2016.19-24
50. Valk, H., Sebek, L.B.J. and Beynen, A.C., 2002. Influence of phosphorus intake on excretion and blood plasma and saliva concentrations of phosphorus in dairy cows. J. Dairy Sci., 85(10), 2642–2649. Doi.org/10.3168/jds.S0022-0302(02)74349-X
51. Van Knegsel, A. T. M., Brand, H.V. D., Graat, E. A. M., Dijkstra, J., Jorritsma, R., Decuypere, E., Tamminga, S. and Kemp. B., 2007. Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. J. Dairy Sci., 90, 1477–1485. Doi.org/10.3168/jds.S0022-0302(07)71633-8
52. Ward, J.D. and Spears. J.W., 1999. The effect of low-copper diets with or without supplemental molybdenum on specific immune responses of stressed cattle. J. Anim. Sci., 77, 230–237. DOI:10.2527/1999.771230x
53. Wathes, D.C., Cheng, Z., Bourne, N., Taylor, V.J., Coffey, M.P., Brotherstone, S., 2007. Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. Domestic Animal Endocrinology, 33(2), 203–225. DOI:10.1016/j.domaniend.2006.05.004
54. Yokus, B. and Cakir, D. U., 2006. Seasonal and physiological variations in serum chemistry and mineral concentrations in cattle. Biol Trace Elem Res., 109(3), 255–266. DOI:10.1385/BTER:109:3:255.

Figures

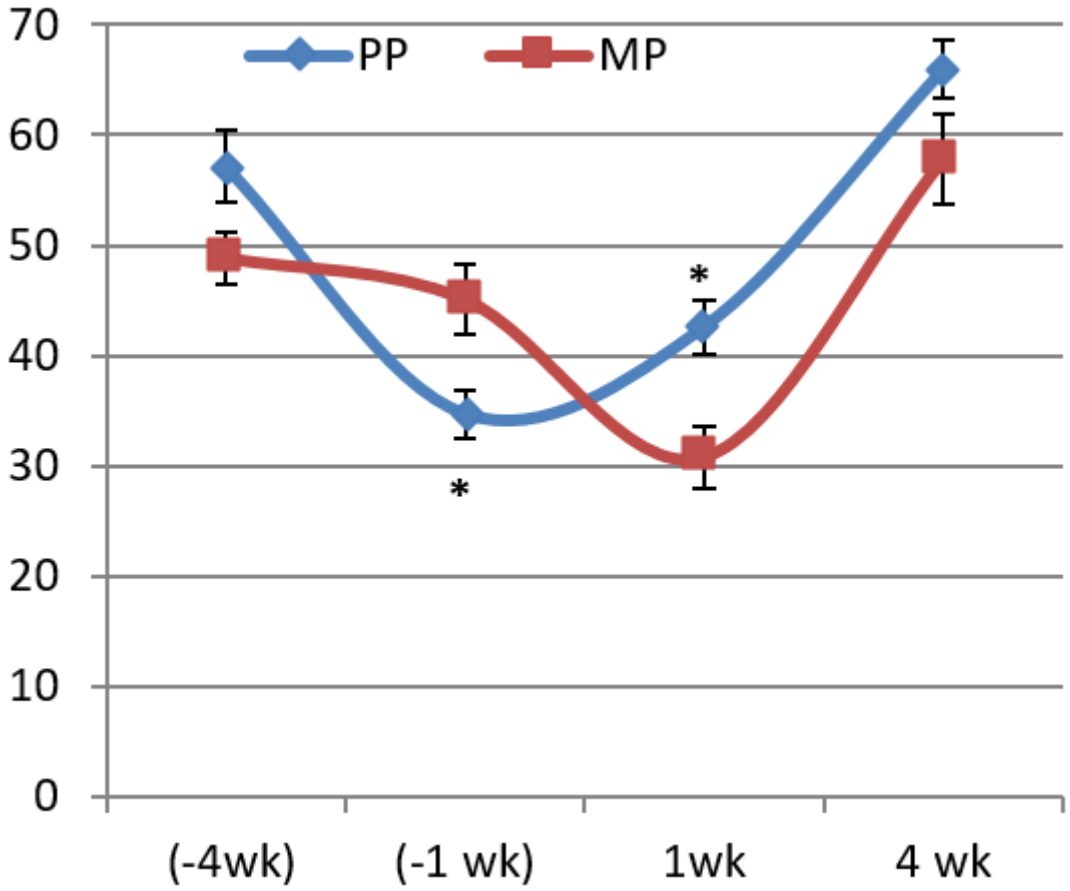

Figure 1

Effect of parity and physiological stage on glucose (mg/dl) concentration in ewes * represent the significant difference between PP and MP.

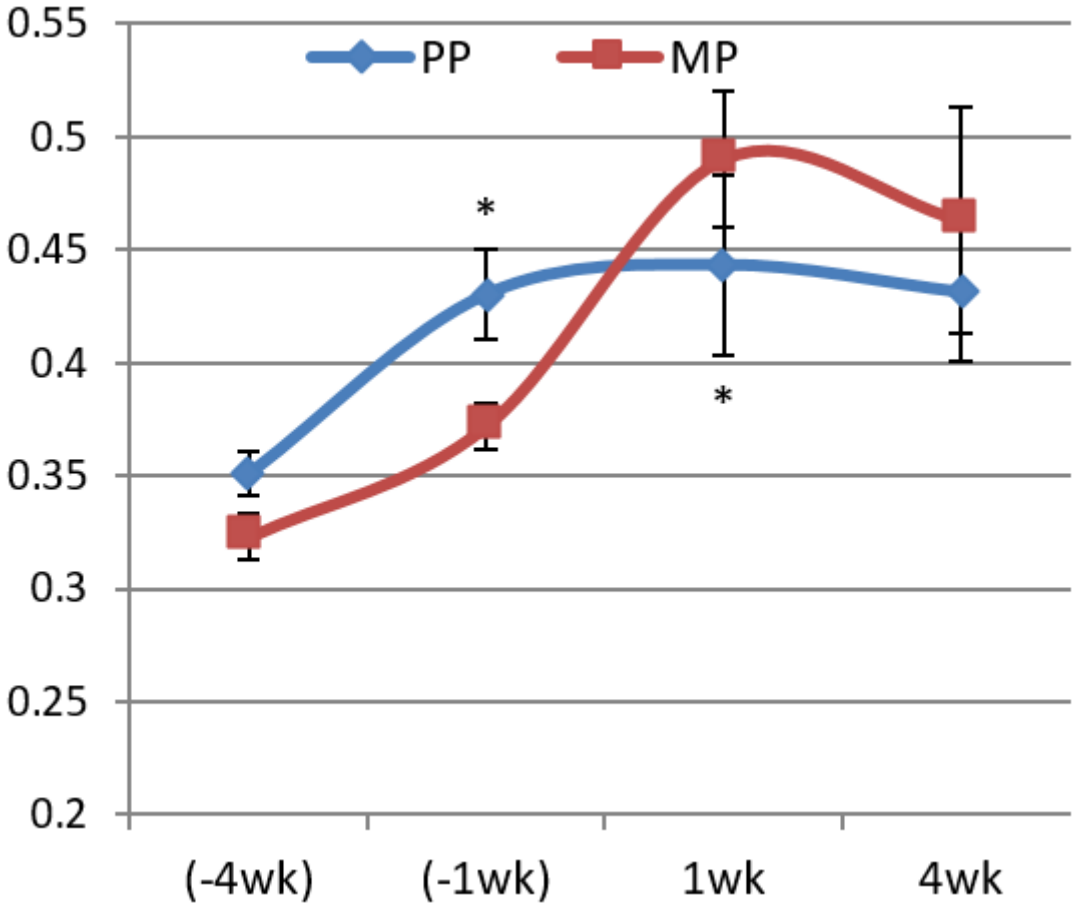

Figure 2

Effect of parity and physiological stage on NEFA (mmol/l) concentration in ewes * represent the significant difference between PP and MP.

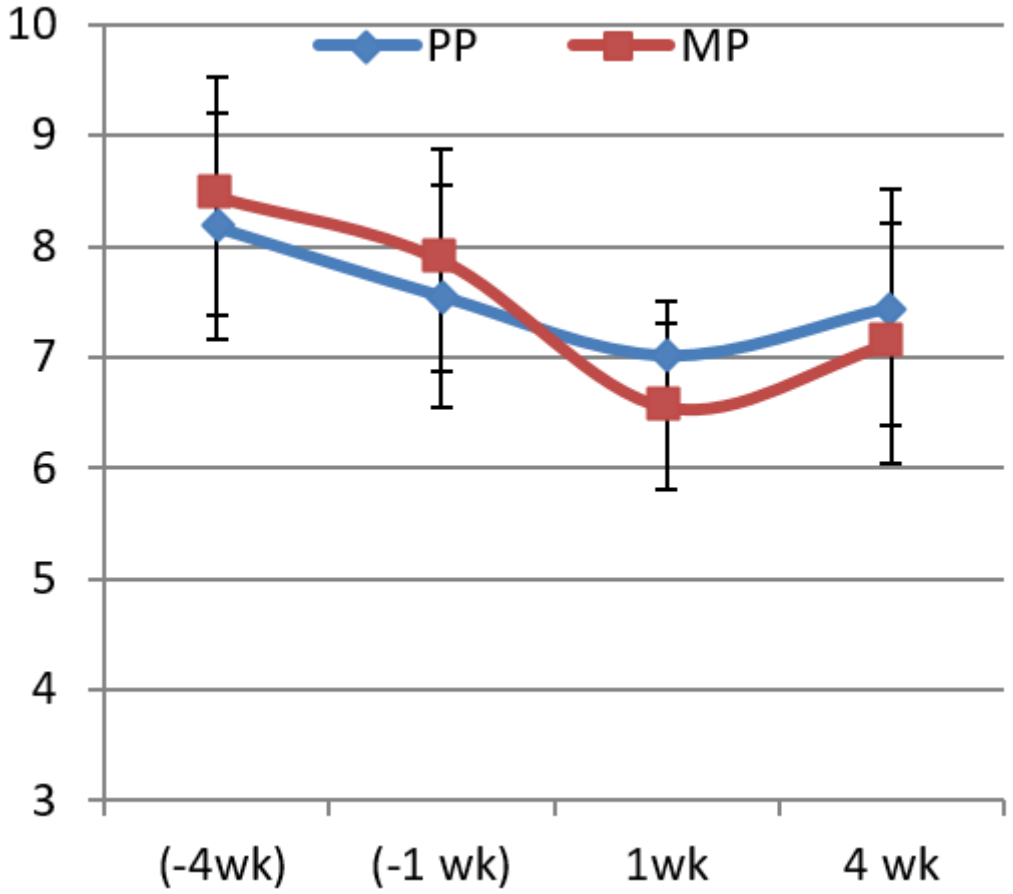

Figure 3

Effect of parity and physiological stage on Total protein (mg/dl) concentration in ewes

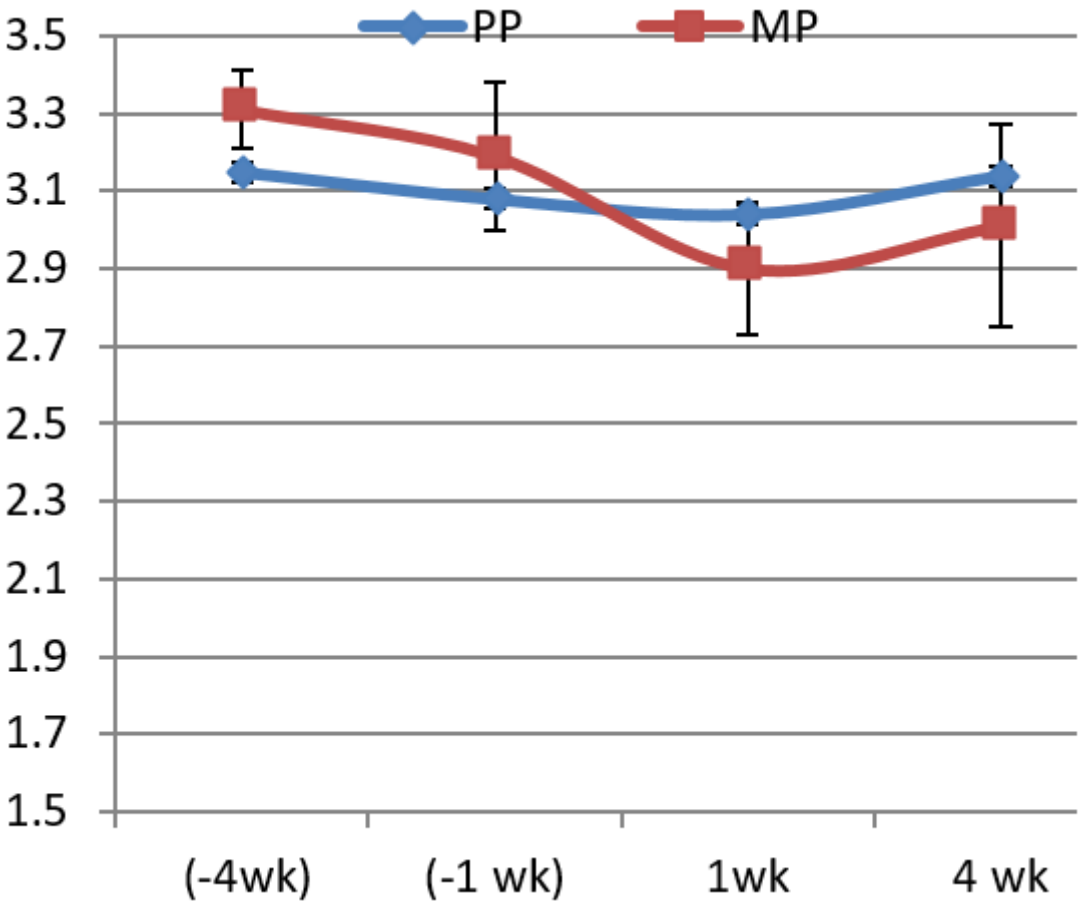

Figure 4

Effect of parity and physiological stage on Albumin (mg/dl) concentration in ewes

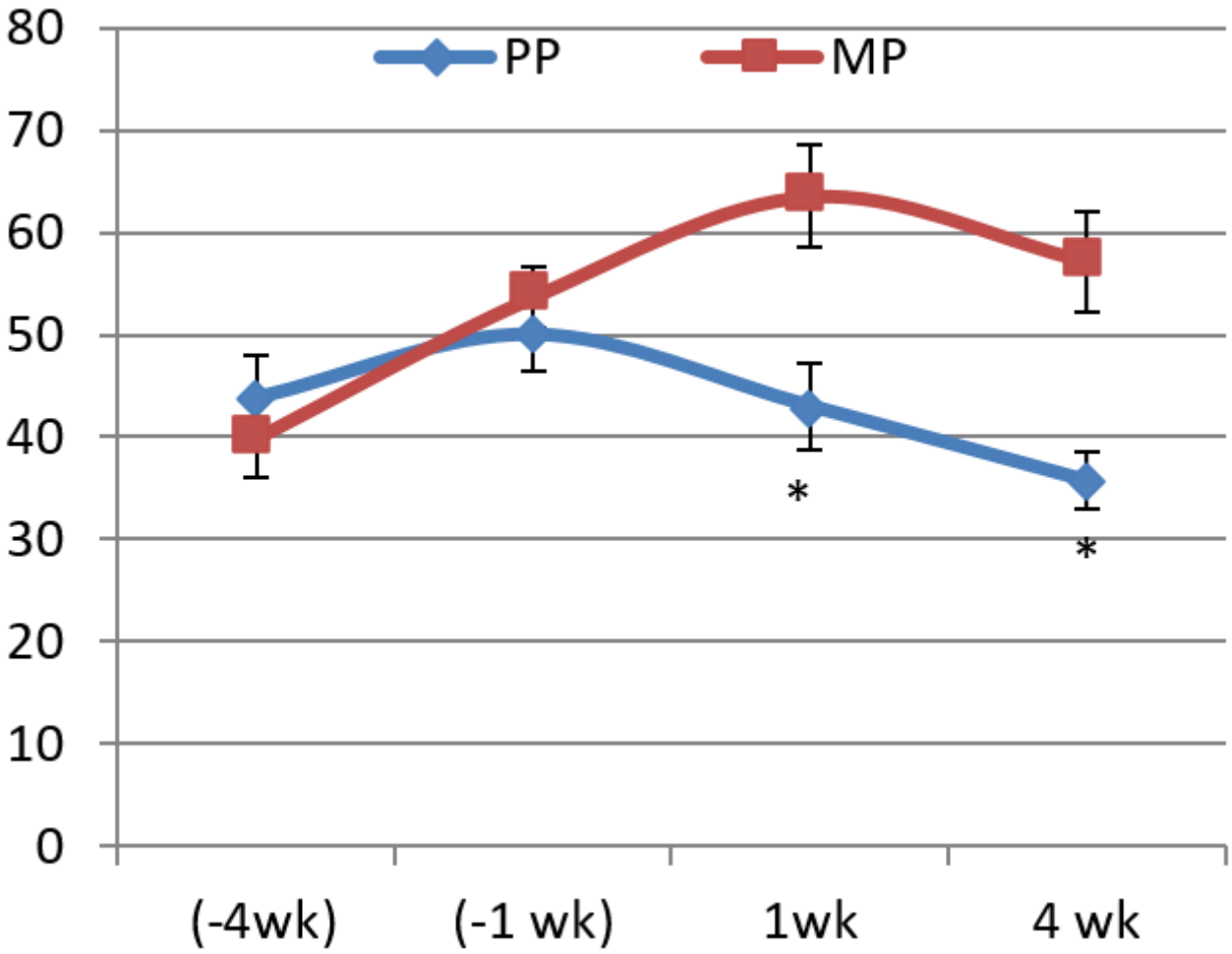

Figure 5

Effect of parity and physiological stage on GGT (IU/l) concentration in ewes * represent the significant difference between PP and MP.

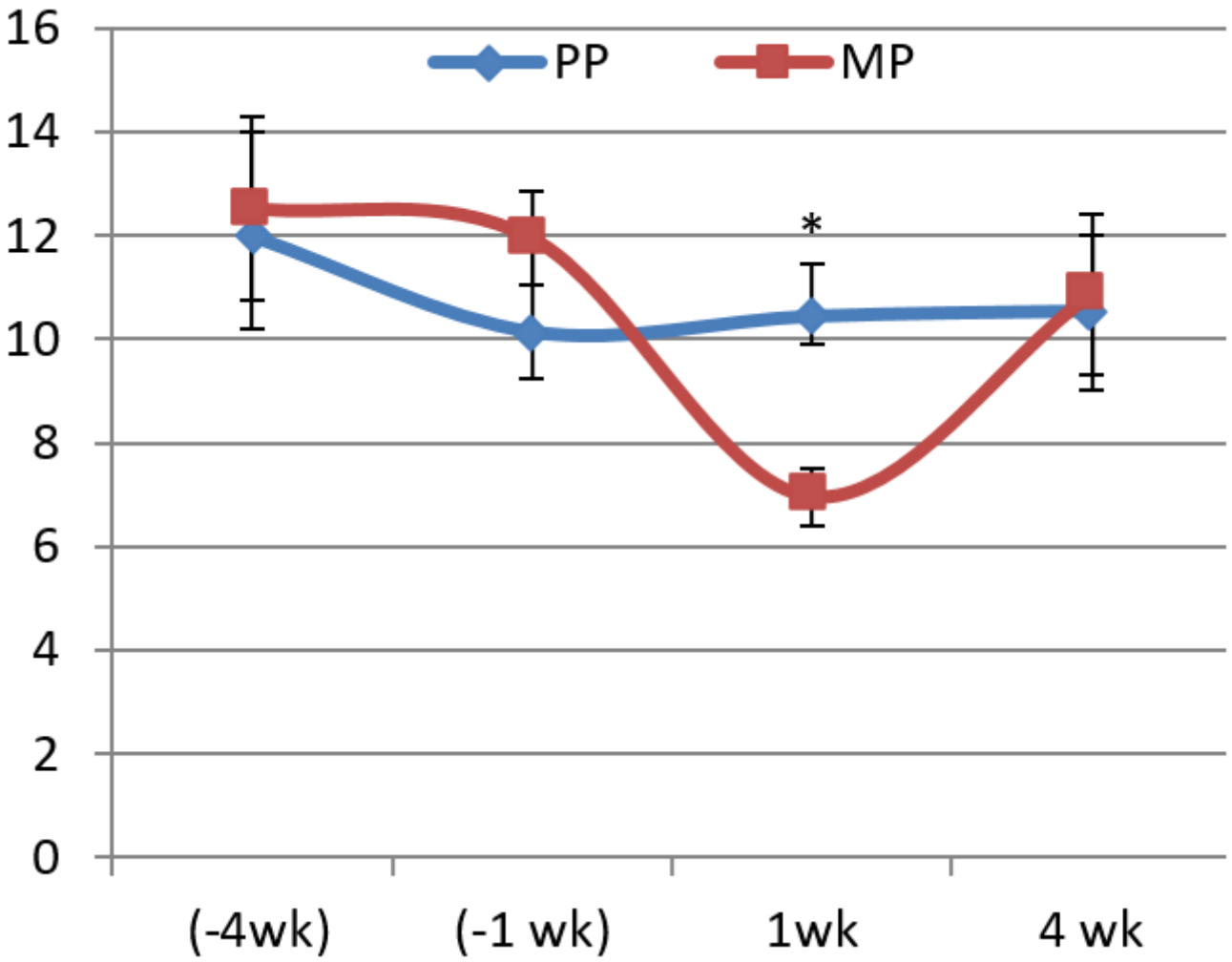

Figure 6

Effect of parity and physiological stage on Calcium (mg/dl) concentration in ewes * represent the significant difference between PP and MP.

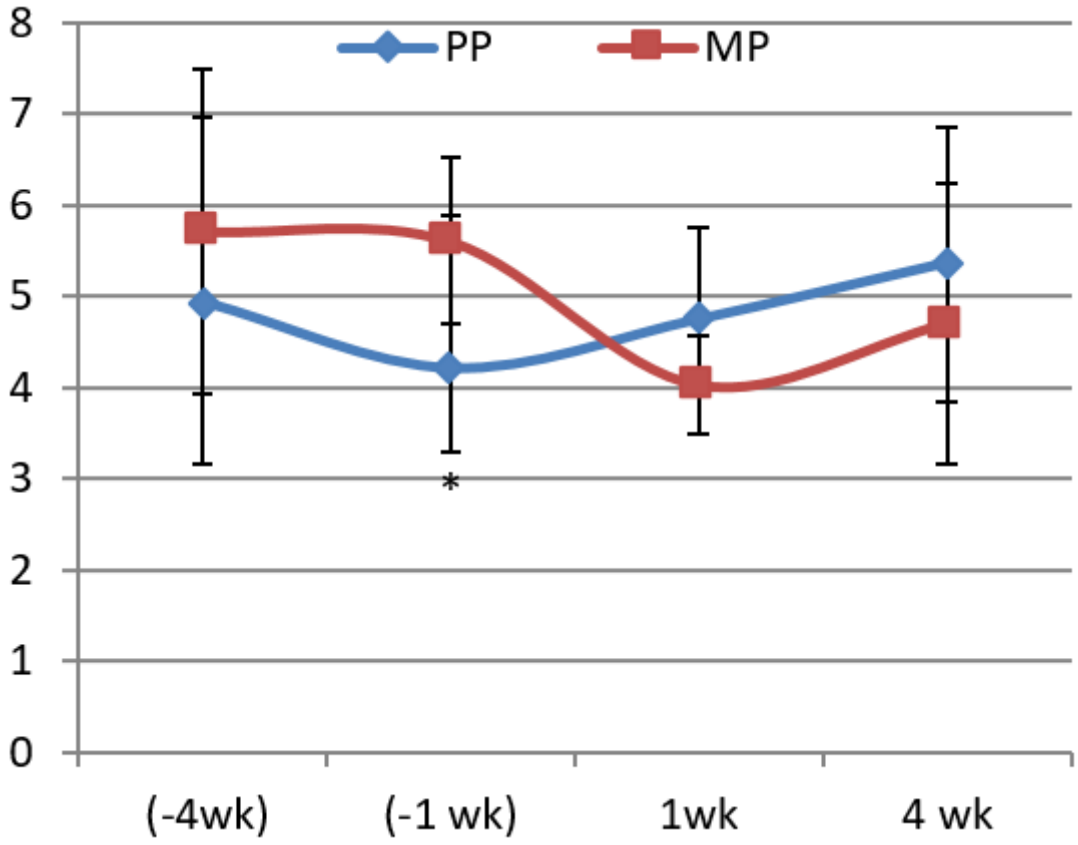

Figure 7

Effect of parity and physiological stage on phosphours (mg/dl) concentration in ewes * represent the significant difference between PP and MP.