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The effect of birth weight on passive transfer of immunity in Kangal Akkaraman breed lambs

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ABSTRACT

Objectives: This study evaluates the influence of birth weight on the passive transfer of immunity (PTI) in Kangal breed lambs, a robust and economically significant Turkish sheep breed.

Materials and Methods: A cohort of 38 healthy neonatal lambs was assessed to elucidate the associations between birth weight and key indicators of PTI, including serum Optical Brix percentage, total protein (TP) concentration, and gamma-glutamyl transferase (GGT) enzyme activity. Blood samples were collected 48–72 hours postpartum and analyzed using cost-efficient and field-applicable methods such as refractometry, automated TP quantification, and enzymatic assays.

Results: The findings demonstrated that lambs with birth weights below 4.53 kg exhibited significantly reduced serum Brix percentages ($P=0.003$), TP concentrations ($P=0.002$), and GGT activity levels ($P=0.028$), indicative of compromised colostral immunoglobulin absorption. Receiver operating characteristic (ROC) analysis identified 4.53 kg as the critical threshold for adequate PTI, with the discriminatory performance primarily supported by TP and GGT ROC analyses, while optical Brix refractometry showed supportive but comparatively weaker discriminatory capacity. Birth weight exhibited a moderate to strong positive correlation with TP ($R=0.351$) and GGT ($R=0.471$) concentrations, whereas Brix refractometry showed a strong correlation with TP ($R=0.880$), indicating good diagnostic agreement. These findings highlight the importance of considering breed-specific PTI thresholds, particularly in breeds such as the Kangal, which are characterized by relatively higher mean birth weights.

Conclusions: In circumstances where direct measurement of immunoglobulin G (IgG) is not feasible, indirect indicators such as Brix refractometry, total protein (TP), and GGT measurements may serve as practical, field-applicable, and economically accessible approaches for evaluating passive transfer under field conditions. Ensuring timely and adequate colostrum intake in lambs below the identified threshold (<4.53 kg) are imperative for mitigating immunological deficits and enhancing neonatal survival outcomes in Kangal sheep husbandry.

Keywords: Passive transfer of immunity, Birth weight, Colostrum, Serum brix refractometry, Neonatal lamb health

INTRODUCTION

Since the synepitheliochorial placental structure in sheep prevents the transfer of maternal antibodies to the fetus, antibodies provided through colostrum

are necessary for the defense of newborn lambs against early infections (Weaver et al., 2000). Maternal antibodies that are transmitted via colostrum play a critical role in helping newborn lambs defend against infections during the early



stages of life. Passive immunity acquired through colostrum post-birth is a key factor in ensuring the newborn's successful adaptation to its external surroundings (Hernández-Castellano et al., 2015; Godden et al., 2019; Kara ve Ceylan, 2021). Colostrum is a rich source of immunoglobulin (Ig) proteins and includes additional immune elements such as maternal leukocytes and cytokines (Şahal et al., 2018). The absorption of colostrum Immunoglobulin G (IgG) is most effective within the first 24 hours after birth and occurs through a non-selective process known as endocytosis (Sangild, 2003). Several methods are used to assess passive transfer of immunity (PTI) in ruminants. Among these, radial immunodiffusion (RID) and enzyme-linked immunosorbent assay (ELISA) are recognized as the gold-standard techniques for determining serum IgG levels directly. These tests typically require the transfer of samples to a laboratory setting. Additionally, they demand specialized technical proficiency and can be financially burdensome. Conversely, alternative methods provide an indirect assessment of serum Ig concentrations in lambs, facilitating the evaluation (Deelen et al., 2014). In agricultural environments, PTI can be assessed through the measurement of serum total protein (TP) levels. Serum TP is composed of albumin and globulins; while albumin concentrations in newborns typically remain constant, globulin levels are directly linked to the absorption of colostrum IgG. As such, globulin concentrations can act as a reliable indicator of colostrum ingestion (Hernandez et al., 2016; Aydoğdu et al., 2019).

Moreover, the elevated gamma-glutamyl transferase (GGT) activity present in colostrum provides an additional means of determining colostrum consumption in calves through serum GGT activity (Britti et al., 2005). After colostrum intake, significant increases in TP levels and GGT activity are observed, closely correlating with IgG concentrations. Research has demonstrated that lambs consuming colostrum exhibit serum GGT activity levels approximately 60 times higher than those of their mothers on the first day of life. Similarly, another study found that nursing lambs possess serum GGT enzyme activity up to 140 times greater than that of healthy adult sheep. Furthermore, a strong positive correlation has been identified between neonatal serum immunoglobulin concentrations and GGT enzyme activity (Pauli, 1983; Braun et al., 2010).

More recently, the measurement of serum Brix percentages has emerged as a widely used alternative test. The high correlation between serum IgG concentrations and Brix percentages ($r^2=0.93$) has enhanced its reliability. Brix percentages can be measured using optical or digital refractometers, offering a practical and efficient approach (Deelen et al., 2014; Elsohaby et al., 2015; Giammarco et al., 2021; Sarica and Aydoğdu, 2024).

The Kangal sheep, officially registered as a distinct breed originating from the Akkaraman sheep, is recognized as the largest and most robust member of the Akkaraman lineage (Tarım ve Orman Bakanlığı, 2020). While its breeding spans 13 provinces across Turkey, it is predominantly concentrated in the Sivas region (Kandemir and Taşkin, 2022). Mature males typically weigh around 95 kg, while females average 65 kg (Tarım ve Orman Bakanlığı, 2020). In 2012, the population of Kangal sheep across Turkey was approximately 500,000, with breeding activities distributed over 13 provinces (Resmi Gazete, 2012). By 2020, this number had risen significantly, with an estimated 650,000 sheep recorded in Sivas alone, the majority of which were identified as Kangal sheep (Kandemir and Taşkin, 2022). Consequently, the Kangal sheep plays a vital role in supporting both the regional and national economy (Kivrak et al., 2022).

Passive transfer of immunity through colostrum is a critical determinant of neonatal health and survival in ruminants, and several studies have investigated this process in lambs and calves of different breeds. In lambs, the efficiency of passive immunity transfer has been evaluated by comparing different colostrum feeding methods, demonstrating that both colostrum intake method and timing significantly influence serum immunoglobulin concentrations and neonatal immune status (Egdir & Ocal, 2023). In sheep, colostrum quality has been assessed using practical field tools such as the Brix refractometer, and variations in colostrum quality between breeds have been reported (Sarica & Aydoğdu, 2024). Breed-related differences in colostrum quality have also been documented between Merino and Akkaraman ewes, highlighting the influence of genetic background on colostrum immunoglobulin content (Kara & Şenel, 2025). In cattle, comparisons between indigenous Turkish breeds and imported breeds have similarly revealed differences in colostrum quality parameters, suggesting that breed characteristics may play an important role in

passive immunity transfer (Kara et al., 2020). However, despite these studies conducted in various sheep and cattle breeds in Türkiye, there is currently no study specifically evaluating colostrum quality and failure of passive transfer in the Kangal sheep breed, indicating a clear gap in the literature.

The aim of this study is to investigate the impact of litter size, birth weight, and sex on passive immunity in Kangal sheep and evaluate the diagnostic efficacy of Brix refractometry (BR), TP, and GGT enzyme activity measurements as cost-effective and practical alternatives to expensive and complex methods like ELISA and RID, which are challenging to implement under field conditions.

MATERIALS and METHODS

Animal design

The study encompassed 38 healthy neonatal Kangal lambs, with their sex, birth weight, and singleton or twin status systematically documented. Blood samples (5 mL) were collected into anticoagulant-free tubes via venipuncture (V. Jugularis) within 48 to 72 hours postpartum from lambs). These samples were allowed to rest at ambient temperature for 30 minutes before being centrifuged at 4000 rpm for 10 minutes to isolate the serum. The separated serum was subsequently stored in labeled microtubes at -80°C until analysis.

Measurement method

Serum TP concentrations and GGT enzyme activity were assessed with commercial kits using an autoanalyzer (Mindray, BS200, China). Brix percentages were measured with an Optical Brix refractometer (ATO, BRM-%0-32, China) on serum samples equilibrated to room temperature. Before initiating the refractometer analyses, the instrument was calibrated to zero with a calibration solution, followed by the execution of the measurements.

Statistical analysis

The data were expressed as mean \pm standard error of the mean (Mean \pm SEM). The Kolmogorov-Smirnov test was used to assess the normality of the data distribution, confirming that all variables followed a normal distribution. Differences between groups were evaluated using an independent t-test. Pearson correlation analysis was performed to determine the strength of relationships between lamb birth weight, serum Optical Brix percentages, TP concentrations, and GGT activities.

To identify the threshold birth weight associated with sufficient PTI, ROC analysis was performed. The analysis used the following reference values: 450 U/L for GGT activity (Gokce et al., 2021), %9 for Brix percentage (Hamer et al., 2023), and 6.8 g/dL for TP concentration (Khan et al., 2006). P of <0.05 was considered statistically significant.

Ethics approval

The study protocol was approved by the Animal Experiments Local Ethics Committee of Sivas Cumhuriyet University (Approval number 97; Approval date, 07/11/2017).

RESULTS

Descriptive data regarding the sex, litter size, and birth weights of the newborn Kangal lambs are presented in Table 1. Among the lambs included in the study, 14 were males and 24 were females. The litter distribution indicated that 19 lambs were twins and 19 were singletons. Regarding the birth weight threshold for adequate passive transfer of immunity, defined as 4.53 kg, 15 lambs fell below this threshold, while 23 lambs had birth weights above it.

Table 1. Descriptive data on sex, litter size, and birth weight of newborn Kangal lambs (n=38).

Variable	Number of Lambs (n)
Sex	
Male	14
Female	24
Litter Size	
Singleton	19
Twin	19
Birth Weight	
<4.53 kg	15
\geq 4.53 kg	23

The mean, standard deviation, minimum, and maximum values for birth weight, serum Optical Brix percentage, TP, and GGT levels of the newborn Kangal lambs are detailed in Table 2. The lambs had an average birth weight of 4.89 kg, with recorded values ranging from a minimum of 2.16 kg to a maximum of 8.84 kg.

The effect of sex on birth weight, Optical Brix percentage, TP, and GGT levels in newborn Kangal lambs is presented in Table 3. No statistically significant differences were observed between male and female lambs regarding birth weight, Optical Brix, TP, and GGT values ($p>0.05$).

Table 2. Mean, standard error, minimum, and maximum values for birth weight, serum optical brix, TP, and GGT levels in newborn Kangal lambs (n=38).

Parameter	Mean ± SEM	Min	Max
Body Weight (kg)	4.89±0.22	2.16	8.84
Optical Brix (%)	10.06±0.18	7.0	12.8
TP (g/dL)	6.63±0.13	4.09	8.05
GGT (U/L)	648.50±48.89	230	1325

Table 3. The effect of sex on birth weight, serum optical brix, TP, and GGT levels in newborn Kangal lambs.

Parameter	Male (n=14)	Female (n=24)	p-value
Body Weight (kg)	5.30±0.44	4.65±0.24	0.165
Optical Brix (%)	9.64±0.30	10.31±0.22	0.078
TP (g/dL)	6.42±0.28	6.75±0.14	0.238
GGT (U/L)	645.79±75.72	650.08±64.79	0.967

Table 4. The effect of litter size on birth weight, serum optical brix, TP, and GGT levels in newborn Kangal lambs.

Parameter	Singleton (n=19)	Twin (n=19)	p-value
Body Weight (kg)	5.31±0.35	4.47±0.24	0.057
Optical Brix (%)	9.95±0.28	10.18±0.24	0.533
TP (g/dL)	6.65±0.23	6.61±0.15	0.890
GGT (U/L)	616.68±75.90	680.32±62.87	0.523

Table 5. The effect of birth weight on serum optical brix, TP, and GGT levels in newborn Kangal lambs.

Parameter	<4.53 kg (n=15)	≥4.53 kg (n=23)	P-value
Optical Brix (%)	9.41±0.30	10.49±0.19	0.003
TP (g/dL)	6.32±0.17	7.12±0.15	0.002
GGT (U/L)	562.87±58.92	779.80±74.87	0.028

Table 6. Correlations between birth weight, serum optical brix, TP, and GGT levels in newborn Kangal lambs.

	Variable	Birth Weight	Optical Brix	TP	GGT
Birth Weight	Pearson Correlation	1	0,257	0,351*	0,471**
	Sig. (2-tailed)		0,119	0,031	0,003
Optical Brix	Pearson Correlation			0,809**	0,437**
	Sig. (2-tailed)			<0,001	0,006
TP	Pearson Correlation				0,546**
	Sig. (2-tailed)				0,000
GGT	Pearson Correlation				1
	Sig. (2-tailed)				

*Significant at the 0.05 level (2-tailed). **Significant at the 0.01 level (2-tailed).

Table 7. AUC, cut-off, sensitivity, and specificity values for birth weight in newborn Kangal lambs.

Parameter	Cut-off Value	AUC	Standard Error	%95 CI for AUC	p-value	Sensitivity (%)	Specificity (%)
GGT (450 U/L)	4.53	0.783	0.084	0.617 - 0.948	0.004	73.3	82.6
Optical Brix (9%)	4.53	0.670	0.084	0.506 - 0.834	0.227	80.0	66.7
TP (6.8 g/dL)	4.53	0.729	0.091	0.552 - 0.907	0.016	66.7	85.0

AUC: Area under the curve.

Table 4 indicates that although no statistically significant difference was observed in birth weight between singleton and twin lambs, twin lambs exhibited slightly lower body weights compared to singletons (p=0.057). The influence of birth weight on serum Optical Brix percentage, TP, and GGT levels in newborn Kangal lambs is presented in Table 5.

Based on previously established thresholds for adequate passive transfer, Brix percentage, TP, and GGT levels, this study identified 4.53 kg as the critical birth weight threshold for optimal passive

immunity transfer in Kangal lambs. Lambs with birth weights below 4.53 kg demonstrated significantly lower serum Optical Brix (p=0.003), TP (p=0.002), and GGT (P=0.028) levels compared to those with birth weights exceeding this threshold.

The relationships between birth weight, Optical Brix percentage, TP, and GGT levels are presented in Table 6. Birth weight was found to have a weak positive correlation with Optical Brix (r= 0.296, p>0.05), a moderate positive correlation with TP levels (r=0.351, p<0.05), and a strong positive correlation with GGT levels (r=0.471, p<0.001).

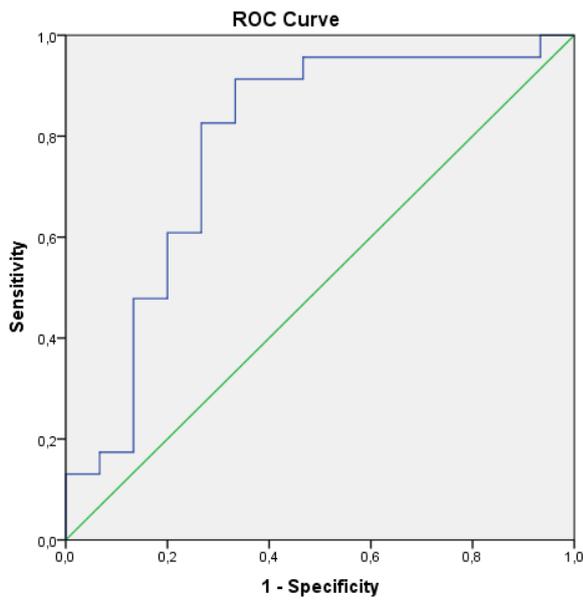


Figure 1. Roc curve for birth weight based on GGT analysis results in newborn Kangal lambs.

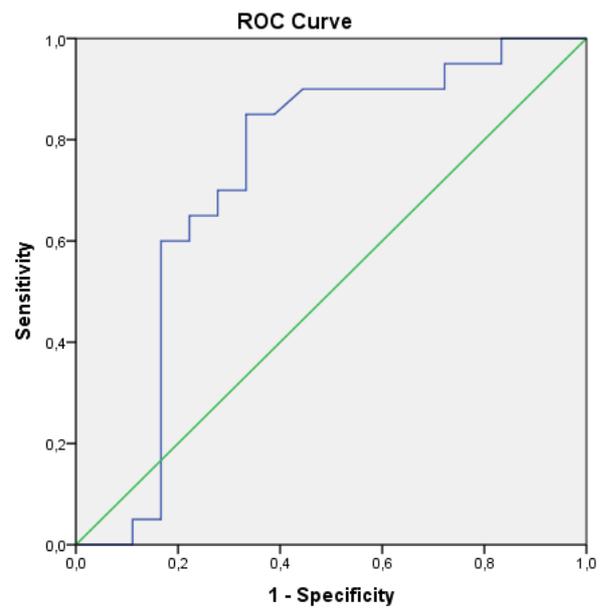


Figure 3. Roc curve for birth weight based on TP analysis results in newborn Kangal lambs.

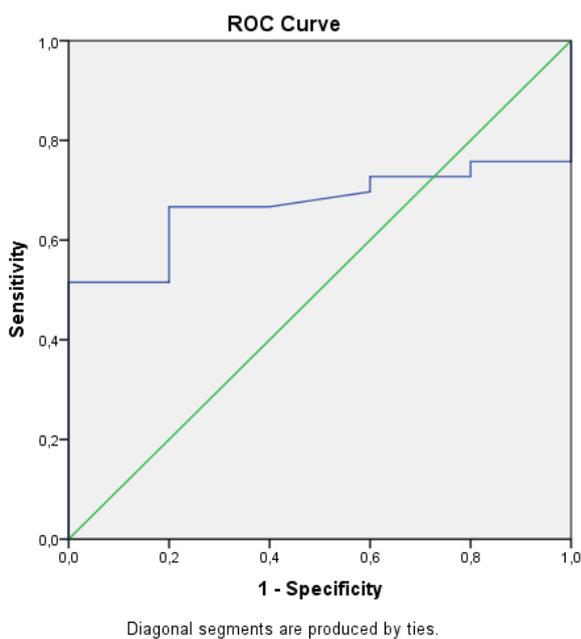


Figure 2. Roc curve for birth weight based on brix analysis results in newborn Kangal lambs.

Regarding the relationship between Optical Brix and the other parameters, a strong positive correlation was observed with TP levels ($r=0.880$, $p<0.001$), while a moderate positive correlation was identified with GGT levels ($r=0.376$, $p<0.05$). Furthermore, a strong positive association was detected between TP and GGT levels ($r=0.546$, $p<0.001$).

Figures 1, 2, and 3 depict the ROC curves utilized to establish the optimal birth weight threshold for newborn Kangal lambs based on GGT, Optical Brix, and TP values, respectively. Sensitivity, specificity, and accuracy were optimized to achieve the maximum area under the curve. The calculated sensitivity and specificity for the test are presented in Table 7.

The threshold for PTI progression, as documented in the literature, is 450 U/L for GGT, %9 for Optical Brix, and 6.8 g/dL for TP. All three analyses identified a birth weight threshold of 4.53 kg. At this threshold, GGT activity demonstrated a sensitivity of %73.3 and a specificity of 82.0%; Optical Brix showed a sensitivity of %80.0 and a specificity of %66.7; while TP exhibited a sensitivity of %66.7 and a specificity of %85.0.

DISCUSSION

Various direct and indirect methods are available to assess whether lambs have received sufficient colostrum intake (Souza et al., 2021). While techniques like RID and ELISA directly measure IgG concentrations, their high costs, requirement for specialized expertise, and dependency on laboratory infrastructure limit their feasibility in field settings. As a result, several indirect approaches have been developed, offering cost-effective, user-friendly, and reliable alternatives. These methods include measuring serum TP levels, Brix, and evaluating GGT (Aydođdu et al., 2019). In this study, indirect measurement techniques—

serum TP levels, Brix, and GGT—were selected over direct methods due to their affordability, longer reagent stability, minimal infrastructure requirements, and proven reliability and practicality for on-site applications as evidenced by previous research.

Male lambs are generally heavier at birth than females due to the influence of sex-linked genetic mechanisms regulating fetal growth. The presence of the SRY gene on the Y chromosome initiates male gonadal differentiation and contributes to sex-specific patterns of fetal development through downstream gene activation pathways (Demirtaş & Pişkin, 2009; McGovern et al., 2020). In addition to genetic factors, prenatal growth is also affected by uterine capacity and maternal resource allocation. In polytocous species such as sheep, the uterine environment represents a limited space and nutrient supply, resulting in increased intrauterine competition as litter size increases. Consequently, multiple pregnancies are typically associated with reduced fetal growth and lower birth weights compared with singleton pregnancies (Gardner et al., 2007; Mellor & Murray, 1985).

Birth weight and litter size are important determinants of neonatal viability and early immune competence in lambs. Lambs born with lower birth weights often exhibit reduced vigor, delayed standing and suckling behavior, and decreased colostrum intake, all of which may negatively affect the efficiency of passive transfer of immunity (PTI) (Nowak & Poindron, 2006; Hernandez-Castellano et al., 2015). Similarly, twin or multiple-born lambs may experience increased competition for colostrum, which can further compromise the acquisition of adequate immunoglobulin concentrations during the critical early postnatal period (Weaver et al., 2000; Hernandez-Castellano et al., 2014). Sex-related differences in neonatal physiology have also been reported, with male lambs generally displaying higher birth weights and greater early growth potential compared with females, although the magnitude of this difference may vary depending on breed, maternal nutrition, and management conditions (Mukasa-Mugerwa et al., 1994; Ahmad et al., 2000; Chniter et al., 2009).

In the present study, litter size and sex were identified as potential sources of variation in birth weight. Although the differences were not statistically significant, female lambs tended to have lower birth weights than males, and twin lambs had lower birth weights than singletons.

These findings are consistent with previous studies reporting that fetal growth is influenced by both sex and intrauterine competition among littermates (Mukasa-Mugerwa et al., 1994; Ahmad et al., 2000; Chniter et al., 2009; Takci et al., 2023). The absence of statistically significant differences in the current study may be attributed to factors such as sample size, management conditions, or breed characteristics. Nevertheless, the observed trends support the well-established biological relationship between litter size, fetal growth, and neonatal physiological development reported in the literature. Birth weight is a frequently studied factor in research on PTI. While some studies have demonstrated a strong association between birth weight and serum IgG concentrations in lambs (Khan et al., 2006; Oldham et al., 2011; Gökçe et al., 2013), others have reported no significant relationship (Cabello and Levieux, 1981; Massimini et al., 2006; Boucher, 2014).

Research indicating that birth weight affects IgG levels suggests that lambs with lower birth weights are often physically weaker, reducing their ability to consume sufficient colostrum during the critical early stages of life. This inadequate intake leads to lower IgG concentrations in their serum (Ahmad et al., 2000; Christley et al., 2003; Khan et al., 2006; Gökçe et al., 2013). Consistent with this research, the present study exhibits that low birth weight may play a significant role in influencing PTI occurrence.

In this study, the threshold birth weight indicating adequate passive immunity in Kangal lambs was determined using established parameters for passive transfer: 450 U/L GGT (Gökçe et al., 2013), 6.8 g/dL TP (Khan et al., 2006), and %9 Brix (Hamer et al., 2023). ROC analysis of these parameters identified 4.53 kg as the critical threshold. Lambs with birth weights below this value exhibited significantly lower levels of serum Optical Brix ($p=0.003$), TP ($p=0.002$), and GGT ($p=0.028$) compared to those above the threshold.

Previous research has suggested a birth weight threshold of 3 kg, indicating that lambs born below this weight are at a higher risk of passive immunity transfer failure (Mukasa-Mugerwa et al., 1994; Khan et al., 2006; Gökçe et al., 2013). For instance, Dwyer et al. (2016) reported that the optimal birth weight for Scottish Blackface lambs ranges between 3 and 5 kg. Similarly, Nowak and Poindron (2006) recommended a birth weight range of 3 to 5.5 kg as optimal for lambs. However, these thresholds vary by breed (Gilbert et al., 1988; Dwyer et al., 2016).

The average birth weight of Kangal lamb has been reported as approximately 5 kg (Takci et al., 2023). Comparatively, Khan et al. (2006) found that Pak-Karakul lambs had birth weights of 3.84 kg for males and 3.51 kg for females, while Tahalli lambs weighed 3.58 kg and 2.93 kg for males and females, respectively. Similarly, Gökçe et al. (2013) reported that Akkaraman lambs had average birth weights of 4.1 kg for males and 3.8 kg for females.

These results highlight the need to consider breed-specific differences when determining birth weight thresholds for PTI. Based on the findings of this study, 4.53 kg has been identified as the threshold birth weight for PTI in Kangal lambs, providing a valuable reference for future research.

This study is the first to define a birth weight threshold for identifying PTI failure in Kangal lambs using Optical Brix refractometry on serum samples, focusing on this native Turkish breed. The findings indicate that low birth weight is a crucial factor in assessing PTI management in Kangal sheep operations. Lambs weighing less than 4.53 kg at birth are at a greater risk of PTI failure, highlighting the necessity of ensuring sufficient colostrum intake immediately after birth for these lambs.

Limitations of the Study

Several limitations of this study should be acknowledged when interpreting the findings. First, the relatively small sample size may limit the statistical power and reduce the generalizability of the results. In addition, the study was conducted on animals originating from a single farm/location, which may restrict the applicability of the findings to other management systems, environmental conditions, or sheep populations. Another important limitation is the lack of direct measurement of serum immunoglobulin G (IgG) using reference methods such as radial immunodiffusion (RID) or enzyme-linked immunosorbent assay (ELISA); therefore, the indirect indicators used in this study could not be validated against a recognized gold standard. Furthermore, the cross-sectional design of the study provides only a single time-point assessment and does not allow evaluation of temporal changes in passive transfer status. Finally, the absence of longitudinal follow-up data prevented the investigation of potential associations between passive transfer indicators and subsequent health outcomes in lambs. Despite these limitations, acknowledging these factors enhances the

transparency of the study and strengthens the interpretation of the results within the appropriate scientific context.

CONCLUSION

The results also reveal that even in breeds like Kangal sheep, known for their typically high birth weights, dropping below a specific threshold can increase vulnerability. This highlights the need for breed-specific birth weight thresholds to guide PTI evaluation and improve the care of low-birth-weight lambs.

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