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Research Article

Metabolic evaluation on Sakiz ewes with still and live births without an etiological diagnosis

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ABSTRACT

This study aimed to compare the differences in the metabolic status of grazing ewes with live and stillbirth lambs for which no etiological infectious agent could be isolated. Group 1 (live and stillbirth lambs) and group 2 (live lambs) consisted of 20 multiparous Sakiz ewes. Blood samples were collected two times on the parturition day and the day fifteenth after parturition. In the first sampling time, triglyceride levels of group 2 were determined significantly ($p<0.001$) higher than group 1. In the second sampling time, levels of magnesium ($p<0.05$), total protein ($p<0.01$), cholesterol ($p<0.01$), and triglyceride ($p<0.05$) levels were found significantly higher in group 2 than group 1. However, significant decreases were observed in both calcium and triglyceride levels in group 2 between the two sampling times. The beta-hydroxybutyric acid (BHBA) ($p<0.05$) levels were found significantly higher in the second sampling time than in the first sampling time only in group 1. Unlike the BHBA levels, a significant decrease was determined in both cholesterol and aspartate aminotransferase (AST) levels ($p<0.05$) in group 1 as the lactation progressed. Finally, we concluded that the metabolic status of group 2 is better than group 1, and it is important to the evaluation and follow-up of the metabolism in ewes with stillbirth without any etiological diagnosis that couldn't be determined.

INTRODUCTION

Small ruminants, mainly ewes and goats, have a prominent role both economically and socially in the daily life of many people. Generally, small ruminants are housed in developing countries, and %90 of them are reared in the rural side with a lack of new techniques and improper management skills. Inadequate and inappropriate management practices and diverse pathogens have been reported as vital causes in decreasing productivity and reproductive failure in small ruminant breeding (Ali et al., 2019).

Ovine abortion and stillbirth are significant problems in many ewes-producing countries worldwide. These problems are not only causing economic loss but also threaten public health due to zoonotic microorganisms (Yılmaz et al., 2002; Dun, 2019; Clune et al., 2021). The primary reproductive diseases, abortions, and stillbirths mainly occur by the infections such as brucellosis, leptospirosis, toxoplasmosis, listeriosis, campylobacteriosis (Ali et al., 2019). Although the microbial agents are the most important cause of abortions and stillbirths in ewes, non-infectious causes such as pregnancy toxaemia, poor nutrition, stress-related factors like heat stress or handling, plant or drug-related poisonings may also have an important role in the pathology (Mearns, 2008).

The peripartum period is very important for ewes due to the frequent observation of many infectious diseases and metabolic diseases such as pregnancy toxaemia, mastitis, and hypocalcemia (Mavrogianni and Brozos, 2008). Generally, studies that performed previously focused on the lactation period. However, metabolic investigations have been performed focused on grazing ewes both in the lambing period and at the beginning of the lactation less often. Serum biochemical tests are commonly used to diagnose many animal diseases, particularly metabolic diseases that can cause economic losses in decreased fur, wool, and milk production (Kiran et al., 2012). The metabolic profile test can be beneficial to evaluate the adaptation of ewes to conditions in extensive or semi-extensive production systems (Cabiddu et al., 2019). In lactating animals, blood biochemical parameters such as total protein, triglycerides, free fatty acids, and urea are important and commonly measured indicators of metabolic activity (Karapehlivan et al., 2007). In the condition of a disturbed energy balance, the serum levels of the previously mentioned biochemical parameters may change (Ulcar and Celeska, 2010).

Determining an aetiological diagnosis for investigations related to ovine abortions and stillbirth can be difficult. Because having for precise diagnosis relies heavily on the safety of laboratory methods, the quality of submitted samples, and the availability of diagnostic tests (Clune et al., 2021). In parallel, Kirkbride (1992) and Clune et al. (2021) reported that an aetiological diagnosis was able to determine in only 32% and 57% percent of investigations, respectively. Infectious agents, which are frequently observed and cause abortion, were detected in 81% of cases with etiological diagnoses. Additionally, it was

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reported that the remaining cases were caused by a wide range of infectious agents and non-infectious diseases (Clune et al., 2021). Making an aetiological diagnosis is very important not only for preventing economic loss but also for the protection of public health. Therefore, this study aimed to determine the differences in the metabolic profiles of the ewes who had healthy lamb/s and clinically healthy grazing ewes who had at least one stillbirth lamb, but no etiological diagnosis could be made.

MATERIAL and METHODS

This study was performed in February at the Sariova/Burdur (37_587627N, 30_399818E; 1.532 m above sea level) which has a semi Mediterranean climate and an average rainfall of 472.1 mm yearly. There were sixty ewes in the flock and there was no overcrowding in the flock, shelter, and building conditions, and they were grown in facilities that met the species' specific welfare standards. All ewes were vaccinated and treated for ecto- and endoparasites regularly.

Animal and Feeding Management

Twenty clinically healthy, multiparous Sakiz crossbreed ewes whose ages ranged between 24 and 60 months old were included in the study. They were separated into two groups as in an equal number. The ewes in group 2 were selected randomly from the same flock. The ewes were only in group 1 had at least one stillbirth lamb. All the lambs were born in three days, and the lambs in both groups were completely healthy, except the stillbirth lambs were born from the ewes in group 1.

All ewes were mainly grazing-free. Additionally, they were fed with a formulated diet of nearly 700 gr per ewes daily (Table 1)

Table 1. Ingredient composition of the concentrate feeding

Item	Ingredient
Analytical composition %	
Dry matter	88.88
Crude protein	15.5
Crude cellulose	10.28
Crude ash	8.68
Crude oil	2.44
Calcium	1.25
Phosphorus	0.74
Sodium	0.45
Vitamins	
Vitamin A (IU/kg)	9.000
Vitamin D3 (IU/kg)	3.000
Vitamin E (mg/kg)	40
Trace Elements mg/kg	
Mangan	50
Ferrous	50
Zinc	100
Copper	10
Iodine	0.8
Cobalt	0.1
Selenium	0.3

Blood Samples and Biochemical Analysis

Blood samples were collected from jugular veins into tubes from the ewes in both groups, on the parturition day after parturition, the fifteenth day in the postpartum period and twenty-one days after the birth. Blood samples were centrifuged at 3000 rpm for 15 minutes after the collection, and all the serum samples were stored at -20 °C until laboratory analysis. Urea, magnesium, beta-hydroxybutyric acid (BHBA), gamma-glutamyl transferase (GGT) triglyceride, total protein, inorganic phosphorus, aspartate aminotransferase (AST), calcium, cholesterol, and albumin were measured using Randox Monaco-5002 via the spectrophotometric method.

Microbiological and Serological Analysis

In this study, the most common microbiological agents that cause abortion and stillbirth in the territory where the sheep lives were investigated.

Blood samples were taken on the 21st day after birth were used to test for *C. abortus, C. burnetii,* Maedi-Visna and Bluetongue diseases. Chlamydia abortus and Coxiella burnetii were tested using commercial ELISA kits according to the manufacturer's instructions (IDEXX Chlamydiosis total Ab test and IDEXX Q fever Ab test). Maedi-Visna and Bluetongue diseases were investigated of ID Screen MVV/ CAEV Indirect, and IDEXX Bluetongue Competition Ab tests were used according to manufacturers' instructions. Also, placental tissue samples were collected for microbiological cultivation, and Brucella ovis was investigated using Brucella selective agar from these samples.

Statistical Analysis

All statistical analyses were performed using the SPSS 25.0 program. Distribution analysis of data was performed with the Shapiro-Wilk test. When comparing groups from the same sample period, a t-test was employed for parameters with a normal distribution and a Mann-Whitney U test for values with a non-normal distribution. In this study, the results were expressed as the mean ± standard error of mean (SEM). Differences in $p<0.05$ value were considered statistically significant.

RESULTS

The sampling times of the obtained biochemical parameter measurement data and the statistical comparison results between the groups are given respectively (Table 2, Table 3). Triglyceride levels were significantly higher in group 2 than group 1 in the first sampling time ($p < 0.001$). No significant difference was observed in the other parameters between the groups in the first sampling time. In the second sampling time magnesium ($p < 0.05$), triglyceride ($p < 0.05$), total protein ($p < 0.01$), and cholesterol ($p < 0.01$) levels were determined significantly higher in group 2 than group 1. It was observed that BHBA levels were significantly ($p < 0.05$) higher in the second sampling time than the first sampling time in group 1. However, cholesterol ($p < 0.05$) and AST ($p < 0.05$) levels were significantly higher in the first sampling time than in the second sampling time in group 1. In group 2, both triglyceride ($p < 0.01$) and calcium ($p < 0.01$) levels were determined significantly higher in the first sampling time than in the second sampling time. All lambs born in the group 2 were alive. In both groups, the total number of live and dead lambs per ewes was 2.3 in group 1 and 1.8 in group 2 (Table 4).

All the microbiological and serological analyses were negative for all ewes included in both groups. There were no pathological lesions observed macroscopically in both stillbirth lambs and placentas.

DISCUSSION

It can be considered normal the stillbirth-abortion rate is less than 5% in a season (Dun, 2019). In this study, there were no abortion cases but the stillbirth rate was 26%, compatible with the result of Ali et al. (2019), and also it can be suggested that this is a significant loss for the flock. Having a precise diagnosis in abortion-stillbirth cases is very important for preventing economic loss. Although diagnostic success in abortions and stillbirths is 10-15% better in ovine than for bovine Kirkbride (1992) it may be impossible to always have an exact diagnosis in small ruminants. However, Kirkbride (1992) and (1993) reported that 67% and 56% of cases had no exact diagnosis in the total of 8962 and 1784 ovine and bovine abortion and stillbirth cases, respectively. Similarly, Clune et al. (2021) reported that in 529 cases in Australia between 2000-2018, there was no etiological diagnosis made in 43% of total cases of ovine abortions and stillbirths. In this study, no infectious agents were found after microbiological and serological investigation. It can be suggested that although no abortion was observed in this study, the rate of stillbirth is compatible with previous reports.

The postpartum period is important not only for cows but also for ewes. Because of this reason, many investigations particularly related to energy metabolism, were performed on the transition period in ewes (Marutsova and Marutsov, 2018; Cabiddu et al., 2019). However, according to our search not

Table 2. Comparison of the groups on days 0th. and 15th.

Parameter	Group 1	Group 2	P value
0th day			
Urea (mg/dL)	25.58±3.9	24.50±1.45	NS
Magnesium (mg/dL)	2.36±0.1	2.27±0.6	NS
BHBA (mmol/L)	0.60±0.07	0.50±0.06	NS
Triglyceride (mg/dL)	17.05±0.86	36.45±3.72	***
T.Protein (g/dL)	6.21±0.25	6.46±0.19	NS
In. Phosphorus (mg/dL)	3.59±0.65	4.61±0.77	NS
GGT (U/L)	27.29±2.14	30.11±2.64	NS
Calcium (mg/dL)	11.72±0.71	11.30±0.31	NS
Cholesterol (mg/dL)	63.31±3.14	64.29±4.87	NS
AST (U/L)	91.35±4.47	79.43±4.54	NS
Albumine (g/dL)	2.55±0.60	2.41±0.60	NS
Calcium/Magnesium	5.07±0.3	5.0±0.2	NS
15th day			
Urea (mg/dL)	27.89±1.67	28.77±1.97	NS
Magnesium (mg/dL)	2.17±0.05	2.42±0.09	*
BHBA (mmol/L)	0.83±0.08	0.65±0.07	NS
Triglyceride (mg/dL)	15.08±0.62	19.64±1.65	*
T.Protein (g/dL)	6.00±0.13	6.77±0.18	**
In. Phosphorus (mg/dL)	5.00±0.22	4.57±0.34	NS
GGT (U/L)	30.75±2.64	32.44±3.93	NS
Calcium (mg/dL)	11.24±0.61	9.84±0.29	NS
Cholesterol (mg/dL)	52.84±2.51	63.63±2.68	**
AST (U/L)	77.76±4.13	80.59±3.67	NS
Albumine (g/dL)	2.38±0.89	2.59±0.64	NS
Calcium/Magnesium	5.22±0.3	3.86±0.7	**

*:p<0.05 **:p<0.01 ***:p<0.001 and NS:Not Significant, BHBA:betahydroxybutiric acid, T.Protein:Total Protein, In. Phosphorus: Inorganic Phosphorus, GGT:Gammaglutamyl transferase, AST:Aspartate amino transferase

Table 3. Comparison of the days 0th. and 15th. in the groups

Group 1			
Parameter	**0th**	**15th**	**P value**
Urea (g/dL)	25.58±2.05	27.89±1.67	NS
Magnesium (mg/dL)	2.36±0.10	2.17±0.05	NS
BHBA (mmol/L)	0.56±0.07	0.83±0.08	*
Triglyceride (mg/dL)	17.05±0.86	15.08±0.62	NS
T.Protein (g/dL)	6.21±0.25	6.00±0.13	NS
In. Phosphorus (mg/dL)	3.59±0.65	5.00±0.22	NS
GGT (U/L)	27.29±2.14	30.75±2.64	NS
Calcium (mg/dL)	11.72±0.71	11.24±0.61	NS
Cholesterol (mg/dL)	63.31±3.14	52.84±2.51	*
AST (U/L)	91.35±4.47	77.76±4.13	*
Albumine (g/dL)	2.55±0.06	2.38±0.89	NS
Calcium/Magnesium	5.07±0.3	5.22±0.3	NS
Group 2			
Urea (mg/dL)	24.50±1.45	28.77±1.97	NS
Magnesium (mg/dL)	2.27±0.06	2.42±0.09	NS
BHBA (mmol/L)	0.50±0.06	0.65±0.07	NS
Triglyceride (mg/dL)	36.45±3.72	19.64±1.65	**
T.Protein (g/dL)	6.46±0.19	6.77±0.18	NS
In. Phosphorus (mg/dL)	4.61±0.77	4.57±0.34	NS
GGT (U/L)	30.11±2.64	32.44±3.93	NS
Calcium (mg/dL)	11.30±0.31	9.84±0.29	**
Cholesterol (mg/dL)	64.29±4.87	63.63±2.68	NS
AST (U/L)	79.43±4.54	80.59±3.67	NS
Albumine (g/dL)	2.41±0.66	2.59±0.64	NS
Calcium/Magnesium	5.0±0.2	3.86±0.7	***

*:p<0.05 **:p<0.01 ***:p<0.001 and NS: Not Significant, BHBA:betahydroxybutiric acid, T.Protein:Total Protein, In. Phosphorus: Inorganic Phosphorus, GGT:Gammaglutamyl transferase, AST:Aspartate amino transferase

Table 4. Comparison of the days 0th. and 15th. in the groups

Group 1		
Number of ewes (n)	Stillbirth lamb (n)	Live lamb (n)
7	1	1
2	1	2
1	2	1
Group 2		
Number of ewes (n)	Stillbirth lamb (n)	Live lamb (n)
4	-	2
2	-	3
4	-	1

enough data is presented in the literature related to evaluating the energy metabolism of ewes with stillbirth in the postpartum period. Nazifi et al. (2002) reported a decrease in triglyceride levels during the first fifteen days of lactation. Although they didn't find any significant decrease in the triglyceride levels between the days first and seventh, they found a significant decrease between the days seventh and fifteenth. Similarly, Carcangiu et al. (2007) also reported a significant decrease in triglyceride levels during the first thirty days after parturition. In the present study, in triglyceride levels, although, there was no significant decrease determined in group 1 between the two sampling times, a statistically significant decrease was determined in group 2**.** In the present study, the number of lambs in lactation was 1.8 and 1.1 in group 2 and group 1, respectively. It may result from that triglyceride, used in the mammary gland to form milky fats during lactation (Carcangiu et al., 2007). It can be suggested that our results of both groups are compatible with the previous results (Nazifi et al., 2002; Carcangiu et al., 2007). During the lactation period, insulin stimulation of lipogenesis may not be as efficient as the pregnancy period. Both serum levels of triglycerides and total cholesterol show a decrease at the beginning of the lactation compared with the pregnancy period (Piccione et al., 2009). Nazifi et al. (2002) found a statistically significant decrease in cholesterol levels between the parturition day and the second week of lactation in healthy Iranian ewes. Moreover, Ramos et al. (1994) found similar changes with Nazifi et al. (2002) in serum cholesterol levels in healthy Mountain Breed ewes. Conversely, Cabiddu et al. (2019), Carcangiu et al. (2007), and Antunovic et al. (2011) found an increase in cholesterol levels in healthy ewes, and only one of them was not statistically significant (Carcangiu et al., 2007). This difference may be that the intervals between the sampling times in the postpartum period, which were longer compared to our study, and the samples were collected in the later stages of lactation. Additionally, many studies performed in domestic animals showed prominent variations in serum concentration of triglyceride, cholesterol, and lipoproteins between different species, even within the same species (Khaki et al., 2012). In the present study, a decrease in the cholesterol levels of both groups was observed as the lactation progressed, and the decrease between the two sampling times was found statistically significant only in group 1. Similar to triglyceride levels, a decrease was observed in the cholesterol levels at the first two weeks of lactation that was compatible with previous reports (Nazifi et al., 2002; Antunovic et al., 2011).

Serum BHBA and NEFA levels are one of the important blood indicators of lipomobilization in ruminants (Gonzalez et al., 2011), and blood BHBA concentration is being used commonly evaluating negative energy balance in dairy animals, so it is a diagnostic marker for subclinical and clinical ketosis (Sordillo et al., 2012). Duffield (2000) suggested that while evaluating the energy balance of animals, NEFA and BHBA are mostly used in the prepartum and postpartum periods. However, Dzadzovski et al. (2015) was found BHBA serum levels in non-pregnant ewes 0.69 mmol/L. Additionally, Pancarci et al. (2007) was found higher BHBA levels in healthy ewes on the seventh day after parturition compared to the parturition day. In this study, we found an increase in the level of the BHBA concentrations in both groups in time, and it can be suggested that our results are compatible with the previous studies. However, Raoofi et al. (2013) reported a gradual decrease in the BHBA levels during the first two weeks of the lactation period, unlike our study. On the other hand, the mean serum BHBA levels of the twin-bearing ewes (0.89 mmol/L) were found higher than the single-bearing ewes (0.51 mmol/L) in the peripartum period, and the difference between the two groups was statistically significant. Raoofi et al. (2013) reported that half of their ewes were single bearing and they fed all the ewes with concentrated feed. However, no single-bearing ewes were included in our study, and all ewes were mainly fed grazing. We thought these differences between the studies are important for the difference in the BHBA levels.

Carcangiu et al. (2007) reported an increase in serum calcium levels as the lactation progressed in ewes, although there was no significant difference between the days 7th and 30th. Similarly, Kadzere et al. (1997) reported an increase between the parturition day and the seventh day of parturition. Additionally, Gürgöze et al. (2009) found a significant increase in serum calcium levels of ewes days from the 7th to 14th after parturition. Conversely, in the present study, a decrease was determined as the lactation period progressed. Moreover, a significant decrease was found in the calcium levels of ewes in group 2. Many researchers have reported different results about Ca levels during pregnancy and lactation (Treacher and Caja, 2002; Raoofi et al. 2013). The differences can occur from feeding, differences of regional practice, or the number of lamb per ewes. Generally, there is no information stated in the studies on the number of single- or twin-bearing ewes. However, Raoofi et al. (2013) reported that the calcium levels of the twin-bearing ewes significantly decreased compared to the single-bearing ewes. In parallel with this study, Treacher and Caja (2002) reported that the requirement of Ca in twin-bearing ewes is higher than the single-bearing ewes, and twin-bearing ewes are more susceptible to hypocalcemia than single-bearing ewes. So, it can be suggested that our results are normal when the differences between the previous studies and the number of lambs in the lactation period between the two groups of the present study are considered.

Magnesium is a very important mineral for the continuation of pregnancy. Borella et al. (1990) reported that an increased Ca/Mg ratio is associated with abortion and gestational pathologies. Altura et al. (1983) determined vasospasm in certain blood vessels in vitro caused by an increased Ca/Mg ratio. They suggested this is responsible for the spasm of umbilical and placental vessels at the end of the pregnancy. In the present study, although the Ca/Mg ratio was determined higher in group 1 than in the control group, no statistically significant difference was found in the first sampling time. However, a statistically significant difference was found between the groups on the fifteenth day, and this condition may be caused by the decrease of the magnesium levels of group 1. It can be suggested that changes in magnesium levels of both groups in two sampling times were in the reference ranges and compatible with previous results (Gürgöze et al., 2009; Omidi et al., 2015).

When biochemical parameters of the two groups were compared in the same sampling time, triglyceride levels of group 2 were found significantly higher than group 1 on the two sampling times, particularly in the first sampling time. Lipid fractions are very changeable. For example, NEFA and phospholipid levels increase during under- and over-nutritional conditions, respectively. But triglyceride levels increase in both under- and over-nutritional conditions and infectious cases (Caldeira et al., 1991; Aytekin et al., 2015). In the present study, changes in triglyceride levels are compatible with previous studies (Nazifi et al., 2002; Carcangiu et al., 2007). Similarly, both groups' total protein and cholesterol levels were found compatible with previous studies, and both parameters levels of group 2 were significantly higher than the other group on the second sampling time. As the lactation progressed, increase and decrease were observed in albumin levels of groups 2 and 1,. We thought that the difference between the total protein levels of both groups on the second sampling time might be caused by this phenomenon. When considering the results of the previous studies (Cabiddu et al., 2019; Caldeira et al., 2007; Karapehlivan et al., 2007), it can be suggested that the metabolical functions of ewes in group 2 were better than ewes in group 1 in terms of both energy balance and protein metabolism.

CONCLUSIONS

The number of metabolical investigations on stillbirth and abortion cases in ewes is not enough. Because of this reason, the present study is important due to the presentation of metabolical evaluation and differences between grazing ewes with unknown causes of stillbirth, and grazing ewes that had healthy lambs. It can be suggested that the ewes included in group 2 have better metabolical conditions than the ewes included in group 1. In conclusion, more studies about energy and protein metabolisms are needed in ewes with stillbirth and abortions in which no etiological agent can be isolated.

DECLARATIONS

Ethics Approval

All procedures were approved by the Animal Ethics Committee (AEC) at Burdur Mehmet Akif University, Turkey (No: 95/826, 17.11.2021)

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author contribution

Idea, concept and design: FK, GB

Data collection and analysis: GB

Drafting of the manuscript: FK, GB

Critical review: FK, GB

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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