



The Effect of Twinship on Mineral Matter, Immunoglobulin G and Lamb Birth Weight in Late Pregnant Ewes and Their Newborn Lambs

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ABSTRACT

The aim of this study was to investigate the effects of twinship on the mineral matter, immunoglobulin G (IgG), and lamb birth weight in late pregnant ewes and their lambs. The material of the study consisted of the 18 Kıvrıkcık breed ewes, which were 130-150 days pregnant, and their 27 newborn lambs. The ewes included in the study were divided into two groups by ultrasound as those carrying a single pregnancy (n=9) and a twin pregnancy (n=9). Body Condition Scores (BCS) were determined and then their blood samples were taken. The lambs born from these ewes were weighed with precision scales as soon as they were born, and blood samples were taken 24 hours after birth. Mineral substance determination from blood samples was made by ICP-OES, and IgG determination was made by ELISA. It was found that serum IgG and mineral matter levels were not affected by twinship in ewes (p>0.05). Calcium (Ca) level was significantly higher in twin lambs compared to single lambs (p<0.05). There was no statistical difference between single and twin lambs in terms of other minerals and IgG concentrations (P>0.05). Total protein and globulin values were significantly higher in single lambs than twin lambs (P <0.05). It was determined that single-born lambs were significantly heavier than twins (P<0.05), and while male lambs were heavier than females, the difference between them was not statistically significant (P>0.05). As a result, it was determined that twinship and BCS did not affect the mineral substance, total protein, albumin, globulin and IgG levels in late pregnant ewes, while twinship in lambs affected lamb birth weight, total protein, globulin and Ca values, but not other mineral and IgG levels. Significantly low total protein and globulin concentrations in twin lambs suggest that these animals cannot absorb enough colostrum.

Keywords: Immunoglobulin, Late pregnant, Mineral, Twin.

ÖZ

İkizliğin İleri Gebe Koyunlar ve Bunların Kuzularında Mineral Madde, İmmunglobulin G ve Kuzu Doğum Ağırlığı Üzerine Etkisi

Sunulan çalışmada, ileri gebe koyunlar ve bunlardan doğan kuzularda ikizlik durumunun mineral madde, İmmunglobulin G (IgG) ve kuzu doğum ağırlığı üzerine etkilerini araştırmak amaçlandı. Çalışmanın materyalini gebeliğin 130-150. günleri arasında Kıvrıkcık ırkı 18 adet koyun ile bunların yeni doğan 27 adet kuzusu oluşturdu. Çalışmaya alınan koyunlar ultrason ile tek gebelik (n=9) ve ikiz gebelik (n=9) taşıyanlar olarak iki gruba ayrıldı. Vücut Kondüsyon Skorları (VKS) belirlendi ve kan örnekleri alındı. Bu koyunlardan doğan kuzular doğar doğmaz hassas terazi ile tartıldı ve doğumdan 24 saat sonra kan örnekleri alındı. Alınan kan örneklerinden mineral madde tayini ICP-OES, IgG tayini ELISA ile yapıldı. Koyunlarda serum IgG ve mineral madde düzeylerinin ikizlikten etkilenmediği görüldü (P>0.05). İkiz kuzularda Kalsiyum (Ca) düzeyi tek kuzulara göre önemli derecede yüksekti (P<0.05). Diğer mineraller ve IgG konsantrasyonu bakımından tek ve ikiz kuzular arasında istatistiki açıdan fark yoktu (P>0.05). Tek kuzularda total protein ve globulin değerleri ikiz kuzulara göre anlamlı derecede yüksek bulundu (P<0.05). Tek doğan kuzuların ikizlere göre anlamlı derecede ağır olduğu (P<0.05), erkek kuzuların dişilere göre daha ağır olmakla birlikte aralarındaki farkın istatistiki açıdan önemli olmadığı (P>0.05) bulundu. Sonuç olarak; ileri gebe koyunlarda ikizliğin ve VKS'nin mineral madde, IgG, total protein, albumin ve globulin düzeylerini etkilemediği, kuzularda ise ikizliğin kuzu doğum ağırlığı, total protein, globulin ve Ca değerlerini etkilerken, diğer mineraller ve IgG düzeylerini etkilemediği tespit edildi. İkiz kuzularda total protein ve globulin konsantrasyonlarının anlamlı derecede düşük olması bu hayvanların yeterince kolostrum ememediğini düşündürmektedir.

Anahtar Kelimeler: İmmunglobulin, İkiz, İleri gebe, Mineral.



INTRODUCTION

Mineral substances are inorganic elements having great importance in the nutrition of living creatures, participating in the structure of many tissues in the body, and acting as cofactors of various enzymes. These substances are essential nutrients for the healthy development, growth, reproduction, and productivity of animals. Their deficiencies lead to disruption in body functions. The body cannot produce these substances and should take them from external sources (Corah 1996; Kaneko et al. 2008). Physiological conditions such as season, pregnancy, and lactation in animals may affect serum mineral levels (Teixeira et al. 2013). It has been studied by some researchers considering that twin pregnancy in ewes may also affect the mineral levels. (Yıldız et al. 2005; Gürdoğan et al. 2006; Abdollahi et al. 2013). But further research is needed.

Since the placenta does not allow the passage of immunoglobulins in ruminants, the offsprings are born with a low immunoglobulin level; and their immunoglobulin levels increase within 24 hours after colostrum intake (Quigley 2005). Therefore, an adequate colostrum intake immediately after birth is crucial for transferring passive immunity and increasing survival rates (O'Doherty and Crosby 1997; Morales-dela Nuez et al. 2011; E Hernandez-Castellano et al. 2014). It is reported that lambs receiving an insufficient amount of colostrum in the first hours of life are more susceptible to diseases, and their mortality rate increases (Ahmad et al. 2000; Nowak and Poindron 2006). Serum IgG levels of newborn lambs have been reported to be affected by IgG levels in the ewes' sera and colostrum and can be increased by improving the serum IgG level of the ewes in the last 15 days of pregnancy (Vatankhah 2013). However, it is not yet clear how twin pregnancy affects IgG concentration in ewes and lambs.

Birth weight was reported to affect the survival rates of the lambs in the neonatal period and growth rate in the postnatal period (Morris et al. 2000; Christley et al. 2003). The effect of twinship on lamb birth weight in different ewe breeds has been studied and found significant (Gardner et al. 2007; Corner et al. 2013; Vural 2013). However, further studies are necessary on the Kivircik Breed ewes. It was reported that fetal development accelerates in the last six weeks of gestation in ewes, and it is a critical period because preparations are made for lactation (Abdelrahman 2008). However, during this period, the effects of twinship on the mother and offspring are not clear enough. Therefore, in this study, it was investigated whether twin pregnancy has a reducing effect on mineral matter, IgG and lamb birth weight in late pregnant ewes and their lambs.

MATERIALS AND METHODS

The material of the study consisted of 18 Kivircik breed ewes, which were between 3-5 years old, weighing 55.61 ± 0.76 kg and 130-150 days pregnant, and their 27 newborn lambs. This study was approved by the Local Ethics Board for Animal Experiments of Tekirdağ Namık Kemal University, Turkey (2018/02). The animals were provided by a private enterprise in the Silivri district of Istanbul. The animals used in the study were administered antiparasitic drugs at regular intervals of six months and were housed in the same free kennel. All animals were fed by a concentrated commercial feed that contains 14% crude protein, 15% fiber, 3% fat, 2500 kcal/kg metabolic

energy twice a day 1 kg per animal, and oat grass, twice a day 2 kg per animal. Drinking water and lick stone containing minerals (Ca, Mg, P, K, Na, Mn, Cu, Zn, and Co) was held ready in front of the animals. Table 1 shows the analysis of the ration content and ration nutrient given to animals.

Table 1. Ration Content and Ration Nutrient Analysis (%).

Ration Content	Ration Nutrient Analysis
Oats hay %	62
Barley %	19.5
Corn %	8
Cottonseed meal %	2.2
Sunflower meal %	1
Molasses	2
Limestone %	0.6
Salt %	3.2
Dry matter kg	2.81
Crude protein %	13.9
Metabolized energy Mcal	2.56
Ca %	0.72
P %	0.42
Mg %	0.36

Ca: Calcium, P: Phosphorus, Mg: Magnesium.

Sampling

The ewes included in the study were examined with a 5 MHz ultrasonography device and divided into two groups that contain single pregnant ewes (n=9) and twin pregnant ewes (n=9). Body condition scores (BCS) of ewes in both groups were determined using the 5-point scale reported by Thompson and Meyer (1994). On the same day, 10 ml blood samples were collected from the jugular vein of all ewes and transferred to tubes without anticoagulant. All of the lambs born from these ewes were weighed with sensitive scales as soon as they were born. The lambs were kept with their mothers for 72 hours in 1.0 x 2.0-meter compartments to get enough colostrum and were allowed to suck their mothers within one hour of birth. Blood samples were taken from the jugular veins of lambs 24 hours after birth.

Blood Analysis

The blood samples without anticoagulant were centrifuged at 3000 rpm for 15 minutes, and their sera were separated. Sera were stored in Eppendorf tubes at -80 C until analysis. Determination of calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo) in the sera was made by ICP-OES device (Spectro Spectro Blue SOP Model) in Tekirdağ Namık Kemal University Central Research Laboratory (NABİLTEM) according to the method reported by Al-Jameil (2014). IgG concentrations were measured on the ELISA device (EPOCH, BioTek) using the commercial IgG kit (ewes IgG ELISA Kit) according to the manufacturer's instructions (Sunred Biological Technology Co., Ltd.). Total protein, albumin, globulin levels were determined by measuring with an automatic biochemical analyzer (Biotechnica Instruments BT 3500, Rome, Italy).

Statistical Analysis

Statistical analyses were performed with SPSS 22 package program. The findings were given as mean value \pm standard deviation. Shapiro-Wilk test (n<50) was used to check whether the continuous variables in the study were

normally distributed and when seeing the variables were not normally distributed then non-parametric tests were performed. Mann-Whitney U test was used to compare the mineral matter, IgG, total protein, albumin, globulin, BCS and lamb birth weights. $P < 0.05$ value was considered statistically significant. Spearman correlation coefficients were calculated to examine the relationships between total protein, albumin, globulin, IgG, and BCS in ewes and lambs included in the study. The statistical significance level was set at 5% in the calculations ($P < 0.05$).

RESULTS

Serum Ca, Zn, Se, Mo levels in single pregnant ewes, and Mg, P, K, Cu, Na levels were high in twin pregnant ewes, but the difference was not statistically significant. Serum IgG concentration was higher in ewes carrying twin offsprings than that of ewes carrying single. However, it was not statistically significant ($P > 0.05$) (Table 2).

There was not statistically significant difference between serum Mg, P, K, Cu, Zn, Se, Mo and Na levels between single-born lambs and twin born lambs ($P > 0.05$) (Table 3).

In terms of lamb birth weight, single lambs (5.824 ± 0.964) were determined to be born significantly heavier compared to twin lambs (4.792 ± 0.622) ($P < 0.05$).

In terms of gender, male lambs (5.44 ± 0.839) were heavier than females (4.936 ± 0.97), but the difference was not statistically significant ($P > 0.05$) (Table 4).

When the total protein, albumin, globulin, IgG, and BCS values were compared between the single and twin ewe groups, no significant difference was observed between the two groups ($P > 0.05$) (Table 5). As to the lambs, total protein and globulin values were significantly higher ($P < 0.05$) in single lambs compared to twin lambs, but no significant difference was observed between albumin and IgG values ($P > 0.05$) (Table 5).

A statistically significant positive correlation was found between albumin and total protein, IgG, and globulin measurements in single pregnant ewes and between globulin and total protein measurements in twin pregnant ewes ($P < 0.05$). No statistically significant correlation was found between BCS and total protein, albumin, globulin, and IgG measurements in both ewe groups ($P > 0.05$) (Table 6). A statistically significant positive correlation was found between globulin and total protein measurements in single-born lambs and between total protein and IgG measurements in twin-born lambs ($P < 0.05$) (Table 7).

Table 2. Some mineral concentrations in single and twin pregnant ewes.

Mineral/IgG	Single (n=9)	Twin (n=9)	p-value
Mg (mg/dl)	2.615±0.400	2.678±0.463	0.289
Ca (mg/dl)	8.919±1.051	8.837±0.939	0.906
P (mg/dl)	6.497±2.207	8.307±1.667	0.077
K (mg/dl)	27.049±3.004	31.383±5.240	0.059
Cu (µg/ml)	0.692±0.12	0.756±0.139	0.346
Zn (µg/ml)	0.912±0.150	0.898±0.169	0.724
Se (µg/ml)	0.427±0.118	0.339±0.073	0.126
Mo (µg/ml)	0.150±0.061	0.147±0.025	0.480
Na (mg/dl)	309.451±26.60	328.151±30.094	0.409

Values were presented as Mean ± SD. Statistical significance level was set as $P < 0.05$. Mg: magnesium, Ca: calcium, P: phosphorus, K: potassium, Cu: copper, Zn: zinc, Se: selenium, Mo: molybdenum, Na: sodium.

Table 3. Some mineral concentrations in single and twin lambs.

Mineral	Single (n=9)	Twin (n=18)	p-value
Mg (mg/dl)	2.009±0.134 ^a	2.063±0.275 ^a	0.995
Ca (mg/dl)	8.479±2.066 ^a	10.454±1.245 ^b	0.034
P (mg/dl)	13.973±2.743 ^a	12.058±3.448 ^a	0.346
K (mg/dl)	44.673±34.085 ^a	41.100±10.772 ^a	0.409
Cu (µg/ml)	0.368±0.052 ^a	0.480±0.127 ^a	0.077
Zn (µg/ml)	1.076±0.223 ^a	1.067±0.224 ^a	0.906
Se (µg/ml)	0.350±0.078 ^a	0.359±0.051 ^a	0.724
Mo (µg/ml)	0.152±0.022 ^a	0.130±0.034 ^a	0.068
Na (mg/dl)	246.680±43.736 ^a	284.964±35.084 ^a	0.126

Values were presented as Mean ± SD. a,b There is a significant difference between the values in the same column with no common letters ($P < 0.05$). Mg: magnesium, Ca: calcium, P: phosphorus, K: potassium, Cu: copper, Zn: zinc, Se: selenium, Mo: molybdenum, Na: sodium.

Table 4. Birth weight in single and twin lambs.

	Single (=9)	Twin (n=18)	p-value	Female (n=13)	Male (n=14)	p-value
Birth weight (kg)	5.824±0.964 ^a	4.792±0.622 ^a	0.036	4.936±0.97 ^b	5.44±0.839 ^c	0.308

Values were presented as Mean ± SD. Statistical results set to (P<0.05). IgG: Immunoglobulin G. b,c There is a significant difference between the values in the same column with no common letters (P<0.05).

Table 5. Total protein, albumin, globulin, IgG, BCS levels in all animals.

	Ewes			Lambs		
	Single (n:9)	Twin (n:9)	p-value	Single (n:9)	Twin (n:18)	p-value
Total Protein(g/L)	68.2±5.0 ^a	63.0±5.0 ^a	0.150	62.6±4.9 ^b	52.3±6.5 ^c	0.030
Albumin(g/L)	38.0±3.0 ^a	35.9±2.7 ^a	0.152	26.0±2.4 ^b	27.9±3.3 ^b	0.420
Globulin(g/L)	30.3±2.70 ^a	27.0±2.70 ^a	0.065	36.5±6.0 ^b	24.4±6.9 ^c	0.020
IgG(mg/ml)	10.11±4.03 ^a	11.17±5.24 ^a	0.689	5.92±1.61 ^b	4.74±2.32 ^b	0.328
BCS	3.50±0.45 ^a	3.0±0.45 ^a	0.096			

Statistics were presented as Mean ± SD, Statistical results set to P<0.05 were considered as significant. BCS: Body condition score. b,c There is a significant difference between the values in the same column with no common letters (P< 0.05).

Table 6. Correlations between measurements in single and twin pregnant ewes.

		Single Ewes				Twin Ewes			
		Total P	Alb	Glb	IgG	Total P	Alb	Glb	IgG
Alb	r	,829*				,486			
	p.	,042				,329			
Glb	r	,429	,257			,943*	,257		
	p.	,397	,623			,005	,623		
IgG	r	,441	,177	,883*		,493	-,116	,406	
	p.	,381	,738	,020		,321	,827	,425	
BCS	r	-,120	,000	,000	,185	,000	-,478	,120	,485
	p.	,822	1,000	1,000	,726	1,000	,338	,822	,329

Statistics were presented as Mean ± SD, Statistical results set to P<0.05 were considered as significant. BCS: Body condition score.

Table 7. Correlations between measurements in single and twin lambs.

		Single Lambs			Twin Lambs		
		IgG	TOTAL P	ALB	IgG	TOTAL P	ALB
TOTAL P	r	,294			,883*		
	p.	,571			,020		
ALB	r	,194	-,203		,353	,257	
	p.	,713	,700		,492	,623	
GLB	r	-,029	,829*	-,667	,471	,771	-,257
	p.	,956	,042	,148	,346	,072	,623

Statistics were presented as Mean ± SD. Statistical results set to P<0.05 were considered as significant. BCS: Body condition score.

DISCUSSION AND CONCLUSION

It has been reported that the plasma Cu, Zn, and Se concentrations decreased in the late periods of the pregnancy in ewes, and it was lower in twin pregnant ewes compared to those carrying a single pregnancy (Gürdoğan et al. 2006; Abdollahi et al. 2013). The decline reason has been attributed to the fetus' increased demand in advanced pregnancy (Hostetler et al. 2003). Similar to these studies, in the present study as well, serum Zn and Se concentrations were lower in twin pregnant ewes and their lambs, compared to single pregnant ewes and their lambs, even though it was not statistically significant ($P>0.05$). It has been suggested that this decrease in twins occurred because of the increase in need depending on the number of offspring. However, in our study, Cu concentration was found to be higher in twin pregnant ewes and their lambs compared to single pregnant ewes and their lambs, although it was not statistically significant ($P>0.05$). According to these results, it is thought that the demand for Cu increases in twins.

Molybdenum metabolism in small ruminants has rarely been studied. The values of this study and the previous researches reporting serum Mo levels in ewes (Ryssen and Stielau 1981; Marques et al. 2011; Silva et al. 2018) are consistent. It was higher in single pregnant ewes and their lambs than twin pregnant ewes and their lambs in the present study, but there was no statistical difference between them ($P>0.05$). These results suggest that the need for molybdenum increases in twin pregnancy.

Kenyon et al. (2007) reported that the serum calcium levels were lower in triplet-pregnant ewes than twin-pregnants, but the difference was not statistically significant. Similarly, Yıldız et al. (2005) reported that the calcium level of twin pregnant ewes in late pregnancy is lower than those carrying a single fetus. In the present study, serum Ca concentration was found to be higher in single pregnant ewes compared to twin pregnant ewes ($P>0.05$). Ca concentration was statistically significantly higher in twin-born lambs than those in single-born lambs ($P<0.05$). High Ca levels in twin-born lambs and low calcium levels in their mothers may be related to meeting the needs of twin offsprings.

It has been reported that serum Mg concentration decreases significantly with the late pregnancy in ewes, it is lower in twin pregnant ewes than in single pregnant ewes, but there is no statistical difference between them (Yıldız et al. 2005). Kenyon et al. (2007) reported no significant difference between twin and triplet pregnant ewes and their lambs. Similar to these studies, in our study, there was no difference in Mg concentrations between single pregnant ewes and twin pregnant ewes, single-born lambs, and twin-born lambs ($P>0.05$). However, the Mg values of the presented study were higher than the values obtained from Yıldız et al. (2005) and Kenyon et al. (2007). This difference is thought to be due to Mg supplied in the diet.

It is reported that the serum Na and K concentrations increase in the late pregnancy stage of the ewes (Dakka and Abd-El-Aal 1992; Kulcu and Yur 2003). Yıldız et al. (2005) as well, mentioned an insignificant increase in serum Na and K concentrations in the late pregnancy stage of twin and single pregnant ewes, also reported no difference between single pregnant and twin pregnant ewes. In our study, Na and K concentrations of the twin-pregnant ewes were higher than that of single pregnant ewes. But the difference between them was not significant

($P>0.05$). The higher values in twin pregnant ewes may be a sign of the adaptation of ewes to twinship. Serum Na concentration was higher in twin lambs than single-born lambs, and K concentration was higher in single-born lambs than twins, but there was no statistical difference between them ($P>0.05$).

Sansom et al. (1982) and Roubies et al. (2006) reported that P concentrations in ewes decreased during pregnancy compared to the post-pregnancy period. The serum P concentration was reported to be significantly lower in twin pregnant ewes than single pregnant ewes. The decrease in P concentrations in ewes was attributed to the increased demand for skeletal development of the fetus (Yıldız et al. 2005). However, El-Tarabany (2012) reported that the P concentration in twin pregnant ewes was significantly higher than single pregnant ewes. Similarly, also in the present study, the serum P concentration was found higher in twin pregnant ewes than single pregnant ewes, but the difference was not significant. Serum P concentration of single-born lambs was higher than the twin-born lambs, but the difference was not significant. Differences in findings suggest that P homeostasis in the body is complex, and new studies are needed.

In newborn lambs, the humoral immunity is dependent on the passive transfer of IgG taken from colostrum and is determined by measuring serum IgG concentration (Massimini et al. 2006). It has been reported that serum IgG levels in newborn lambs were associated with the health status of the animals and were higher in healthy lambs than those who were sick and died (Ahmat et al. 2000; Gökçe and Atakişi 2019). As Ahmat et al. (2000) reported that gender did not affect IgG levels in lambs, Alves et al. (2015) informed that twin pregnancy in ewes and gender in lambs did not cause changes in IgG levels. Turquino et al. (2011) reported that twinship in lambs did not affect IgG, total protein, and globulin concentrations, whereas Nunes (2006) stated that IgG concentrations in single-born lambs were higher than twins. On the other hand, Boland et al. (2005) reported that lambs born from ewes receiving mineral vitamin supplements (Ca, P, Mg, Na, Zn, Se, I, Mn, Co, and Vitamin E) in the last two months of pregnancy had lower IgG levels than lambs of ewes without mineral vitamin supplements. In the present study, IgG levels were found higher in twin pregnant ewes compared to single pregnant ewes, but the difference between them was insignificant ($P>0.05$). IgG levels were higher in single-born lambs than the twins, however, the increase was not statistically significant ($P>0.05$). The IgG findings of the presented study are consistent with Ahmad et al. (2000), Boland et al. (2005), Alves et al. (2015) and Turquino et al. (2011) while differing from Nunes (2006). That mineral supplements, which were in the form of licking stones, were supplied to the ewes in the study, confirms the thesis of Boland et al. (2005) that mineral supplement in the late pregnant ewes decreases IgG absorption and serum concentration in lambs. Different results might be obtained due to differences in colostrum's IgG level, the time of administration, and the mode of administration.

Vatankhah (2013) has reported that BCS does not affect IgG and total protein concentrations. Al Sabbagh et al. (1995) and Alves et al. (2015) reported that they did not observe a correlation between BCS and colostrum IgG levels in ewes. Similarly, in the current study, no statistically significant relationship was found between BCS and total protein, albumin, globulin, and IgG measurements in ewes ($P>0.05$).

Vatankhah (2013) revealed a correlation between IgG and total protein in ewes and lambs. Similarly, in the present study, a high positive correlation was found between IgG and globulin in single pregnant ewes and IgG and total protein in twin-born lambs ($P < 0.05$). In addition, a high positive correlation was found between globulin and total protein in single lambs and twin pregnant ewes ($P < 0.05$). The current study determined that the total protein, albumin, globulin, IgG, and BCS values in ewes were not affected by twinning. However, the total protein and globulin values were observed significantly lower in the twin lambs. The variations between the studies could have resulted from ewes' genetic differences, nutritional differences, and the different extraction methods of IgG and total proteins.

Lamb birth weight is significant in terms of mother production power and lamb growth rate during the postpartum period. It is reported that gender and birth type (single, twin) affect the birth weight of lambs, the male gender is heavier than the female gender, and single born lambs are born heavier than the twin lambs (Ekiz and Altinel 2007; Ceyhan et al. 2010). In the present study, those single lambs were born significantly heavier than twin lambs in points of lamb birth weight. In terms of gender, male lambs were heavier than females, but the difference was not significant. Similar to the findings of the presented study, Yaralı and Karaca (2004) reported that the effect of gender on lamb birth weight was insignificant, and the twinning was significant. In Ramlıç ewes, Ceyhan et al. (2010), in Zom ewes, Koncagül et al. (2013) reported that both twinning and gender significantly affected lamb birth weight. The mean lamb birth weights determined in the present study were found higher than the studies reported above. It can be considered that the effects of race, care, feeding, and year can cause variations in the values.

As a result, it was determined that twinning in late pregnant ewes could cause changes in mineral matter and IgG levels in the form of increase or decrease, but these levels were insignificant. Furthermore, it was determined that twinning had a significant effect on birth weight, total protein, globulin and Ca values in lambs, it did not affect other minerals and IgG levels. It was determined that BCS and twinning in ewes did not affect IgG, total protein, albumin, and globulin levels, whereas twinning in lambs affected total protein and globulin levels. Significantly low concentrations of total protein and globulin in twin lambs suggest that these animals cannot absorb enough colostrum.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

Idea/Concept: KY

Supervision/Consultancy: SK

Data Collection and/or Processing: KY, SA

Analysis and/or Interpretation: KY, SA

Writing the Article: KY

Critical Review: HA

REFERENCES

- Abdelrahman MM (2008).** The effect of high calcium intake by pregnant Awassi ewes at late gestation on minerals status and performance of ewes and newborn lambs. *Livestock Sci*, 117(1), 15-23.
- Abdollahi E, Kohram H, Shahir MH (2013).** Plasma concentrations of essential trace (microminerals and thyroid hormones during single or twin pregnancies in fat-tailed ewes. *Small Rum Res*, 113(2-3), 360-364.
- Ahmad R, Khan A, Javed MT, Hussain I (2000).** The level of immunoglobulins in relation to neonatal lamb mortality in Pak-Karakul sheep. *Veterinarski Arhiv*, 70 (3), 129-139.
- Al-Jameil N, Tabassum H, Al-Mayouf H, et al. (2014).** Analysis of serum trace elements-copper, manganese and zinc in preeclamptic pregnant women by inductively coupled plasma optical emission spectrometry: a prospective case-controlled study in Riyadh, Saudi Arabia. *International J Clin Exp Path*, 7(5), 1900-1910.
- Al-Sabbagh TA, Swanson LV, Thompson JM (1995).** The effect of ewe body condition at lambing on colostrum immunoglobulin G concentration and lamb performance. *J Anim Sci*, 73(10), 2860-2864.
- Alves AC, Alves NG, Ascari IJ, et al. (2015).** Colostrum composition of Santa Inês sheep and passive transfer of immunity to lambs. *J Dairy Sci*, 98(6), 3706-3716.
- Boland TM, Keane N, Nowakowski P, Brophy PO; Crosby TF (2005).** High mineral and vitamin E intake by pregnant ewes lowers colostrum immunoglobulin G absorption by the lamb. *J Anim Sci*, 83(4), 871-878.
- Ceyhan A, Sezenler T, Yıldırım M, Erdoğan İ (2010).** Reproductive performance and lamb growth characteristics of Ramlıç sheep. *Kafkas Univ Vet Fak Derg*, 16, 213-216.
- Christley RM, Morgan KL, Parkin TDH, French NP (2003).** Factors related to the risk of neonatal mortality, birth-weight and serum immunoglobulin concentration in lambs in the UK. *Preventive Vet Med*, 57(4), 209-226.
- Corah L (1996).** Trace mineral requirements of grazing cattle. *Anim Feed Sci Tec*, 59(1-3), 61-70.
- Corner RA, Mulvaney FJ, Morris ST, et al. (2013).** A comparison of the reproductive performance of ewe lambs and mature ewes. *Small Rum Res*, 114(1), 126-133.
- Dakka AA, Abd-EL-Aal TS (1992).** Studies on minerals picture in the blood sera of Egyptian sheep. *Assiut Vet Med J (Egypt)*, 28, 242-249.
- E Hernandez-Castellano LM, Almeida A, Castro N, Arguello A (2014).** The colostrum proteome, ruminant nutrition and immunity: a review. *Cur Prot Pep Sci*, 15(1), 64-74.
- Ekiz B, Altinel A (2007).** The Growth and Survival Characteristics of Lambs Produced by Commercial Crossbreeding Kıvrıkcık Ewes with F₂ Rams with the German Black-Headed Mutton Genotype. *Turk J Vet Anim Sci*, 30(6), 507-512.
- El-Tarabany AA (2012).** Physiological changes in ewes conceived single or twins fetuses related with survivability of lambs. *Arab J Nuc Sci App*, 45(3), 1-1.
- Gardner DS, Buttery PJ, Daniel Z, Symonds ME (2007).** Factors affecting birth weight in sheep: maternal environment. *Reproduction*, 133(1), 297-307.
- Gökçe E, Atakışi O (2019).** Interrelationships of Serum and Colostral IgG (Passive Immunity) with Total Protein Concentrations and Health in Lambs. *Kafkas Univ Vet Fak Derg*, 25(3), 387-396.
- Gürdoğan F, Yıldız A, Balıkcı E (2006).** Investigation of serum Cu, Zn, Fe and Se concentrations during pregnancy (60, 100 and 150 days) and after parturition (45 days) in single and twin pregnant sheep. *Turk J Vet Anim Sci*, 30(1), 61-64.
- Hostetler CE, Kincaid RL, Mirando MA (2003).** The role of essential trace elements in embryonic and fetal development in livestock. *The Vet J*, 166(2), 125-139.
- Kaneko JJ, Harvey JW, Bruss ML (2008).** Clinical Biochemistry of Domestic Animals. Academic Press, USA.
- Kenyon PR, Stafford KJ, Jenkinson CMC, Morris ST, West DM (2007).** The body composition and metabolic status of twin-and triplet-bearing ewes and their fetuses in late pregnancy. *Livestock Sci*, 107(2-3), 103-112.
- Koncagül S, Vural ME, Karataş A, Akca N, Bingöl M (2013).** Reproductive performance of ewes and growth characteristics of lambs in Zom sheep reared in Karacadağ District. *Kafkas Univ Vet Fak Derg*, 19(1), 63-68.
- Kulcu R, Yur F (2003).** A study of some serum mineral levels before and during pregnancy and during lactation period of sheep and cattle. *Biol Trace Elem Res*, 92(3), 275-279.

- Marques AVS, Soares PC, Riet-Correa F, et al. (2011).** Teores séricos e hepáticos de cobre, ferro, molibdênio zinco em ovinos e caprinos no estado de Pernambuco. *Pesqui Vet Bras*, 31(5), 398-406.
- Massimini G, Britti D, Peli A, Cinotti S (2006).** Effect of passive transfer status on preweaning growth performance in dairy lambs. *J Am Vet Med Ass*, 229(1), 111-115.
- Morris CA, Hickey SM, Clarke JN (2000).** Genetic and environmental factors affecting lamb survival at birth and through to weaning. *New Zealand J Agri Res*, 43(4), 515-524.
- Nowak R, Poindron P (2006).** From birth to colostrum: early steps leading to lamb survival. *Reprod Nut Dev*, 46(4), 431-446.
- Nunes ABV (2006).** Estudo da transmissão da imunidade passiva e da mortalidade em cordeiros mestiços de Santa Inês, na região Norte de Minas Gerais. Dissertação (Mestrado em Medicina Veterinária), Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
- O'Doherty JV, Crosby TF (1997).** The effect of diet in late pregnancy on colostrum production and immunoglobulin absorption in sheep. *Anim Sci*, 64(1), 87-96.
- Roubies N, Panousis N, Fytianou A, et al. (2006).** Effects of age and reproductive stage on certain serum biochemical parameters of Chios sheep under Greek rearing conditions. *J Vet Med*, 53(6), 277-281.
- Ryssen V, Stielau WJ (1981).** Effect of different levels of dietary molybdenum on copper and Mo metabolism in sheep fed on high levels of Cu. *British J Nut*, 45(1), 203-210.
- Quigley J (2005).** Managing variation in calf and heifer programs. In: Proc. 20th Annual Southwest Nutrition and Management Conference, Tempe, Arizona.
- Sansom BF, Bunch KJ, Dew SM (1982).** Changes in plasma calcium, magnesium, phosphorus and hydroxyproline concentrations in ewes from twelve weeks before until three weeks after lambing. *British Vet J*, 138(5), 393-401.
- Silva TR, Soares PC, Dantas AF, et al. (2018).** Serum and liver copper, iron, molybdenum and zinc concentration in goats and sheep in the state of Paraíba, Brazil. *Pesqui Vet Bras*, 38(7), 1313-1316.
- Teixeira IAMA, Resende KT, Silva AMA, et al. (2013).** Mineral requirements for growth of wool and hair lambs. *R Bras Zootec*, 42(5), 347-353.
- Thompson JM, Meyer HH (1994).** Body condition scoring of sheep. Corvallis, OR: Extension Service, Oregon State University.
- Turquino CM, Flaiban KMC, Lisbôa JAN (2011).** Transferência de imunidade passiva em cordeiros de corte manejados extensivamente em clima tropical. *Pesqui Vet Bras*, 31, 99-205.
- Vatankhah M (2013).** Relationship between immunoglobulin concentrations in the ewes serum and colostrum, and lambs serum in Lori-Bakhtiari sheep. *Iran J Appl Anim Sci*, 3(3), 539-544.
- Vural ME (2013).** Reproductive Performance of Ewes and Growth Characteristics of Lambs in Zom Sheep Reared in Karacadag District. *Kafkas Univ Vet Fak Derg*, 19(1), 63-68.
- Yarah E, Karaca O (2004).** Kıvrıkcık koyunları farklı senkronizasyon uygulamalarında kuzu üretimi ile kuzuların canlı ağırlık ve belgözü ultrasonik ölçüm parametreleri. In: 4. Ulusal Zootekni Bilim Kongresi, Isparta, Türkiye.
- Yıldız A, Balıkcı E, Gurdogan F (2005).** Serum mineral levels at pregnancy and postpartum in single and twin pregnant sheep. *Biol Trace Elem Res*, 107(3), 247-254.