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Passive immunity in lambs: Colostral and serum γ-glutamyltransferase as a predictor of IgG concentration and related to the diseases from birth to 12 weeks of life

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Abstract: The main goal of this study was to find a link between colostrum and the 1-day-old lamb serum γ-glutamyltransferase (GGT) activity and immunoglobulin G (IgG) concentration and their relation with neonatal diseases and beyond. Further, to set a linear relationship between the serum GGT activity (SGGTA) and the IgG concentration (SIgGC) in different days of the neonatal period, thereby determining the feasibility of the GGT activity in the prediction of the colostrum quality and passive immunity and to define a cut-off point for the SGGTA associated with an increased risk of illness or death in lambs. For this purpose, blood samples were obtained from the lambs before the colostrum intake (day 0) and on different days (1, 2, 4, 7, 14 and 28) in the neonatal period. The colostrum was collected from the respective ewes (n = 254) related to the lambs. The most accurate (R^2 = 0.652) model for predicting the SIgGC or passive immune status was the multiple regression model developed to calculate ln[IgG] from ln[GGT] in healthy neonatal lambs using the serum GGT and IgG values of day 0, 1, 2, 4, 7, 14 and 28. The In[GGT] activity at 24 h after birth in lambs that died or became ill during the neonatal period accounted for approximately 77% and 88% of the variation in the ln[IgG] concentration at 24 h after birth, respectively. The study revealed that SGGTA-24 > 500 IU may be considered as a critical cut-off point for the adequate colostral passive transfer. This study also disclosed that the colostral GGT activity might be used as an indicator to determine the colostrum quality.

Keywords: colostrum quality; cut-off value; GGT; IgG; neonatal lamb health

Diseases and deaths experienced in the neonatal period of lambs result in marked economic losses (Gokce et al. 2013; Voigt et al. 2019) and, thus, raise concern about this period as being critical for sheep farmers (Swarnkar et al. 2018).

In ruminants, syndesmochorial placentation blocks the transmission of immunoglobulins (Ig) so the offspring are born with negligible serum concentrations of immunoglobulin G (IgG). Therefore, the acquisition of passive immunity by the immedi-

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ate ingestion and absorption of maternal Ig from the colostrum is critical in neonatal lambs (Nowak and Poindron 2006; Loste et al. 2008; Hine et al. 2019). The colostrum also contains nutrients, minerals, trace elements, hormones, growth factors, and other non-specific immunologic substances (Nowak and Poindron 2006; Loste et al. 2008; McGrath et al. 2016), necessary for normal development. An adequate amount of colostrum must be ingested as soon as possible since the intestinal absorption time of Ig takes up to 24 hours (Alves et al. 2015; Voigt et al. 2019). Thus, the first day after birth is the most critical time for lambs, since the failure of passive transfer (FPT) occurs at this time. FPT is a secondary immunodeficiency condition that predisposes newborn ruminants to the development of common neonatal diseases and adversely influences their performance later in life (Massimini et al. 2006; Gokce et al. 2013; Gokce et al. 2014; Lopreiato et al. 2017). Failure of passive transfer (FPT) and the resulting hypogammaglobulinemia have also been linked to an increased risk of neonatal illnesses and deaths (Britti et al. 2005). Therefore, the early determination of the colostrum quality and passive immune status is imperative in making timely management and therapeutic decisions in order to reduce the risk of neonatal diseases.

Numerous previous studies established an association between neonatal diseases and the FPT in animals and, thus, advocated the significant role of IgG in the prevention of infections and improving the growth performance in neonates (Massimini et al. 2006; Voigt et al. 2019). The diagnosis of FPT and colostrum quality requires direct or indirect measurements of the IgG concentrations in the lamb's serum and colostrum. The single radial immunodiffusion (SRID) and Enzyme-Linked Immunosorbent Assay (ELISA) (Massimini et al. 2006; Gokce et al. 2019) are reported to directly measure the IgG concentrations. However, these two methods are time consuming, costly, labour intensive and of limited use on site, so a need for alternative methods that are fast, readily-available, inexpensive and suitable for bedside use on the farm by farmers or veterinarians has arisen (Zarrilli et al. 2003; Lee et al. 2008). Therefore, screening with alternative indirect methods that are capable of validating SRID or ELISA results could be a much more effective way to detect FPT in individual herds (Lee et al. 2008; Aydogdu and Guzelbektes 2018). Several indirect methods, such as serum total protein concentration assays, the 10% glutaraldehyde reagent test and the zinc sulfate turbidity (ZST) test are commonly used (Massimini et al. 2006; Lee et al. 2008; Aydogdu and Guzelbektes 2018; Gokce et al. 2019). These tests vary in terms of accuracy, speed, cost and suitability for field use (Parish et al. 1997; Hogan et al. 2015). Obtaining fast and accurate test results on the farm is critical in making timely management and treatment decisions as well as making clinical decisions, especially for the prognosis and designing treatments of neonatal diseases (Parish et al. 1997; Massimini et al. 2006). However, the accuracy of these tests has only been studied in healthy neonatal lambs (Massimini et al. 2006).

The intestinal absorption is nonselective in neonatal ruminants, so calves and lambs are able to absorb many proteins, including macromolecular substances, within the first 24 h to 48 h after birth (Britti et al. 2005; Hine et al. 2019). If colostrum intake is ensured within an appropriate timeframe, colostral enzymes are also able to cross the intestinal barrier via the nonselective mechanism as immunoglobulins and could be used as markers of the passive transfer status (PTS). The colostral GGT activity (CGGTA) is approximately 470 times greater than the serum activity in some studies (Maden et al. 2003). Measuring the serum GGT activity (SGGTA) in the early neonatal period may be a useful predictor of the passive transfer (Aydogdu and Guzelbektes 2018), as the GGT activity was reported to be consistent with the FPT (Maden et al. 2003; Zarrilli et al. 2003). However, these studies utilised a limited number of colostrum samples and the feasibility of using CGGTA to predict the passive immune status has not been detailed.

Studies in calves (Parish et al. 1997; Wilson et al. 1999; Aydogdu and Guzelbektes 2018) and lambs (Britti et al. 2005) have demonstrated the usefulness of the serum GGT activity as an indicator of FPT. The link between SGGTA and SIgGC has also been revealed in healthy lambs only (Britti et al. 2005; Massimini et al. 2006). In previous studies, regression models were generated to predict the PTS as a function of the age and SGGTA for lambs < 15 days in healthy lambs (Maden et al. 2003). In addition, the feasibility of using SGGTA to predict the passive immune status in clinically ill or dead lambs has not previously been assessed.

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Consequently, data regarding the SGGTA threshold values that are associated with increased risk of sickness and death in lambs during the neonatal and subsequent periods or the accuracy of these threshold values is not yet known.

This study is designed to determine the correlation and linear relationships between the GGT and IgG concentrations in healthy lambs and to evaluate the GGT's potential for predicting disease through the measurement of the serum GGT and IgG concentrations at 24 h after birth in healthy, sick and deceased lambs in the neonatal period and the period covering 5–12 weeks of life. It is also aimed at disclosing the relationship between the colostral GGT and IgG concentrations in terms of the passive immunity, colostrum quality and lamb health.

MATERIAL AND METHODS

Animals

Details of the study design (animals, farm selection, farm management practices) were given previously (Gokce et al. 2014). In brief, 301 Akkaraman crossbreed ewes and 347 lambs born to them on two neighbouring farms with similar farm management practices and feeding regimens were included in the current study. The lambing was monitored by farmers throughout the parturition season and lambs were allowed to get the colostrum through suckling by themselves, within 24 h of birth. The lambs and their mothers were kept in an individual pen up to seven days, and then the lambs were moved to a group pen and allowed to suckle twice a day (in the morning and evening) and they were also provided only hay after the first week of life for three weeks and straw and commercial growth feed (Bayramoglu AS, Turkey) in addition to the hay for three months. The lambs that reached three months of age were turned out to graze on a pasture during the day time and given hay and commercial feed during the night.

Blood and colostrum sample collections

Blood samples were collected from all the lambs at 24 ± 1 h of life by a jugular vein puncture into serum clot tubes. In order to monitor the GGT and IgG concentrations over a month period, a total of 41 lambs, observed as healthy in the neonatal period, were also blood sampled before (precolostral time = 0 h) and after the colostrum intake (2, 4, 7, 14 and 28 days). All the lambs (n = 300) received the colostrum by naturally suckling within the first 24 h after birth and were not assisted. The serum was harvested by centrifugation at 2 000 g for 5 min and stored at –20 °C until the analyses.

The colostrum samples were obtained within 0–4 h after parturition from the ewes (n = 254) that gave birth to the lambs tested in this study. The collected samples were centrifuged at 2 000 g for 30 min to remove the fat and sediments and the supernatant was stored in 1.5 ml micro centrifuge tubes at –20 °C until the analysis. During the analysis, the colostrum samples obtained from the supernatants were diluted 100-fold and the solid phase was removed twice by using a nylon66-Syringe Filter. The transparent samples were used for the analysis.

IgG and GGT enzyme activity assays

The serum IgG levels were determined by a commercial ELISA kit (Bio-X Competitive ELISA Kit for Ovine blood serum IgG Assay-BIO K 350; Bio-X Diagnostics, Rochefort, Belgium). The colostrum IgG level was also measured with the same kit using a bovine colostrum calibrator (Bio-X Elisa Kit for Bovine Immunoglobulin Assays-BIO K 165; Bio-X Diagnostics, Rochefort, Belgium). The serum and colostral GGT activity was spectrophotometrically measured using a commercial kit (TML, Istanbul, Turkey).

Clinical examination

All the lambs were subjected to routine clinical examinations as previously described where, for each clinical entity (diarrhoea, pneumonia, suspected septicaemia, fatigue-anorexia syndrome, other or unknown), a case definition was designated (Gokce and Erdogan 2009). The health status of the lambs was monitored on the farms on daily visits during the neonatal period (0–28 days) and every two days after the neonatal period (29–84 days) until the 12th week of life. Those lambs exhibiting one or more signs such as a poor suckling reflex, ano-

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rexia, depression, lethargy, fever, nasal discharge, abnormal lung sounds and high respiratory and heart rate, coughing, diarrhoea or watery faeces, at least two signs of dehydration (skin elasticity, sunken eye, etc.) on clinical examination and died due to reasons other than trauma on necropsy were considered ill.

Statistical analysis

The lambs were classified as healthy or sick as a result of the clinical examination. In addition, sick lambs were also defined as dead or recovered. The period of concern covered the neonatal period (the first week and the following three weeks) and the period from 5 to 12 weeks of life to compare the morbidity, mortality and their relations with the serum IgG concentrations and GGT activity determined at 24 h after the birth and the dam's colostral IgG concentrations and GGT activity. Those animals whose IgG and GGT concentrations were not determined for any reason were excluded from the analyses.

The data were collected and entered into a database (Microsoft Access). The mean ± SE values for the serum and colostrum IgG concentrations or the GGT activities were calculated. The serum IgG concentration and GGT activities on days 0, 1, 2, 4, 7, 14 and 28 in the healthy lambs (n = 41) were compared by use of one-way repeated-measures ANOVA (analysis of variance). When results of the F-test were significant, the time-dependent differences were localised by use of Tukey's HSD test. An independent sample T-test was used to compare the GGT activities or IgG concentrations at 24 h after the birth in the different period of life categorised based on healthy versus sick; healthy versus dead; healthy versus recovered. The GGT activities and IgG concentrations at 24 h after the birth were also compared with each value determined for the ill, dead and recovered versus the different time periods.

The relationship between the serum IgG concentration and the GGT activity were explored by Pearson's correlation and a simple/multiple regression analysis. The accuracy of the serum GGT activity for estimating the serum IgG concentrations on the different days of the neonatal period [0 (before suckling), 1, 2, 4, 7, 14 and 28] in the healthy lambs and on day 1 for those categorised according to the clinical examination (healthy, sick, deceased and recovered in the neonatal period and the period from 5 to 12 weeks of life) was established by using the standard linear regression analysis previously described in detail (Massimini et al. 2006; Gokce et al. 2014). Multivariate regression models were developed that predicted the natural logarithm of the serum IgG concentration (continuous dependent variable) as a function of the natural logarithm of the serum GGT activity (continuous independent variable) and the age of lambs at the time of sampling (categorical independent variable) and the interaction of the sampling times. In addition, the accuracy of the colostral GGT activity for estimating the colostral IgG concentration in sheep was established using the simple linear regression method. The calculations were performed by use of a statistical software package (SPSS, v16.0). The Origin6 program was used to obtain the scatter diagrams illustrations. For all the analyses, values of $P < 0.05$ were considered significant.

RESULTS

Clinical outcomes

The clinical examination revealed that the proportion of lambs that contracted diarrhoea (32/347), pneumonia (6/347) suspected septicaemia (11/347) and Fatigue-Anorexia Syndrome (FAS) (11/347) in the neonatal period was 9.2%, 1.7%, 3.2%, 3.2%, respectively. A total of thirteen lambs died in this period. The most common health problems in the lambs in the period of 5–12 weeks of life were diarrhoea (18.6%, 62/334), pneumonia (7.5%, 25/334), suspected septicaemia (1.2%, 4/334), pneumo-enteritis (3.5%, 11/334) and other/unknown causes (1.8%, 6/334) and the number of lambs that died during this period was fifteen.

Correlation between serum GGT and IgG concentrations in the neonatal period

The study evaluated 300 lambs and the mean ± SEM serum IgG and GGT concentrations at the 24th h after birth were 21 038 ± 668 mg/l and 2 320 ± 86 IU, respectively, and there was a significant (R = 0.745, P = 0.000) correlation between these two parameters.

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The SGGTA was significantly greater on day 1, 2, 4, 7 and day 14 of the neonatal period when compared with that of day 0 (before the colostrum intake) in the healthy lambs while the SIgGC was significantly higher in all the samples taken after the colostrum intake (Table 1). There was a significant positive correlation between the SGGTA and SIgGC determined on day 1, 2, 4, 7 and 14 of the neonatal period that existed in the serum of the healthy lambs (Table 2).

The variables having a linear correlation were subjected to simple and multiple regression models (Table 3). A positive curve linear relationship was detected between the serum IgG concentration and the serum GGT activity. Logarithmic transformations were used because the preliminary graphic depictions of the data suggested the presence of curvilinear relationships between the serum GGT activity and the initial serum IgG concentration, and the age and the initial serum IgG concentration. The models that did not employ the natural logarithm transformations were screened and rejected because these models had a substantially lower correlation coefficient.

The simple regression models showed that the natural logarithm (ln) of the serum GGT activity (ln[GGT]) was linearly and significantly ($P < 0.05$ to $P < 0.001$) associated with the estimated ln of the serum IgG concentration (ln[IgG]) only on day 1, 2, 4 and 7 during the first week of life in the healthy lambs. The accuracy of these models in predicting the ln[IgG]as a function of the ln[GGT] fluctuated between 24% and 65%. The multiple regression models showed that the ln[GGT] was linearly and significantly ($P < 0.001$) associated with the estimated ln[IgG] on the different days during the neonatal period in the healthy lambs. These multivariate models were moderately accurate in estimating the IgG concentration (R^2 = 0.342 to 0.652).

The most accurate result for predicting the SIgGC using simple regression models was that of day 1 (R^2 = 0.509 to 0.644) (Table 3, Figure 1A). Similarly, the most accurate (R^2 = 0.652) results for predicting the SIgGC was the multiple regression model developed to calculate the ln[IgG] from the ln[GGT] in the healthy neonatal lambs using the serum GGT and IgG values of day 0 (before the colostrum intake), 1, 2, 4, 7, 14 and 28 (Table 3).

Relationship between serum GGT concentrations and healthy status

The serum GGT activity at 24 h after birth (SGGTA-24) of the sick and dead lambs were markedly lower than that of the healthy lambs in the first week of life and the neonatal period as a whole. Similar results were determined for the IgG concentrations at 24 h after birth (SIgGC-24). The SGGTA-24 and SIgGC-24 were lower in the recovered lambs than in the healthy lambs in both periods, but it was only significant in the first week of life (Table 4). However, the lamb mortality was the greatest (11/13) in the first week after birth.

The serum GGTA-24 and SIgGC-24 of the clinically healthy lambs had significant positive correlations between (R = 0.571, P = 0.000 01) the diseased (R = 0.820, P = 0.000 01), dead (R = 0.785, P = 0.001) and recovered (R = 0.750, P = 0.000 01) lambs during the neonatal period. Additionally, in each of these groups of lambs, there was a strong linear relation-

Table 1. Serum GGT activity (IU) and IgG (mg/l) concentrations (mean ± SE) in the healthy lambs during the neonatal period (n = 41)

Days	0	1 (± 1th)	2	4	7	14	28
GGT	38 ± 2.19	2 679 ± 281.10**	914 ± 121.8**	395 ± 40.62**	188 ± 17.34**	70 ± 4.37*	54 ± 2.34
IgG	278 ± 25	25 596 ± 2 053**	26 095 ± 2 093**	2 198 ± 1 725**	1 629 ± 12 812**	1 007 ± 61**	898 ± 538**

Independent sample T-test used. Significantly different from day 0 (*$P < 0.05$, **$P < 0.001$)

Table 2. The correlations between the GGT and IgG concentrations in the healthy neonatal lambs (n = 41)

Day	0	1	2	4	7	14	28
Pearson's correlation	0.019	0.767**	0.635**	0.544**	0.493**	0.405**	0.067
Sig. (2-tailed)	0.906	0.000 01	0.000 01	0.000 01	0.001	0.009	0.678

**$P < 0.01$

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Table 3. Regression models between the GGT (IU) and IgG (mg/l) concentrations in the lamb blood serum samples

Simple regression analysis				
Days	*n*	formulas	R^2	*P*
0	41	ln(IgG) = 2.089 + [0.298 × ln(GGT)]	0.037	0.228 2
1	41	ln(IgG) = 2.651 + [0.658 × ln(GGT)]	0.644	0.000 01
2	41	ln(IgG) = 5.316 + [0.373 × ln(GGT)]	0.365	0.000 01
4	41	ln(IgG) = 5.016 + [0.442 × ln(GGT)]	0.330	0.000 01
7	41	ln(IgG) = 4.924 + [0.461 × ln(GGT)]	0.240	0.001 1
14	41	ln(IgG) = 5.250 + [0.378 × ln(GGT)]	0.112	0.032 4
28	41	ln(IgG) = 6.384 + [0.087 × ln(GGT)]	0.003	0.751 2
Multiple regression analysis				
Days	*n*	formulas	R^2	*P*
0,1,2,4,7,14,28	41 × 7 = 287	ln(IgG) = 0.906 + [0.964 × ln(GGT)] + (0.930 × day)	0.652	0.000 01
1,2,4,7,14,28	41 × 6 = 246	ln(IgG) = 5.811 + [0.282 × ln(GGT)] + (–0.007 × day)	0.499	0.000 01
1,2,4,7,14	41 × 5 = 205	ln(IgG) = 5.724 + [0.293 × ln(GGT)] + (–0.003 × day)	0.442	0.000 01
1,2,4,7	41 × 4 = 164	ln(IgG) = 5.145 + [0.354 × ln(GGT)] + (0.060 × day)	0.342	0.000 01
1,2,4	41 × 3 = 123	ln(IgG) = 4.568 + [0.188 × ln(GGT)] + (0.401 × day)	0.359	0.000 01
1,2	41 × 2 = 82	ln(IgG) = 3.446 + [0.477 × ln(GGT)] + (0.599 × day)	0.467	0.000 01
Simple regression analysis according to the results of the clinical examinations				
Neonatal period				
1	300*	ln(IgG) = 1.276 + [0.824 × ln(GGT)]	0.758	0.000 01
1	243[a]	ln(IgG) = 3.633 + [0.522 × ln(GGT)]	0.509	0.000 01
1	57[b]	ln(IgG) = 0.100 + [0.966 × ln(GGT)]	0.879	0.000 01
1	44[c]	ln(IgG) = 2.388 + [0.668 × ln(GGT)]	0.869	0.000 01
1	13[d]	ln(IgG) = [1.064 × ln(GGT)] – 754	0.767	0.000 01
The period covering with 5 to 12 weeks of life				
1	312*	ln(IgG) = 3.132 + [0.585 × ln(GGT)]	0.625	0.000 01
1	190[a]	ln(IgG) = 3.385 + [0.566 + ln(GGT)]	0.580	0.000 01
1	97[b]	ln(IgG) = 2.957 + [0.599 + ln(GGT)]	0.667	0.000 01
1	82[c]	ln(IgG) = 3.063 + [0.582 + ln(GGT)]	0.690	0.000 01
1	15[d]	ln(IgG) = 2.431 + [0.683 + ln(GGT)]	0.571	0.001

*General (without any evaluation of the clinical examination); [a]healthy, [b]ill, [c]survived, [d]died

ship between their ln[GGT] and the ln[IgG] at 24 h after birth (ln[GGT-24] and ln[IgG-24], respectively). The ln[GGT-24] in the healthy, sick, dead and recovered lambs during this period accounted for approximately 51%, 88%, 77%, and 87% of the variation in the ln[IgG-24], respectively (Figure 1, Table 3).

The sick and recovered lambs had a significantly lower SGGTA-24 than the healthy lambs in the period of 5–12 weeks of life. However, there was no significant difference between the dead and healthy lambs' SGGTA-24 in this period. Similar results were found for the SIgGC-24 (Table 4). A significant correlation between the serum SGGTA-24 and the SIgGC-24 in the healthy (R = 0.709, P = 0.000), sick (R = 0.715, P = 0.000), dead (R = 0.689, P = 0.005) and recovered (R = 0.738, P = 0.000) lambs was observed in this period. Additionally, in these groups of lambs, there were significant linear relationships between their ln[GGT-24] and the ln[IgG-24]. The ln[GGT-24)] in the healthy, sick and dead and recovered lambs during this period, accounted for approximately 58%, 67%, 69% and 57%, of the variation in ln[IgG-24], respectively (Table 3).

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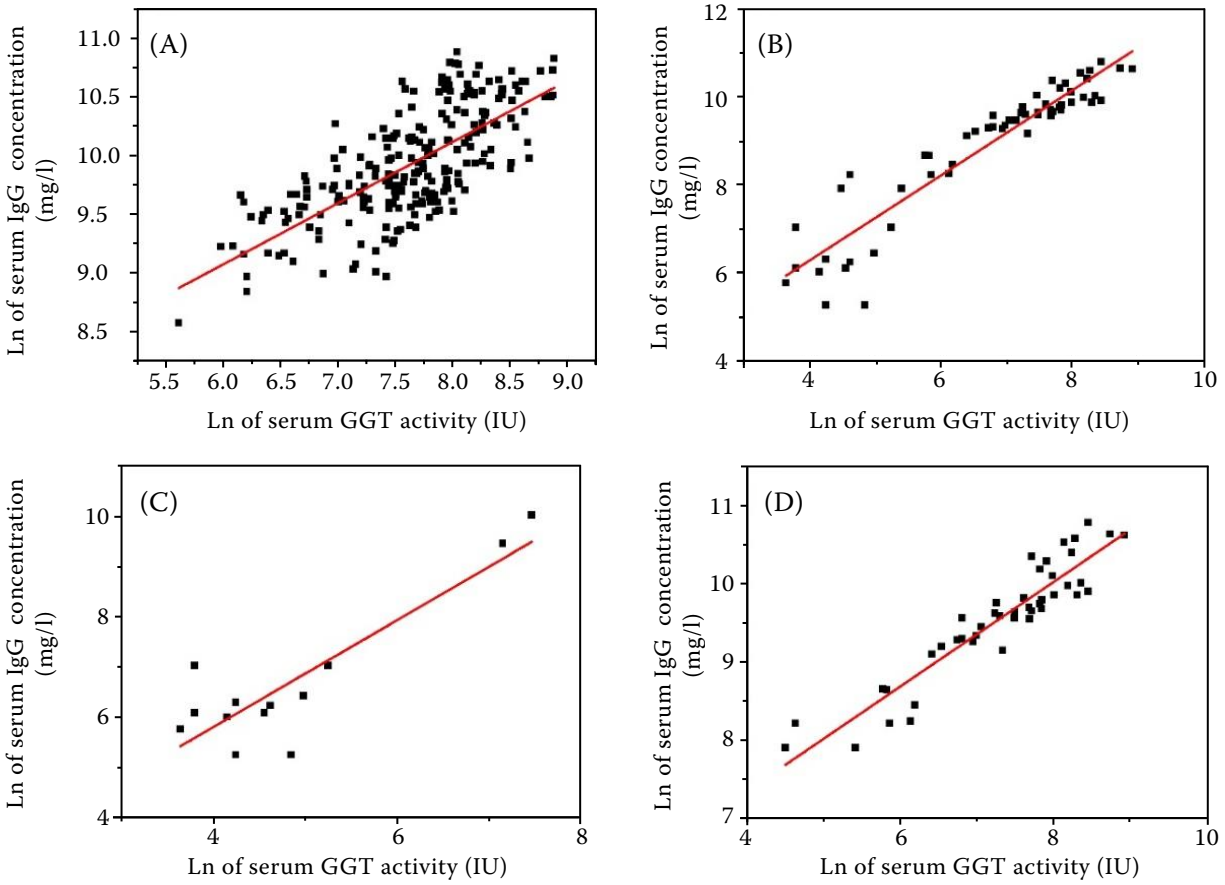

Figure 1. Scatter diagrams illustrating the associations between the ln[GGT-24] and the ln[IgG-24], observed in 243 healthy (A), 57 sick (B), 13 deceased (C), 44 recovered (D) lambs in the neonatal period. In each graph, the solid line represents the best fit for the data, as determined by the means of the simple linear regression

Table 4. Serum GGT enzyme activity and IgG concentrations at 24 h after birth in the lambs categorised as sick and healthy during the first 12 weeks of life[a]

Periods		Health status			
		healthy	ill	outcome after the illness	
				died	recovered
1st week of neonatal period	IgG	22 384 ± 648	4 762 ± 1 071[b***]	543 ± 9.8[b***]	8 622 ± 1 227[b***,c***]
	GGT	2 470 ± 87	512 ± 136[b***]	90 ± 14[b***]	898 ± 206[b***,c**]
n		277	23	11	12
2nd to 4th week of neonatal period	IgG	22 593 ± 642	20 963 ± 2 351	17 692 ± 2 078	21 167 ± 3 342
	GGT	2 458 ± 85	2 553 ± 347	1 516 ± 100	2 618 ± 490
n		243	34	2	32
Neonatal period (1–4 weeks)	IgG	22 594 ± 679	14 432 ± 1 621[b***]	3 194 ± 1 898[b***]	17 764 ± 1 748[b**,c***]
	GGT	2 458 ± 91	1 729 ± 222[b**]	310 ± 151[b***]	2 149 ± 83[c***]
n		243	57	13	44
5th to 12th week	IgG	23 296 ± 682	19 032 ± 1 627[b**]	23 793 ± 1 883	18 165 ± 1 755[b***]
	GGT	2 550 ± 91	2 138 ± 222[b*]	2 340 ± 151	2 101 ± 83[b*]
n		190	97	15	82

[a]Data are presented as the mean ± SE; [b]Significantly different from the healthy lambs ([b***]$P < 0.000\,1$, [b**]$P < 0.01$, [b*]$P < 0.05$); [c]Significantly different from the dead lambs ([c***]$P < 0.001$, [c**]$P < 0.01$)

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Table 5. Lamb morbidity and mortality associated with the various categories of the serum GGT enzyme activity at 24 h after birth (SGGTA-24)

GGT (IU)	Periods							
	neonatal				5 to 12 weeks			
	morbidity		mortality		morbidity		mortality	
Categories	n^1/n^2	%	n^1/n^2	%	n^1/n^2	%	n^1/n^2	%
< 200	13/13	100	11/13	84.6	1/2	50.0	0/2	0.0
201–500	6/14	42.9	0/14	0	10/14	71.4	1/14	7.1
1–500	19/27	70.3	11/27	40.7	11/16	68.8	1/16	6.3
501–1 000	5/31	16.1	0/31	0	10/31	32.3	1/31	3.2
1 001–1 500	7/37	18.9	1/37	2.7	18/36	50.0	3/36	8.3
1 501–2 000	4/49	8.2	1/49	2.1	12/48	25.0	2/48	4.1
2 001–2 500	7/44	15.9	0/44	0	19/44	43.1	1/44	2.3
> 2 500	15/112	13.4	0/112	0	27/112	24.1	7/112	6.3

n^1 = number of diseased or dead lambs in this category; n^2 = total number of lambs in this category

The SGGTA-24 was divided into various categories (Table 5). The neonatal morbidity rates in the lambs with an SGGTA-24 < 500 IU was 3.7 to 8.6 times higher than those with an SGGTA-24 > 500 IU (Table 5). The majority of the lambs with SGGTA-24 levels ≤ 200 IU died (84.6%) while those with levels higher than 200 IU in the neonatal period generally survived (97.9% to 100%) (Table 5). The percentage of the neonatal mortality in the lambs with an SGGTA-24 < 200 IU was 84.6% while this figure varied from 0% to 2.7% for the lambs with an SGGTA-24 above 200 IU. As the SGGTA-24 concentrations increased, the rate of morbidity and mortality decreased for the neonatal period.

The critical threshold of the SGGTA-24 for increased risk of mortality and morbidity in the neonatal period was < 200 IU and < 500 IU, respectively.

The morbidity rates during the 5th–12th week period in the lambs with an SGGTA-24 < 500 IU was 1.4 to 3 times higher than rates in lambs > 500 IU (Table 5). However, no specific SGGTA-24 level associated with an increased risk of death in this period was identified (Table 5).

Effect of colostral GGT activity on health status and passive immunity in lambs

The colostral IgG concentration (CIgGC) (n = 166) varied from 13 886 mg/l to 128 783 mg/l (mean ± SEM, 60 836 ± 1 942.8 mg/l) and the colostral GGT activity (CGGTA) (n = 274) ranged from 1 821 IU to 41 054 IU (15 166 ± 459.8). The correlation between these two parameters were significantly positive (R = 0.261, P = 0.001). Similarly, a significant (P = 0.001) but poor (R^2 = 0.068) linear relationship (Figure 2) between the CIgGC and CGGTA was noted (Regression model):

$$\text{CIgGC} = 4\,809 + 0.083\,7 \times \text{CGGTA} \quad (1)$$

The colostral GGT activity did not significantly differ between the healthy and diseased lambs in both periods of concern (P > 0.05) (Table 6).

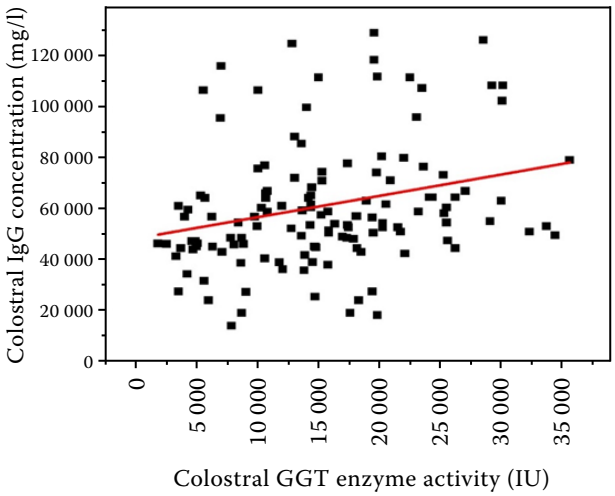

Figure 2. Scatterplots of the colostral IgG and GGT concentrations observed in 166 sheep. In each graph, the solid line represents the best fit for the data, as determined by the means of the simple linear regression

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Table 6. Effect of the colostral GGT (IU) activity (mean ± SE) on the health status of the lambs

Period	Clinical examination			
	healthy	ill	outcome after the illness	
			died	survived
Neonatal	15 483 ± 499	13 255 ± 1 145	11 722 ± 2 318	13 715 ± 1 343
n	235	39	9	30
5 to 12 weeks	15 422 ± 583	15 012 ± 787	15 692 ± 1 854	14 865 ± 874
n	175	90	16	74

DISCUSSION

Appropriate preventative measures rely on the accurate and timely determination of the passive immune status in neonatal lambs and, therefore, they are important in the reduction of morbidity and mortality rates (Gokce and Erdogan 2009; Gokce et al. 2013; Lopreiato et al. 2017; Voigt et al. 2019). This study has demonstrated the accuracy of SGGTA, a test that can be adapted to the field as almost all veterinarians have a blood chemistry analyser for determining the passive immunity.

The present study revealed a relationship between the serum GGT activity and the IgG concentrations in lambs. The mean SGGTA prior to the colostrum intake (0 h), day 1, 2, 4, 7, 14 and 28 in our study were comparable, either lower or higher than those reported for the corresponding days in previous studies (Pauli 1983; Maden et al. 2003; Britti et al. 2005; Massimini et al. 2006; Niine et al. 2018). These different figures found in the SGGTA during the neonatal period in healthy lambs could be attributed to the different measuring methods (Maden et al. 2003; Massimini et al. 2006), the colostral management practices exercised (i.e., the feeding time, amount of colostrum received, quality of colostrum), the sample size, the health status of the lambs (Tessman et al. 1997; Maden et al. 2003; Massimini et al. 2006) in the studies mentioned above. A significant positive correlation existed between the SGGTA and SIgGC determined on day 1, 2, 4, 7 and 14 of the neonatal period in the healthy lambs (Table 2).

Due to marked decline over time, it has been reported that SGGTA is only significant in determining the passive immunity on the first three days of the neonatal period (Maden et al. 2003). In newborn lambs, SGGTA-24 has been reported to be approximately 75 to 145 times greater than the levels prior to the colostrum intake (Britti et al. 2005). Our study also found that the SGGTA-24 levels were 65 times higher than levels prior to the colostrum absorption.

Previously published studies exploring the linear relationship between the SIgGC and SGGTA had limitations. These studies only examined the lambs in the first few days after birth (Britti et al. 2005; Massimini et al. 2006). The accuracy of the regression models created for this purpose were either moderate or insufficient to permit owners and practitioners to predict the PTS in lambs of varying ages (Tessman et al. 1997; Maden et al. 2003). Especially, all of the studies mentioned above only evaluated a small number of healthy lambs (Maden et al. 2003; Britti et al. 2005). However, this study evaluated a large number of lambs and used thorough and frequent clinical examinations over a long period.

Previous studies reported that the ln[GGT] was closely and linearly correlated with the ln[IgG], and that the ln[GGT] accounted for approximately 92% (Massimini et al. 2006), 88% (Britti et al. 2005), 40% (Maden et al. 2003), 52% (Tessman et al. 1997) of the variation in the ln[IgG] in 1-day-old healthy lambs.

In our study, the most accurate result for predicting the SIgGC using the simple regression models was that of day 1 (R^2 = 0.509 to 0.644) in the healthy lambs during the neonatal period (Table 3, Figure 1A). The simple regression models (Table 3) were moderately accurate in predicting the serum IgG concentrations (R^2 = 0.34 to 0.64) and the accuracy of our model in calculating the SIgGC based on the SGGTA was similar to that reported by Tessman et al. (1997), but comparatively higher than that reported by Maden et al. (2003). However, it is fairly lower compared to the study by Massimini et al. (2006). Similarly, the strength (R^2 = 0.77) of the linear relationship between the ln[GGT] and the ln[IgG] in 2-day-

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old lambs was determined by Britti et al. (2005). However, this linear relationship between the two variables was poor (R^2 = 0.37) in the 2-day-old lambs and the 3-day-old lambs (R^2 = 0.23) in our study (Table 3) and in the study by Maden et al. (2003). On the other hand, it is well known that the lamb's small intestine rapidly loses its ability to absorb the colostrum enzyme intact such as the GGT between 24 and 48 h after birth. The reason for the rapid decrease in the serum GGT activity is probably due to its degradation or deactivation (Maden et al. 2003; Britti et al. 2005). A strong correlation between the serum GGT activity and the serum IgG concentration could only be detected within 24 h of the birth in our study. The study by Britti et al. (2005), reported a strong association between the GGT and IgG in 2-day-old lambs; this decrease was observed while the serum GGT activity in day 2 was fairly high compared to other studies (Pauli 1983; Maden et al. 2003) and to our study as well. Furthermore, Britti et al. (2005) showed that the average serum GGT activity determined on day 2 was not notably different from the levels determined on day 1. This could be the reason that a strong linear relationship was identified in the other study (Britti et al. 2005).

Our study identified a moderate linear relationship (R^2 = 0.47) in the multiple regression model established between the serum GGT and the IgG concentrations measured on day 1 and 2 after birth (Table 3) while the study by Britti et al. (2005) found this relationship to be quite strong (R^2 = 0.89). The SGGTA peaks at 24 h following the colostrum absorption, as it did in our study, fell dramatically in the subsequent days of the neonatal period. However, the IgG concentrations generally remained stable during the first 2 weeks and declining levels were seen later. Therefore, a strong linear relationship between the IgG and GGT concentrations was only noted on day 1 of the neonatal period and this relationship weakens as time passes. In previous studies, regression models were generated to predict the PTS as a function of the age and serum GGT activity for lambs on various days of the first 16 days of the neonatal period (Tessman et al. 1997; Maden et al. 2003). The models that were developed in these studies were moderately (R^2 = 0.51 to 0.52) accurate in predicting the SIgGC. Similarly, the multiple regression models developed in our study to estimate the ln[IgG] as a function of the ln(GGT) by using the results on days 1, 2, 4, 7 and 14 in the clinically healthy neonatal lambs was moderately (R^2 = 0.44) accurate in estimating the SIgGC. However, our study showed that the multiple regression model that was the most accurate (R^2 = 0.65) for predicting the SIgGC from the SGGTA was obtained by using the results of the SGGTA and SIgGC on days 0, 1, 2, 4, 7, 14, and 28 in the healthy neonatal lambs. Therefore, it could be said that this multiple regression model was more accurate than those provided in other studies (Tessman et al. 1997; Maden et al. 2003).

The fact that the simple or multiple regression models established between these two variables (SGGTA and SIgGC) in the healthy lambs exhibited extensive or wider variation in our study and other studies could not be explained. However, factors, such as the management practices being used, the difference in sample size, the measurement methods employed, the health status of the lambs at the sampling time of 24 h, and the clinical examination procedures used when creating the groups upon which the regression analysis was performed could explain these different findings. The present study used clinical observations made during daily visits for the duration of the neonatal period to identify the healthy group. Researchers indicated that the diagnostic performance of the ELISA method was better than the SRID for measuring the IgG concentrations. These findings might be explored to explain the differences noted between the studies conducted on this matter.

The relationship between SGGTA and neonatal diseases or death has not been explored in detail previously, and there is no SGGTA threshold used for the risk of morbidity or mortality in lambs. However, this issue has extensively been investigated in calves, thus several threshold cut-off points for the serum GGT activity, ranging from 50 IU to 300 IU, have been suggested for the identification of the FPT in calves (Parish et al. 1997; Wilson et al. 1999). In our study, a linear relationship was determined and these findings disclose that an SGGTA-24 < 500 IU was associated with an increased risk of morbidity in the neonatal period and, therefore, this cut-off value could be used as a predictive function of the passive immunity.

All of the lambs with an SGGTA-24 < 200 IU and the dead lambs had SIgGC-24 values of less than 2 012 mg/l (Table 4). This level was established as the IgG threshold that is associated with an increased risk of mortality in the neonatal period

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(Gokce et al. 2019). These findings emphasise how an SGGTA-24 < 200 IU could be used as the critical threshold for the passive immunity that indicates a high risk of death in the neonatal period.

No study has been performed to calculate the SIgGC-24 based on the SGGTA-24 and, therefore, its accuracy in identifying the passive immunity in sick and dead neonatal lambs. Our study demonstrates that the developed simple regression models used to calculate the ln[IgG-24] from the ln[GGT-24] in the sick and dead lambs in the neonatal period were quite accurate (R^2 = 0.88 and R^2 = 0.77, respectively) and that the GGT enzyme activity could be confidently used as a substitute for the IgG concentrations. In our previous study, we reported that the morbidity risk (95%) in the neonatal period for lambs with an SIgGC-24 < 6 013 mg/l and the mortality risk (100%) in those < 2 012 mg/l was significantly higher than those in a range above these levels and that these levels could safely be used as the risk thresholds for passive immunity that increase the risk of morbidity and mortality (Gokce et al. 2019). In this study, the GGT concentrations were also measured in the same lambs in an effort to determine the passive immunity and its potential for predicting the morbidity. When the SGGTA-24 threshold values that increase the risk of morbidity (< 500 IU) and mortality (< 200 IU) were adapted to the formulas in this study (simple linear regression models calculating the ln[IgG-24] based on the ln[GGT-24] in the lambs that were sick and died during the neonatal period), the equivalent of these same levels in terms of the IgG was calculated to be 4 937 mg/l for the morbidity and 2 692 mg/l for the mortality. This data was similar to the SIgGC-24 risk thresholds in the same lambs in our previous study. There was a close and linear relationship between the GGT activity and the IgG concentrations, the calculation of the SIgGC from the SGGTA based on the formulas obtained from the linear regression analyses appeared to be useful in the sick and dead lambs during the neonatal period. The mortality and morbidity during the neonatal period could be predicted using these formulas by measuring the SGGTA and necessary prevention and control programmes could be developed accordingly.

The colostral GGTA had no marked correlation with the quality of the colostrum, the importance of predicting the passive immune status and the lamb diseases in this study as reported previously (Maden et al. 2003; Britti et al. 2005; Belkasmi et al. 2019). However, some earlier studies reported a highly significant linear correlation between the colostral GGT activity and the IgG concentrations (R^2 = 0.370 to 0.688) in sheep (Maden et al. 2003; Zarrilli et al. 2003; Belkasmi et al. 2019). This was also the fact in our study, but the linear relationship between the colostral GGT and the IgG concentrations was poor (Figure 2). This finding might still point out that the GGT levels could be beneficial in evaluating the colostrum quality. No relationship was established between the CGGTA and the health status of lambs in first 12 weeks of life. However, we previously reported that a low CIgGC was associated with an increased risk of morbidity in this period (Gokce et al. 2019).

SGGTA is often used as an estimate of the circulating IgG concentrations in healthy lambs (Maden et al. 2003; Britti et al. 2005). Therefore, it is useful to assess the PTS of the lambs (Tessman et al. 1997) on the condition that they are not affected with concurrent illnesses or dehydration (Parish et al. 1997). The measurement of the SGGTA is readily available to many practitioners (Tessman et al. 1997). Therefore, it is also adaptable to field use. In addition to this, a direct correlation between the SIgGC and SGGTA in healthy lambs has been reported (Maden et al. 2003; Britti et al. 2005). It has been suggested that SGGTA is a reasonable way to diagnose FPT in individual lambs. The determination of the PTS may help distinguish lambs suffering from FPT due to overwhelming septicaemia from lambs suffering from hypoglycaemia or hypothermia (Parish et al. 1997; Tessman et al. 1997). Our study demonstrates that the SGGTA could be safely used to predict the SIgGC levels 24 h after birth and the passive immune status of both healthy neonate lambs and those that were sick or dead. Such measures taken ahead of time can prevent morbidity and mortality in neonatal lambs caused by FPT. The availability of a quick way to detect lambs with FPT, such as measuring the SGGTA would provide clinicians the opportunity to initiate treatment or take appropriate measures before the intestine's ability to absorb the IgG diminishes and, thus, potentially limit the lamb's exposure to infectious agents. Selecting a sample collection time of 24 h after parturition makes it easy to evaluate the amount of passive transfer and makes treatment possible before infections occur (Massimini et al. 2006). Furthermore, this study has developed a multiple regression model that can

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provide relatively good accuracy (R^2 = 0.65) in calculating the SIgGC from the SGGTA, which varies according to the age throughout the neonatal period. This information is valuable when selecting treatment regimens and making predictions.

In conclusion, this study was the first comprehensive evaluation of the changes in the GGT enzyme activity during the neonatal period in healthy lambs.

Furthermore, the linear regression models that were evaluated with IgG concentrations indicated a model that could calculate the IgG concentrations from the GGT enzyme activity on any day during the neonatal period.

This study identified the potential of serum GGT enzyme activity at 24 h to predict diseases that develops during the neonatal period and the 5th–12th week and the relevant cut-off values. It also achieved good accuracy in determining the SIgGC-24 from the SGGTA in the sick and dead lambs.

Hence, the present study makes useful information available for field veterinarians in terms of confirming an alternative means to measure the serum GGT activity for FPT in individual lambs and the colostrum quality leading to the assistance in the development of passive transfer monitoring programmes.

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Conflict of interest

The authors declare no conflict of interest.

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