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Research Article / Araştırma Makalesi

Investigation of Adiponectin, Leptin and Ghrelin Levels and Evaluation of Metabolic Profiles in the Periparturient Period in Romanov Sheep

Romanov Koyunlarında Geçiş Döneminde Adiponektin, Leptin ve Ghrelin Düzeylerinin Araştırılması ve Metabolik Profillerinin Değerlendirilmesi

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Abstract: The periparturient period is very important especially in terms of pregnancy-related herd-based diseases. In this period, especially for early diagnosis of subclinical diseases, a metabolic profile test is used. In the periparturient period, important changes occur in adipose tissue. Blood samples were collected from 16 Romanov sheep in the periparturient in serum tubes on the 21st, 14th, 7th, and postpartum days, and 7th, 14th, and 21st days after delivery. Metabolic profile and Adiponectin, Leptin and Ghrelin parameters evaluated from the blood samples. In the periparturient period, ELISA analyzes of TNF-α were performed for inflammatory evaluation. As a result of the analyzes, Prenatal increase in NEFA concentrations, postpartum decrease in cholesterol concentrations, postpartum increase and decrease in AST concentrations, postpartum increase in GGT concentrations, prenatal and postnatal increases in total protein concentrations, postpartum increase in BUN and creatinine concentrations, postpartum decrease in magnesium concentrations, Prenatal increase in adiponectin concentrations and postpartum decrease, postpartum decrease in leptin concentrations and prenatal increase in ghrelin concentrations occurred. With the results obtained, it was concluded that the evaluation of metabolic profile and adipose tissue is important in the diagnosis of diseases in the periparturient period.

Keywords: Adiponectin, Ghrelin, Leptin, Metabolic profile, Periparturient period.

Öz: Periparturient dönem, doğumdan önceki 3 hafta ve doğumdan sonraki 3 hafta olmak üzere toplam 1,5 ayı kapsar. Periparturient dönem özellikle gebeliğe bağlı sürü kaynaklı hastalıklar açısından çok önemlidir. Bu dönemde özellikle subklinik hastalıkların erken teşhisi için metabolik profil testi kullanılmaktadır. Periparturient dönemde yağ dokusunda önemli değişiklikler meydana gelir. Çalışma materyali olarak periparturient dönemdeki 16 Romanov koyunundan doğum öncesi 21., 14., 7. ve doğum sonrası 7., 14. ve 21. günlerde serum tüplerine kan örnekleri alındı. Kan örneklerinden metabolik profil Adiponektin, Leptin ve Ghrelin parametreleri değerlendirilmişitir. Periparturient dönemde enflamatuar değerlendirme için TNF-α ELISA analizleri yapıldı. Analizler sonucunda NEFA konsantrasyonlarında prenatal artış, kolesterol konsantrasyonlarında postpartum düşüş, AST konsantrasyonlarında postpartum artış ve düşüş, GGT konsantrasyonlarında postpartum artış, total protein konsantrasyonlarında prenatal ve postnatal artış, BUN ve kreatinin konsantrasyonlarında postpartum artış, magnezyum konsantrasyonlarında postpartum düşüş, adiponektin konsantrasyonlarında prenatal artış ve postpartum düşüş, leptin konsantrasyonlarında postpartum düşüş ve ghrelin konsantrasyonlarında prenatal artış meydana geldi. Elde edilen sonuçlar ile periparturient dönemdeki hastalıkların tanısında metabolik profil ve yağ dokusunun değerlendirilmesinin önemli olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Adiponektin, Ghrelin, Leptin, Metabolik profil, Periparturient dönem.

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Introduction

Romanov sheep are known as one of the most productive breeds. This short-tailed sheep breed was first bred in the Yaroslavl region of Russia. It has been reported that Romanov sheep are characterized by an extremely long season of sexual activity and early sexual maturity, which is used by breeders to shorten the mating and thus production interval and increase annual selection progress (Vostry et al., 2018).

Due to the high incidence of disease during transition period of sheep.and the increase in technologies that facilitate behavioural monitoring, researchers have become increasingly interested in investigating the later health and performance relationships of postnatal nutrition, rest, and social behaviour (Sepúlveda-Varas et al., 2012).

In developed countries, metabolic profile testing is applied as the most important criterion, with the control of metabolic diseases and the increase in yield expectation from animals. With this tests, the needs and yields of animals are regulated from the dry period to the end of lactation (Aslan et al., 1993). Evaluation of metabolic profile is considered a reliable tool by veterinarians to evaluate nutrient-productivity relationships (Chalmeh et al., 2017).

Periparturient period (PPD); is defined as the last 3 weeks of pregnancy (3 weeks before birth) and the first 3 weeks after delivery, a total of 1.5 months (Arslan et al., 2012). The three weeks before birth is called the dry period, and the first three-week period after birth is called the lactation period (Tunç ve ark., 2017). The peripartum period; covers three periods: prenatal (prepartum), birth (partum), and postpartum (postpartum). Physiological, metabolic, and physical changes occur to adapt to the transition from pregnancy to motherhood, pregnancy, birth, and breastfeeding (Sepúlveda-Varas et al., 2012). In the PPD, in addition to major changes in the placenta, breast, liver, adipose tissue, muscle tissue, and gastrointestinal system in ruminants, weakening of the immune system and some metabolic and hormonal changes occur (Merhan and Özcan, 2010). Farm animals that become ill shortly after birth are more likely to die young and are less productive throughout their lives (Sepúlveda-Varas et al., 2012).

In many species, changes occur in the energy balance and adipose tissue during pregnancy and lactation (Uyanık et al., 2009). It has been reported that the function of adipose tissue is to provide energy in the form of fatty acids to other organs, especially in the first week after birth, when dietary intake cannot meet the high requirements needed for the rapid increase in milk production (Rukkwamsuk et al., 1998).

Adiponectin is primarily secreted by adipose tissue but is also expressed in cardiomyocytes and skeletal muscles (Kabara et al., 2014). Data obtained from clinical studies support the role of hypoadiponectinemia in the development of obesity-related metabolic and cardiovascular diseases, liver diseases, and some types of cancer (Altıner et al., 2016). Leptin is mainly secreted by adipose tissue and is a hormone found in both primary and secondary lymphoid organs and has an important metabolic and immunomodulatory role (Matarese et al., 2005). The primary role of leptin in metabolic homeostasis is to provide hypothalamus information about body fat amount, thereby modulating central nervous system (CNS) functions that regulate late food intake and energy balance (Proloa et al., 1998). Ghrelin, an acylated peptide consisting of 28 amino acids, has been identified as an endogenous ligand of the growth hormone secretagogue receptor (GHS-R). Ghrelin acts through its receptor, GHS-R. Recently, it has been shown that the GHS-R exhibits a high signal of constitutive activity between meals, with efficacy of approximately 50%, providing the endpoint for food intake between meals (Popovic, 2006).

In this study, it was aimed to evaluate the levels of non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHBA), Adiponectin, Leptin, and Ghrelin secreted from the digestive

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system, especially mobilized from adipose tissues during the transition period in sheep breeding, and to recommend the use of metabolic profile testing more widely in sheep. It is thought that it will make an important and innovative contribution to the literature for the pathogenesis of the transition period in sheep.

Material and Method

Ethical Statement

The study protocol was approved by the Animal Ethics Committee for the University of Siirt, (2018/03/05).

Animal Material

The material of the study was started with 20 Romanov breeds aged between 1-4 years of age in Siirt Goat Application and Research Center, which became pregnant beforehand, but was completed with 16 sheep since embryonic death occurred in 4 (four) of the pregnant ewes at the 4th week of pregnancy. All of the animals included in the study were fed in the same housing environment and with the same ration (lentil straw in the morning and evening and 400gr of concentrate feed daily). Clinical examinations of the animals used in the study (body temperature, number of pulsations and respirations, lymph nodes, tracheal palpation, lung auscultation, and percussion) were made in detail.

Collection and Evaluation of Samples

BHBA, NEFA, glucose, albumin, calcium, phosphorus, magnesium, Total protein, BUN, creatinine, cholesterol, triglyceride, GGT, AST, Tumor necrosis factor (TNF-α), adiponectin, leptin, and ghrelin levels were evaluated. Serum samples were taken from the sheep between 11:30 and 14:30 on the 21^{st}, 14^{th}, and 7^{th} days before birth, and at the time of birth and on the 7^{th}, 14^{th}, and 21^{st} days after birth.

Measurement of glucose, triglyceride, calcium, cholesterol, creatinine, albumin, phosphorus, magnesium, AST, GGT, TP, BUN, NEFA, and BHBA values from serum samples was analyzed with an automatic biochemistry analyzer (Randox RX Monaco model sn:5180t240c50485ma) in Burdur Mehmet Akif Ersoy University Veterinary Faculty Animal Hospital Laboratory.

Tumor necrosis factor (TNF-α), Adiponectin, Leptin, and Ghrelin values were measured by ELISA (Enzyme-Linked Immunosorbent Assay) method with commercial kits specific to the species [TNF-α; Sheep Tumor Necrosis Factor (TNF/TNFA/TNFSF2) ELISA Kit (CAT: KTE 110003, Abbkine, China), Adiponectin; Sheep Adiponectin (ADIPOQ) ELISA Kit (CAT: KTE110067, Abbkine, China), Leptin; Sheep Leptin (LEP) ELISA Kit (CAT: KTE110050, Abbkine, China), Ghrelin; Sheep GHRL ELISA Kit (CAT: KTE110062, Abbkine, China)].

Statistical Evaluation

MINITAB program was used for statistical analysis of the data. The distribution of the obtained data was checked by performing a normality test first, and descriptive statistics values were obtained. Repeated Measurements Analysis of Variance (One-Way ANOVA) and Tukey test were used to determine possible differences in the measurement values evaluated on different days for each pair parameter.

Results

Laboratory Findings

Biochemical Findings

In the study, average serum BHBA, NEFA, glucose, cholesterol, triglyceride, AST, GGT, total protein, albumin, BUN, creatinine, calcium, phosphorus, and magnesium concentrations and statistical evaluations of these parameters are shown in Table 1.

No significant difference was found for serum BHBA, glucose, triglyceride, albumin, calcium, and phosphorus concentrations between prenatal, birth, and postpartum days.

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Table 1. Mean serum biochemical values ± standard deviations of animals included in the study (n:16).

Parameters	Prepartum 21st day	Prepartum 14th day	Prepartum 7th day	At Parturition	Postpartum 7th day	Postpartum 14th day	Postpartum 21st day
BHBA (mmol/L)	0.717± 0.553 ^{A}	0.602± 0.498 ^{A}	0.771± 0.827 ^{A}	0.791± 0.720 ^{A}	0.4194± 0.1345 ^{A}	0.4519± 0.1336 ^{A}	0.4813± 0.1202 ^{A}
NEFA (mmol/L)	0.636± 0.518 ^{BC}	0.5388±0.3702 ^{BC}	1.126± 0.978 ^{AB}	1.271± 0.810 ^{A}	0.4156± 0.2452 ^{C}	0.4250± 0.2145 ^{C}	0.564± 0.437 ^{BC}
Glucose (mg/dL)	77.82± 17.46 ^{A}	83.21± 13.14 ^{A}	87.57± 27.92 ^{A}	76.45± 22.33 ^{A}	81.98± 9.95 ^{A}	81.48± 6.82 ^{A}	83.70± 6.11 ^{A}
Cholesterol (mg/dL)	89.92± 9.86 ^{A}	89.50± 15.54 ^{A}	90.01± 19.92 ^{A}	75.73± 10.63 ^{AB}	75.73± 12.73 ^{AB}	70.95± 12.63 ^{B}	78.34± 10.48 ^{AB}
Triglyceride (mg/dL)	32.96± 14.89 ^{A}	42.01± 12.99 ^{A}	39.50± 19.50 ^{A}	45.10± 39.91 ^{A}	33.60± 28.99 ^{A}	46.2± 40.2 ^{A}	41.30± 33.40 ^{A}
AST (U/L)	29.49± 5.52 ^{B}	28.59± 5.87 ^{B}	35.04± 8.11 ^{B}	43.400± 3.704 ^{B}	79.2± 49.4 ^{A}	97.2± 53.8 ^{A}	33.25± 8.56 ^{B}
GGT (U/L)	59.88± 10.84 ^{C}	61.69± 11.48 ^{C}	64.75± 9.97 ^{BC}	60.75± 10.11 ^{C}	80.38± 22.73 ^{AB}	94.19± 25.39 ^{A}	90.50± 21.16 ^{A}
Total Protein (g/dL)	7.071± 0.504 ^{C}	6.761± 0.521 ^{C}	7.883± 0.723 ^{B}	8.098± 0.683 ^{AB}	8.312± 0.492 ^{AB}	8.575± 0.513 ^{A}	8.309 ^{AB}± 0.588
Albumin (g/dL)	3.6713± 0.2586 ^{A}	4.309± 2.329 ^{A}	3.7756± 0.3563 ^{A}	3.7563± 0.2560 ^{A}	3.6300± 0.3161 ^{A}	3.7125± 0.2982 ^{A}	3.6812± 0.2486 ^{A}
BUN (mg/dL)	30.38 ± 6.56 ^{B}	26.00± 5.49 ^{B}	30.75± 9.42 ^{B}	43.63± 17.76 ^{A}	25.38± 8.82 ^{B}	28.13± 8.90 ^{B}	33.25± 8.56 ^{AB}
Creatinine (mg/dL)	1.2569 ± 0.1759 ^{B}	1.2069± 0.1701 ^{B}	1.1969± 0.2223 ^{B}	1.5113± 0.2153 ^{A}	1.1406± 0.1611 ^{B}	1.2212± 0.1565 ^{B}	1.1900± 0.1887 ^{B}
Calcium (mg/dL)	12.779 ± 1.583 ^{A}	12.600± 0.964 ^{A}	12.476± 1.504 ^{A}	12.248± 1.731 ^{A}	13.177± 1.381 ^{A}	13.444± 1.339 ^{A}	13.256± 1.124 ^{A}
Phosphorus (mg/dL)	8.775± 2.219 ^{A}	9.463± 2.389 ^{A}	9.869± 2.600 ^{A}	9.106± 1.917 ^{A}	9.494± 2.382 ^{A}	10.362± 2.886 ^{A}	9.262± 2.983 ^{A}
Magnesium (mg/dL)	3.2212± 0.2978 ^{AB}	3.4744± 0.1914 ^{AB}	3.4850± 0.3535 ^{AB}	3.590± 0.445 ^{A}	3.252± 0.435 ^{AB}	3.513± 0.480 ^{AB}	3.1813± 0.2386 ^{B}

***A, B, C**: The difference between values with different letters on the same line is statistically significant (p <0.05).

Table 2. Mean serum TNF- Alpha. Adinopectin. Leptin and Ghrelin values ± standard deviations of animals included in the study (n:16).

Parameter	*-21th day*	*-21th day*	*-21th day*	*At Parturition*	*7th day*	*14th day*	*21st day*
BHBA (mmol/L)	0.717± 0.553 ^{A}	0.602± 0.498 ^{A}	0.771± 0.827 ^{A}	0.791± 0.720 ^{A}	0.4194± 0.1345 ^{A}	0.4519± 0.1336 ^{A}	0.4813± 0.1202 ^{A}
NEFA (mmol/l)	0.636± 0.518 $_{BC}$	0.5388±0.3702 ^{BC}	1.126± 0.978 ^{AB}	1.271± 0.810 ^{A}	0.4156± 0.2452 ^{C}	0.4250± 0.2145 ^{C}	0.564± 0.437 ^{BC}
TNF-Alpha (pg/Ml)	40.07 ± 12.68 ^{A}	34.86 ± 9.82 ^{A}	50.8 ± 47.30 ^{A}	37.47 ± 10.35 ^{A}	41.18 ± 11.44 ^{A}	38.09 ± 9.90 ^{A}	42.21 ± 12.81 ^{A}
Adiponevtin (ug/L)	44.07 ±90.60 ^{AB}	38.05 ± 8.75 ^{B}	58.2 ± 43.60 ^{A}	37.03 ± 8.83 ^{B}	30.96 ± 7.03 ^{B}	27.68 ± 7.62 ^{B}	25.59 ± 6.25 ^{B}
Leptin (ng/L)	3166 ± 720 ^{AB}	3105 ± 603 ^{AB}	3402 ± 1813 ^{A}	3432 ± 645 ^{A}	3532 ±496 ^{A}	1869.2 ±338.3 ^{C}	2257 ± 459 ^{BC}
Ghrelin (ng/L)	69.39 ±12.33 ^{AB}	57.78 ± 9.15 ^{B}	94.6 ±67.70 ^{A}	68.22 ±11.70 ^{AB}	75.41 ±17.18 ^{AB}	69.41 ±9.67 ^{AB}	75.49 ±19.21 ^{AB}

***A, B, C**: The difference between values with different letters on the same line is statistically significant (p <0.05).

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NEFA concentrations were significantly increased at the time of birth compared to the 21st and 14th days before birth, and a significant decrease occurred on the 7th day after birth. The change in serum NEFA concentrations in the periparturient period was statistically significant (p=0.000).

Serum cholesterol concentrations showed no significant difference between consecutive weeks in the periparturient period. However, there was a significant decrease in the postpartum 14th day compared to the prenatal period (p=0.000).

A significant increase in serum AST concentrations was found at postpartum 7th day and a significant decrease was found at postpartum 21st day. The variation between AST concentrations in the periparturient period was statistically significant (p=0.000).

In the evaluation of serum GGT concentrations, a significant increase occurred on the 7th postnatal day. The variation between serum GGT concentrations in the periparturient period was statistically significant (p=0.000).

Serum total protein concentrations showed a significant increase occurred on the 7th day before birth, and a significant increase occurred on the 14th day after birth compared to the prenatal period. Changes in total protein concentrations in the periparturient period are statistically significant (p=0.000).

It was observed that there was a significant increase in the BUN levels at the time of birth and a significant decrease at the 7th postnatal day compared to the time of birth. Changes in BUN concentrations in the periparturient period are statistically significant (p=0.000).

Serum creatinine concentrations showed a significant increase at the time of birth and a significant decrease on the 7th postpartum day compared to the time of birth (p=0.000).

In the evaluation of serum magnesium concentrations, a significant decrease occurred on the 21st postpartum day compared to the time of birth (p=0.005).

ELISA Findings

In the study, the mean serum TNF-α, Adiponectin, Leptin, Ghrelin concentrations and statistical evaluations of these parameters with the Elisa method are shown in Table 2.

In the evaluation of serum TNF-α concentrations, no significant difference was found between prenatal, birth, and postpartum days (p= 0.450).

Serum adiponectin concentrations were significantly increased at prepartum day 7 and significantly decreased at birth compared to prepartum day 7. The change in serum Adiponectin concentrations in the periparturient period is statistically significant (p=0.000).

Serum leptin concentrations showed a significant decrease on the 14th postpartum day. The change in serum Leptin concentrations during the periparturient period was statistically significant (p= 0.000).

Serum Ghrelin concentrations significantly increase occurred on the 7th day prepartum. The change in serum Ghrelin concentrations in the periparturient period was statistically significant (p=0.028).

Discussion

Prevention of metabolic disorders associated with the periparturient period is an important consideration in the management of ruminant animals (Jones et al., 2018). During the productive lives of sheep, the periparturient period is critical for both animal health and performance. During late pregnancy, sheep are susceptible to serious metabolic diseases (Karagiannis et al., 2014).

Insufficient energy intake causes mobilization of body fats, which increases the concentration of NEFA in the serum, and if the mobilization is excessive, it can cause hepatic lipidosis (Vandehaar et al., 1999). Changes have also been noted in adipose tissue, along with negative energy balance. A study in angora goats stated that serum NEFA concentrations increased in the second week after birth in the periparturient period, and decreased

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continuously in the following weeks, and found that these changes were not statistically significant (Eşki ve ark. 2015). A study conducted on dairy cows reported that blood NEFA level increased at birth, peaked in the first week after birth, and started to decrease after (Yehia and Salem 2015). One study compared the metabolic status of dairy cows in the periparturient period, observed that the NEFA value was significantly higher in cows in the early lactation period than in the late pregnant cows (Djokovic et al., 2013). In Saanen goats, it was reported that serum NEFA concentrations gradually increased 30 days before calving, peaked at birth, after calving gradually decreased until postnatal 35th day, when NEFA reached the lowest level (0.174 mmol/l). In the present study, there was a significant increase in serum NEFA concentrations at the time of birth compared to the 21st and 14th days before birth, and a significant decrease occurred on the 7th day after birth. The increase in NEFA concentrations was thought to be due to the metabolic adaptations that occurred during the transition from late pregnancy to early lactation and the mobilization of fat reserves in the circulation in the form of NEFA to meet the increasing energy demands of lactation.

Small ruminants such as sheep are at risk of ketosis before lambing during late pregnancy, when fetal nutritional demands are highest. BHBA, a ketone substance, is widely used to detect the sheep at risk of developing pregnancy toxemia (Jones et al., 2018). There is a higher BHBA value at the beginning of lactation compared to the last periods of birth and pregnancy in goats (Soares et al., 2018). In Saanen goats, BHBA concentrations increase significantly from 15 days before birth until the 21st day after birth, and then the concentrations decrease (Sadjadian et al., 2013). A study in Makouei sheep reported that the BHBA value increased before birth and decreased after birth (Mohammadi et al., 2016). In the study, no significant difference was found in serum BHBA concentrations during the transition period. Although the changes in BHBA concentrations were not statistically significant, an increase and decrease were observed in parallel with the NEFA concentrations. The increase was attributed to the release of ketone substances by oxidation of fatty acids in the liver to meet the energy needs of sheep entering negative energy balance, especially in the last period of pregnancy and early lactation period.

During the prenatal transition period, most of the maternal glucose supply is used by the near-term fetus, and then after birth, the mammary gland and conditions requiring rapid hepatic changes begin to use the largest portion of the glucose supply. While glucose is regulated under homeostatic controls, a deficiency in dietary carbohydrates or content can lead to inadequate glucose levels (Lager and Jordan, 2012). Boudebza et al. (2016) found that glucose concentrations were significantly higher in the dry period compared to late pregnancy and the beginning of lactation in their study in sheep. One study in Tuj sheep found that serum glucose levels were significantly higher from 1-30 days of lactation to 3 weeks after the dry period. Chalmeh et al. (2017) reported that glucose levels increased before birth, decreased after birth. In the study, no significant difference was found in serum glucose concentrations during the transition period.

In animals other than cattle, it has also been reported that the onset of lactation causes significant changes in hepatic cholesterol metabolism, such as an increase in cholesterol and bile acids in the liver. This adaptation has been suggested to increase the cholesterol level necessary to increase the acid formation and the lipoprotein, cholesterol, and triglyceride levels to support the mammary gland for milk production (Schlegel et al., 2012). Laeger et al. (2013) found no change in cholesterol level during the periparturient period in their study on dairy cows in the periparturient period. Sadjadian et al. (2013) reported that cholesterol decreased during the last two weeks of pregnancy, increased in the first two weeks of lactation, and reached a peak level on the postpartum 42nd day in Saanen goats. Nazifi et al. (2002) observed that serum cholesterol concentrations increased one week before birth

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and remained at their lowest levels 2-3 weeks after birth in sheep. In the present study, no significant difference was found in cholesterol concentrations in the periparturient period between consecutive weeks. However, there was a significant decrease in the postpartum 14th day compared to the prepartum period. It was thought that the decrease may be an indicator of damage to the liver.

Especially in the periparturient period, lipid metabolism is forced to meet the increasing energy demands. Therefore, adipose tissue is mobilized and the liver has to deal with an increased supply of NEFA via oxidation or re-esterification to triglycerides, and fatty liver develops when TG synthesis is increased (Kessler et al., 2014). Boudebza et al. (2016) reported that triglyceride levels were significantly higher in early lactation and dry periods than in the late period of pregnancy in sheep. Celi et al. (2008) observed that the time elapsed after birth did not affect triglyceride concentrations in their study in goats during the periparturient period. Tharwat and Al-Sobayil (2015) reported that there was no significant change in serum triglyceride concentration during the transition period. In the study, parallel to the findings of Celi et al. (2008) and Tharwat and Al-Sobayil (2015), no significant difference was found in serum Triglyceride concentrations during the transition period.

Determination of AST and GGT activities in dairy cows is mostly associated with the fatty liver syndrome, anorexia, and ketosis in dairy cows during early lactation. Increased AST activity in serum is a sensitive indicator of liver damage, even if the damage is subclinical. Seifi et al. (2007) reported that the AST level was measured at the lowest level 22 days before calving and at the highest level 21 days after calving in their study in dairy cows in the transition period. Tharwat and Al-Sobayil (2015) reported that there was a significant increase in the serum AST level from the prepartum 2 weeks to the postpartum 2nd week in their study in goats in the transition period. Soares et al. (2018) found that AST activity in goats during the transition period was relatively higher during the lactation period than during pregnancy. Giuliotti et al. (2014) showed that AST concentrations increased significantly in the postpartum period in their study in Zerasca sheep in the periparturient period. In the study performed, similar to the studies of Soares et al. (2018) and Giuliotti et al. (2014) a significant increase was found at postpartum 7th and 14th days. However, a significant decrease was found on the 21st postpartum day. Since the AST enzyme is not only a liver-specific enzyme, it is also found in muscles, it is not correct to say with certainty whether the increase is related to the liver. For this reason, it would be correct to evaluate especially the CK enzyme. Observation of a parallel increase in GGT level suggested that there may be damage to the liver.

GGT is important as a marker of hepatobiliary system diseases associated with cholestasis and is used in the diagnosis of liver disease (Stojević et al., 2005). Turk et al. (2013) reported that GGT activity increased at birth in their study in cattle. Tharwat et al. (2015) reported that GGT activity in goats decreased significantly only 1 week after birth. Soares et al. (2018) observed an increase in GGT level on the 20th day of lactation in their study in goats. A significant increase occurred in serum GGT concentrations on the 7th postpartum day in the study. It has been concluded that the increase in the GGT enzyme may be the result of damage to the liver.

Serum proteins have generally been used to assess for infections that may occur in the postpartum period and cause a prolonged interval between birth and conception (Piccione et al., 2011). Soares et al. (2018) reported that the total protein value was higher in the last period of pregnancy and the lactation period compared to the birth. In a study on Berari goats, the serum total protein level 14 days before birth showed a significant increase compared to 7 days before birth, the values decreased slightly at birth (day 0), however, the total protein concentration after birth increased from the 7th to the 21st day of birth (Bhoite et al., 2019). They stated that there was a slight increase

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in the amount, but the differences did not significant. Janku et al. (2011) in their study on periparturient goats reported that the TP concentration before and on the day of birth was at the lower limit (or even slightly below) of the physiological range reported in other studies, but began to increase gradually from the 7th day after birth and the highest mean was 71.3 g, measured on the 28th day after birth. Giuliotti et al. (2014) found that the TP value increased significantly in the postpartum period in their study in Zerasca sheep. In the study, there was a significant increase in total protein concentrations on the 7th day before birth, and a significant increase occurred on the 14th day after birth compared to the prenatal period. The increases in total protein concentrations were thought to be due to dehydration.

Decreased albumin levels have been reported as features of liver disease, kidney disease, inflammatory conditions, and malnutrition. It has been shown that serum albumin levels decrease as the severity of fatty liver increases; however, serum levels alone are not an adequate diagnostic tool because albumin can be affected by liver inflammation and other causes of liver disease (Lager and Jordan, 2012). Piccione et al. (2009) reported that albumin increased significantly during the last period of pregnancy and at the end of lactation in their study in sheep. Manat et al. (2016) in their study in Surti goats, reported that the albumin value was the lowest on the postpartum 0th day and reached the highest value on the postpartum 45th day. Tharwat et al (2015) did not find an increase in albumin value after birth in their study on goats. Sadjadian et al. (2013) in their study in Saanen goats, found that albumin was lower in the prepartum period than in the postpartum period and reached the highest level on the 13th and 42nd days postpartum, however, it reached the lowest level 30 days before birth. In this study, unlike other studies, no significant difference was found in serum Albumin concentrations during the transition period.

Turk et al. (2013) found that the concentration of urea in dairy heifers during the transition period was high until the 8th week after birth. Boudebza et al. (2016) reported that the urea concentration in Ouled Djellal sheep was significantly higher in the early lactation period compared to the dry period. Sadjadian et al. (2013) measured the BUN value in Saanen goats at the highest value on the postpartum 21st day and at the lowest value after birth and stated that BUN values were not statistically significant in their study. In the study, it was observed that there was a significant increase in serum BUN concentrations at the time of birth and a significant decrease on the 7th day after birth compared to the time of birth. It was concluded that the increase in BUN concentration was due to the decrease in the glomerular filtration rate due to fluid loss. The decrease in BUN level can be explained by the decrease in feed intake caused by postpartum stress and hormonal changes (Sevinç et al., 1999).

Sevinç et al. (1999) found that although they measured creatinine concentrations within normal limits in the pre and post-partum period in their study in dairy cattle, the values measured in the first 12 hours after birth were high. Piccione et al. (2009) reported that creatinine value increased significantly in the dry period compared to the last periods of pregnancy in sheep. Soares et al. (2018) reported in their study in goats that there was a significant decrease in creatinine after birth and this continued stably throughout lactation. In the study, it was observed that there was a significant increase at the time of birth and a significant decrease on the 7th day after birth compared to the time of birth. It was concluded that the increase in creatinine concentration at the time of birth was due to the decrease in glomerular filtration rate due to fluid loss. The increase in creatinine level at birth can be attributed to the hemodynamic effect of birth stress on the glomerular filtration rate (Sevinç et al., 1999).

The increased demand for Ca by the mammary glands for milk production at the start of lactation draws calcium from the blood and extracellular

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fluids more rapidly, thus depleting the circulating Ca levels of the mother and leading to subclinical or clinical hypocalcemia in periparturient lactating cows and other mammals (Jin et al., 2019). Soares et al. (2018) in their study in goats measured a decrease in total calcium concentration during birth, and values similar to those in pregnancy at the beginning of lactation. Andersen et al. (2005) reported an increase in calcium in the first week of lactation in dairy cattle. Celi et al. (2008) did not record a significant change in calcium levels in late pregnancy and early lactation in their study on goats. Boudebza et al. (2016) reported that the calcium level increased significantly in the late period of pregnancy in Ouled Djellal sheep and there was a decrease in the calcium level in the early period of lactation when compared to the dry period. In the study, similar to the result of Celi et al. (2008), no significant difference was found in serum calcium concentrations during the transition period.

The late stages of pregnancy and early lactation cause changes in both Ca and P metabolism. The increase in bone resorption occurs due to skeletal mineralization of the fetus in late pregnancy and milk production during early lactation. Milk production requires an available source of P, and bone resorption during the first few weeks of lactation is estimated to provide 500 to 600 g of P. Most of the phosphorus mobilized from bone tissue may be a direct result of Ca mobilization for Ca homeostasis in early lactation (Peterson et al., 2005). Tharwat et al. (2015) found that phosphorus concentration increased 2 weeks before and 2 weeks after calving in goats but decreased at birth and one week after birth. Bhoite et al. (2019) measured the lowest value of phosphorus on the 14th prepartum day and the highest value on the postpartum 21st day in Berari goats. In this study, unlike other studies, there was no significant difference in serum Phosphorus concentrations during the transition period.

The magnesium concentration in colostrum is about three times higher than in normal milk, and milk production in lactating cows causes rapid depletion of extracellular magnesium, resulting in hypomagnesemia if not replaced. During the transition period, inadequate serum magnesium levels are adverse metabolic conditions resulting in reduced reproductive performance. Many studies have shown that insufficient blood calcium (clinical or subclinical hypocalcemia) contributes to the incidence of peri and postpartum health problems and impaired fertility (Jeong et al., 2008). Elshahawy and Abdullaziz (2017) found that magnesium levels increased significantly in the third week compared to the first and second weeks after birth in dairy cattle. A study in Berari goats, the magnesium concentration was found to be the lowest on the postpartum 7th day and the highest on the postpartum 21st day (Bhoite et al., 2019). Boudebza et al. (2016) observed an increase in magnesium levels in the last period of pregnancy in Ouled Djellal sheep. In the study, a significant decrease occurred in serum Magnesium concentrations on the 21st postpartum day compared to the time of birth. It was thought that the decrease in magnesium concentration in the postpartum period may be related to milk production.

TNF-α has a variety of immune system functions, including antitumor activity, antimicrobial activity, and inflammation, as well as regulating many physiological functions, including appetite, fever, energy metabolism, and endocrine activity. Factors such as viruses, parasites, other cytokines, and endotoxin lipopolysaccharide (LPS) induce TNF-α production (Kushibiki, 2011). Trevisi et al. (2012) did not record a significant change in TNF-α values in the periparturient period in their study in dairy cattle. In the present study, parallel to the report of Trevisi et al. (2012), no significant difference was found in serum TNF-α concentrations during the transition period.

Adiponectin improves insulin sensitivity and lipogenesis in adipocytes and fatty acids β-oxidation in myocytes and hepatocytes. These effects occur with the activation of their receptors (adipoR1 and adipoR2) expressed in the liver, adipose tissue, and skeletal muscle. Circulating

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adiponectin in dairy cows consists mainly of high molecular weight complexes and its distribution is not affected by the lactation stage. Circulating adiponectin reaches its lowest level soon after calving, then rises between 40 and 70 days until lactation. Circulating adiponectin is inversely related to plasma fatty acids, which are the main lipolysis biomarker in dairy cows (Contreras et al., 2017). Ohtani et al. (2012) reported that there was no significant change in adiponectin concentrations in the periparturient period in Holstein dairy cattle. Komatsu et al. (2007) reported that the level of adiponectin in cattle that were not in the lactation period was two times higher than those in the lactation period. In the study, there was a significant increase in serum Adiponectin concentrations on the 7th day before birth and a significant decrease occurred at the time of birth compared to the 7th day before birth. It was concluded that the contribution of adiponectin to fatty acid oxidation, especially in the liver, could explain this situation.

Leptin is a polypeptide hormone primarily produced by fat cells and has an effective role in modulating feed intake as well as an effective role in energy homeostasis. It also provides a critical link between appetite, energy homeostasis, and reproductive function in the body (Abdelrazek et al., 2018). Rasmussen et al. (2004) reported that leptin levels were higher in the prepartum period than in the postpartum period and decreased in the first few days of birth and lactation in goats. Block et al. (2001) reported that leptin concentration in dairy cattle did not differ significantly during pregnancy, but decreased by half during the lactation period. Nowroozi-Asl et al. (2016) reported that there is a higher leptin concentration in the lactation period compared to the pregnancy period in dairy cattle. Fleming-Waddell et al. (2007) reported in their study in Callipyge sheep that leptin levels decreased in the last period of pregnancy and this situation continued in the birth and early lactation period. In the study, similar to Rasmussen et al. (2004) in their study in goats and Block et al. (2001) in their study in dairy cattle, a significant decrease in the postpartum 14th day was detected. It is thought that the reason why leptin is at a higher level in the last period of pregnancy compared to the postpartum period is to regulate food intake and balance its expenditure due to the increased energy need (Abdelrazek et al., 2018; Enriori et al., 2006).

In response to starvation in ruminants, ghrelin concentrations increase before scheduled meals and feeding suppresses ghrelin secretion. These observations suggest a role for ghrelin in stimulating feed intake, and responses to ghrelin administration supported this hypothesis. In addition to effects on feed intake, ghrelin has been shown to reduce fat oxidation, increase insulin secretion, and affect a wide variety of processes related to energy balance. Finally, as the endogenous ligand for the growth hormone secretagogue receptor, ghrelin provided a new avenue to investigate the regulation of GH (Growth hormone) secretion (Bradford and Allen, 2008). Vargová and Kováč (2016) reported in their study in cattle that ghrelin concentration decreased before birth, increased in the early postpartum period, and reached the highest level 6 weeks after birth. Temizel et al. (2018) reported that ghrelin levels decreased 1 week before birth and tended to increase 1 week after birth. Küçükşen (2017) reported that the ghrelin concentration was lower in the 4th week compared to the 6th week after birth in sheep. In the study, it was found that there was a significant increase in ghrelin concentrations on the 7th day before birth compared to the 14th day before birth. This increase can be explained by the knowledge that Ghrelin levels in ruminants increase to stimulate appetite against hunger (Bradford and Allen, 2008).

Conclusion

In this study, metabolic profile and changes in adipose tissue were evaluated in Romanov sheep. It is thought that Adiponectin, Leptin, and Ghrelin concentrations differ in the periparturient period in Romanov sheep, and this is due to the changes in the adipose tissue during the periparturient period. In the evaluation of metabolic profile,

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changes in NEFA, Cholesterol, AST, GGT, Total protein, BUN, Creatinine, and Magnesium concentrations are thought to be occurred to meet the energy needs in the periparturient period and to tolerate the disorders that may occur before and after birth.

As a result, it was concluded that the evaluation of metabolic profile and changes in adipose tissue in the periparturient period in Romanov sheep may be important in the diagnosis of diseases with a subclinical course.

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