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Comparison of four semi-quantitative tests for evaluation of colostrum quality in Saanen goats

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Abstract

The aim of this study was to investigate the efficacy of Brix refractometry and to compare the Brix value with semi-quantitative indicators such as total protein (TP), total protein in whey (TPw), glutaraldehyde coagulation time (GCT) and gamma-glutamyl transferase (GGT) enzyme activity for determining the quality of colostrum. Colostrum samples were collected from 38 Saanen goats just after parturition and on the first day (24 ± 4 hours) and second day (48 ± 4) after parturition. The Brix value, TP and TPw levels were measured with optic refractometers. The level of GCT was determined with a 10% glutaraldehyde solution. Immunoglobulin G (IgG) concentrations were measured with a goat IgG ELISA. All measurements decreased significantly after parturition. The IgG and Brix values on the day of parturition were 4719.28 ± 107.94 mg/dL and 20.55 ± 0.71, respectively. The TPw levels were lower than TP on all three days and a significant difference was detected on day 2. The IgG concentration was higher in the first-parity and second-parity goats compared with those older does. However, no differences were observed in the other characteristics of the colostrum. Litter size did not affect IgG or the other semi-quantitative tests. Correlation coefficients were higher than 0.8 Tp with TPw, and for the Brix value with both TP and TPw. Brix refractometry could be used to evaluate colostrum quality in Saanen goats and TPw is a more reliable indicator than TP.

Keywords: Brix, glutaraldehyde coagulation time, litter size, parity, total protein
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Introduction

Colostrum contains immunoglobulins, vitamins, fat, proteins, cytokines, growth factors and many more molecules. It is an essential nutrient and is vital for survival of newborns in the first days of their lives (Godden *et al.*, 2019). Because the epitheliochorial placental structure of goats does not allow sufficient immunoglobulin transmission from mother to offspring during pregnancy, goat kids are born hypergammaglobulinemic (Argüello *et al.*, 2004; Castro *et al.*, 2009; Rodriguez *et al.*, 2009; Moreno-Indias *et al.*, 2012; Hamad, 2014). For this reason, the passive transfer of immunity via colostrum consumption is crucial in newborn goat kids (Castro *et al.*, 2011; Dywer *et al.*, 2016). Therefore, the most basic way to transfer sufficient immunoglobulins to goat kids is to ensure that they consume high-quality colostrum (Hamad, 2014). Assessment of quality is based mainly on the measurement of IgG concentration (Bartier *et al.*, 2015), which is established by tests such as RID and ELISA (Moreno-Indias *et al.*, 2012; Godden *et al.*, 2019). However, these tests are costly and require laboratories and skilled staff, so in field conditions it is common to use practical devices such as hydrometers, which provide information about the amount of IgG by measuring colostrum density (Rudovsky *et al.*, 2008; Chigerwe & Hagey, 2014; Godden *et al.*, 2019). As a cost-effective and on-site alternative to measuring IgG in colostrum, indirect tests can be used, such as a Brix refractometry (Bielmann *et al.*, 2010; Quigley *et al.*, 2013), measuring GGT enzyme activities (Yalçın *et al.*, 2010; Thompson *et al.*, 2013; Calamari *et al.*, 2015), and determining TP (Castro *et al.*, 2018) and GCT (Yalcın *et al.*, 2010; Thompson *et al.*, 2013; Batmaz *et al.*, 2019). The Brix refractometer is a tool that is used practically at farm level to establish the colostrum quality of the dam and the passive transfer status of the

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newborn (Bielmann *et al.*, 2010; Quigley *et al.*, 2013; Bartier *et al.*, 2015). In recent years, many studies have been conducted using Brix refractometers (Elsohaby *et al.*, 2015; Hernandez *et al.*, 2016; McCranken *et al.*, 2017; Topal *et al.*, 2018). to determine colostrum quality in cows and passive transfer level in calves and in sheep (Torres-Rovira *et al.*, 2017) and dogs (Mila *et al.*, 2015) . But there is a dearth of studies on the use of Brix refractometry to determine passive transfer in goat kids (Oman, 2018; Batmaz *et al.*, 2019) and to evaluate colostrum quality in goats (Hamad, 2014). In the current literature search no study was found that evaluated colostrum quality in Saanen goats with a Brix refractometer. Therefore, the aim of the present study was i) to investigate the effectiveness colostrum of Brix refractometry to determine colostrum quality at various times (first milking, first day and second day post partum), and ii) to compare the Brix value and some semi-quantitative tests (TP, TPw, GCT tests, and GGT enzyme activity) with direct immunoglobulin measurements (IgG).

Material and Methods

This study was approved by Bursa Uludag University Animal Research Local Ethics Committee (2017-04/01). The animal material consisted of 38 Saanen goats at Uludag University Veterinary Faculty Farm, which were fed according to the normal procedures of the farm. All goats gave birth between 7 March and 15 May and were between 1 and 7 lactations (mean = 1.74).

After cleansing and drying the udders, the first milk jets were discarded and 5 mL colostrum samples were collected manually from both udder lobes. Samples were collected of the first milking colostrum (six hours after parturition, on the first day (24 ± 4 hours after parturition) and on the second day (48 ± 4 hours after parturition), therefore three samples were collected from each goat.

Brix values and total protein levels were assessed with an optical refractometer (Atago Sur-Ne Clinical, Japan) from colostrum samples at room temperature. Colostrum whey was obtained by centrifuging colostrum samples at 4500 rpm for 30 minutes. Total protein levels were determined with the same refractometer from the colostrum whey. Samples were diluted in the measurement of colostrum with TP values greater than 12 g/dL.

Colostrum whey GGT was determined with a dry system chemical analyser (Ref 10745081, Reflotron Plus, Roche Diagnostic GmbH, Germany). When GGT activities in colostrum serums were measured, they were diluted 1/10 and the results were multiplied by 10.

Glutaraldehyde coagulation times were evaluated in colostrum whey. For this purpose, coagulation time was determined by adding 0.1 mL 10% glutaraldehyde test solution (Merck, Germany) to 1 mL colostrum whey, similar to the test performed in blood serum (Yalçın *et al.*, 2010; Thompson *et al.,* 2013; Batmaz *et al.,* 2019). The duration of samples that did not coagulate in 60 minutes was recorded as 60 minutes.

Each sample was stored in a freezer at -20 °C until IgG concentrations were measured. Immunoglobulin G concentrations in goat colostrum were measured with Goat IgG ELISA quantitation set (Bethyl Laboratories, Cat No: E50-104, Montgomery, USA) according to the manufacturer's instructions.

Sigma plot 12 software (Systat Software Inc., California, USA) was used for the statistical analyses in this study. The normality of the data was determined by the Shapiro-Wilk test. Spearman's correlation test was applied to calculate the correlation between data. The one-way ANOVA test was used to determine the significance of differences in test days, litter sizes and parity. For all analysis P <0.05 was regarded as significant.

Results and Discussion

The Brix values and levels of IgG, TP, GCT and GGT at the first milking, and on the first and second day colostrum samples are presented in Table 1. The highest *(P* <0.001) IgG level was found in the first milking colostrum. Similarly, the Brix, TP and GGT values were highest in the first milking colostrum. On the other hand, in parallel with the other characteristics, GCT presented the lowest value in the first milking colostrum as a result of its high density. Colostrum IgG concentrations decreased *(P* <0.001) daily in the first and second days after parturition. Parallel with IgG, the highest Brix value was determined as 20.55 ± 0.71 % in the first milking colostrum and there was a significant difference between the days *(P* < 0.001). The TP value in colostrum was highest in the first milking colostrum, and a significant decrease in colostrum TP concentrations *(P* <0.01) was detected on the consecutive days of the study. Although directly measured colostrum TP concentrations were slightly higher than TP concentrations in colostrum whey, there was a significant difference in the two TP measurements only on the second day *(P* <0.001). Coagulation time of GCT in the first milking colostrum of goats was 4.43 ± 2.16 (Table 1). GCT coagulation time differed between the days of the study *(P* <0.001). Whereas the highest GGT activity in colostrum was detected in the first milking colostrum (1071.96 ± 90.99 IU/L) as in other parameters, GGT activity in colostrum decreased

gradually on day 1 and day 2 after parturition. When compared with first milking colostrum only, day 2 GGT level decreased significantly *(P* <0.01).

Table 1 Immunoglobulin G, Brix value, total protein, glutaraldehyde coagulation time, gamma-glutamyl transferase, and total protein in whey in the colostrum of goats (N = 38) after parturition

Characteristic	First milking	Day 1	Day 2
Immunoglobulin G, mg/dL	4719.28 ± 107.94[a]	3731.55 ± 107.08[b]	3105.23 ± 132.20[c]
Brix value	20.55 ± 0.71[a]	14.79 ± 0.42[b]	12.79 ± 0.25[c]
Total protein, g/dL	14.88 ± 0.72[a]	10.40 ± 0.32[b]	9.49 ± 0.31[c]
Total protein in whey, g/dL	14,12 ± 0.65[a]	9.62 ± 0.33[b]	7.48 ± 0.24[c]
Glutaraldehyde coagulation time, min	4.43 ± 2.16[a]	13.71 ± 3.35[b]	43.28 ± 3.80[c]
Gamma-glutamyl transferase, IU/L	1071.96 ± 90.99[a]	1015.19 ± 69.60[ab]	895.40 ± 47.16[b]

[a,b,c] Within a row, values with a common superscript did not differ with probability P <0.01

Colostrum IgG levels of the 1st and 2nd lactation were significantly higher than those in the 3rd and 4th lactation (Table 2). However, no significant (*P* >0.10) differences were found between lactations for Brix value, TP, TPw, GCT, and GGT.

Table 2 Colostrum content of immunoglobulin G, Brix value, total protein, glutaraldehyde coagulation time, gamma-glutamyl transferase and total protein in whey at the first milking of goats of different parities

Characteristic	Parity (number of goats)			
	1 (n = 9)	2 (n = 11)	3 (n = 9)	≥ 4 (n = 9)
Immunoglobulin G, mg/dL	4952.66 ± 244.62[a]	5130.63 ± 219.68[a]	4348.11 ± 96.49[b]	4354.33 ± 134.64[b]
Brix value	21.25 ± 1.33	19.81 ± 1.17	19.98 ± 1.44	21.33 ± 1.90
Total protein, g/dL	15.44 ± 1.46	13.94 ± 1.17	14.11 ± 1.61	16.25 ± 1.68
Total protein in whey, g/dL	15.26 ± 1.34	13.15 ± 0.89	13.44 ± 1.15	14.86 ± 1.86
Glutaraldehyde coagulation time, min	1.16 ± 0.23	1.90 ± 0.69	1.39 ± 0.29	13.85 ± 8.72
Gamma-glutamyl transferase, IU/L	967.37 ± 101.07	1065.62± 170.68	1226.57 ± 199.94	1046.20 ± 273.42

Colostrum quality was similar (*P* >0.10) in does that produced single and twin kids (Table 3). Thirteen of the does in the study gave birth to a single offspring and 23 had twins. Two does had triplets and were excluded from the statistical evaluation because of the low sample size. The mean Brix value IgG, TP, TPw, GCT and GGT levels of these two goats were 25.5 ± 1.10%, 5071.50 ± 1106.5 mg/dL, 19.8 ± 0.2 g/dL, 16.3 ± 2.7 g/dL, 0.58 ± 0.42 minutes, and 930.0 ± 180 IU/L, respectively. There were no differences in IgG and other parameters in goats with different litter sizes.

Table 4 shows the estimates of correlation between colostrum TP, TPw, Brix value, GGT, and GCT for the 114 samples in this study. IgG was correlated significantly with the other characteristics except for GGT. The highest correlation coefficients were found for Brix value with TPw, Brix value with TP, and TP with TPw.

Table 3 Colostrum content of immunoglobulin G, Brix refractometry, total protein, glutaraldehyde coagulation time, gamma-glutamyl transferase and total protein in whey at the first milking of goats of producing single versus twin kids

Characteristic	Litter size	
	Singleton (n = 13)	Twin (n = 23)
Immunoglobulin G, mg/dL	4699.53 ± 132.78	4699.82 ± 146.91
Brix value	19.70 ± 1.20	20.61 ± 0.91
Total protein, g/dL	13.94 ± 1.24	14.99 ± 0.92
Total protein in whey, g/dL	13.21 ± 0.97	14.45 ± 0.89
Glutaraldehyde coagulation time, min	1.49 ± 0.35	1.27 ± 0.32
Gamma-glutamyl transferase, IU/L	1071.09 ± 135.80	1095.93 ± 128.98

Table 4 Estimates of the correlation between measures of colostrum quality for goats (N = 114)

Characteristics	TP	TPw	Brix	GGT	GCT
IgG	0.34*	0.44*	0.45*	0.01	-0.36*
TP		0.86*	0.89*	0.43*	-0.56*
TPw			0.93*	0.52*	-0.65*
Brix value				0.46*	-0.34*
GGT					-0.63*

IgG: immunoglobulin G, TP: total protein, TPw: total protein in whey, GGT: gamma-glutamyl transferase, GCT: glutaraldehyde coagulation time
*Correlation coefficient differs from zero with probability P <0.01

The IgG concentration in the first milking colostrum in the presented study was close to some studies in which first milking colostrum IgG values were measured by ELISA (Rudovsky *et al.*, 2008) and was higher than other studies (Castro *et al.*, 2006; Morena-Indias *et al.*, 2012; Romero *et al.*, 2013; Hernandez-Castellano *et al.*, 2015). This difference may be because of the breeds of goat used in the studies, the physiological characteristics of the animals, and even the measurement of IgG level in laboratories. Although this decline was evident, the presence of high levels of IgG in the colostrum of goats on the first and second days after parturition indicated that a continuation of the colostrum intake by the goat kids would have had beneficial effects. In many of the aforementioned studies, the goat kids were housed with their dams and allowed to nurse. The current study compared colostrum IgG in the first milking and on day 1 and day 2 with Romero *et al.* (2013). Romero *et al.* (2013) observed a larger decrease over time than was observed in this study. The differences in the decreasing trend of IgG may be related to the breed of goat (Saanen versus Murciano-Granadina). In Argüello *et al.* (2006) the decrease in IgG levels over time was also more pronounced than in the current study.

The average Brix value of colostrum from the first milking was 1% higher than the value obtained by Hamad (2014). The difference between the two studies may have resulted from the breed (Saanen versus local race) used in the studies.

Cow colostrum is considered of good quality when Brix value is greater than 21% (Quigley *et al.*, 2013) and 22% (Bielmann *et al.*, 2010). In the current study, the mean Brix value of goat colostrum was close to that of cow colostrum. This can be explained by the similar consistency of the milk (colostrum) of the two species *(*Park *et al.*, 2007; Park, 2010). Cow colostrum with Brix value that is higher than 22% is accepted as being of high quality (Bielmann *et al.*, 2010), but the slightly lower value in goat colostrum may result from being lighter in colour. As a matter of fact, goat's milk is stated to be whiter because goats convert all of the carotene to vitamin A *(*Park *et al.*, 2007; Park, 2010). Although sheep colostrum contains more protein and fat in the first milking compared with that of goats (Castro *et al.*, 2011), in another study on sheep (Torres-Rovira *et al.*, 2017) most of the Brix values were in the range of 16–23%. It has been

suggested that the Brix value should not be less than 18% and should be 21 - 22%. Similar to cow colostrum (Topal *et al.*, 2018), in the colostrum of goats, the Brix values decreased significantly on day 1 and 2 compared with the first milking.

When TP was evaluated in TPw, even though direct TP measurement was close to TPw in the first milking, colostrum TPw value was significantly lower compared with TP, especially on the second day. Thus colostrum contains substances that are richer in protein than colostrum whey. Similar to the current study, Argüello *et al.* (2006) found that the TP value in colostrum decreased gradually in the hours after parturition.

Although the first-milking colostrum whey coagulated in GCT as fast as 4.33 ± 2.11 minutes, coagulation times were significantly longer because of the decrease in colostrum density on the first and second days, as seen in other parameters.

Similarly, GGT in the presuckling colostrum whey was at a high level, and decrease in GGT levels was slower compared with the other parameters. A significant difference compared with the first milking colostrum was not detected until the second day of the study. This indicated that although the colostrum was rich in GGT, there was a significant amount of GGT in milk (Calamari *et al.*, 2015). Although the GGT activity in milk was relatively stable after the colostral period (Calamari *et al.*, 2015), GGT activity was higher in early lactation than in mid lactation (Mohamed, 2014). In fact, cow's milk has high levels of GGT compared with blood serum (Mohamed, 2014).

To increase the number of samples and strengthen statistical significance, the correlation between the IgG and semi-quantitative tests was evaluated by combining the data of all days. According to this analysis, the correlation coefficient of TPw with IgG was higher than that of TP (Table 4). When all the results were examined, IgG showed the highest correlation, especially with Brix value and TPw. Bielmann *et al.* (2010) estimated a correlation between IgG and the Brix value of 0.96 in colostrum of cattle. This is higher than the 0.45 value the authors obtained in the current study and may be related to the species. In line with the results in the current study, Castro *et al.* (2018) found a significant correlation between TP and IgG.

In studies on goats (Argüello *et al.*, 2006; Hamad, 2014), it was stated that litter size and parity did not affect the quality of colostrum. The present study showed that colostrum IgG levels were higher in the goats that entered the first and second lactations compared with the third and higher lactations. This finding was consistent with the study in which Romero *et al.* (2013) stated that colostrum quality was higher in primiparous goats. In this study, high colostrum IgG concentration in primiparous goats was associated with component concentration because of lower milk yield of primiparous animals. However, in the present study, although IgG levels were higher in animals in the first and second lactation, the expected increase in component concentration in other parameters was not ascertained. This indicated that high IgG levels in the first two lactations may be caused by a factor other than component concentration. Safayi *et al.* (2010) found that in early lactation lumen area, alveolar proportions and the ratio of micro vessel to interstitial tissue in udders were higher in primiparous goats than in multiparous ones. Because IgG is probably transported by transcytosis, these differences in udder tissue may be another factor that could be associated with higher colostrum IgG concentrations in younger animals.

Another parameter in the present study was the relationship between litter size and colostrum quality. In accordance with Argüello *et al.* (2006), no relationship was found between litter size and IgG and other semi-quantitative parameters. In contrast, Hamad (2014) evaluated the quality of colostrum in a domestic goat breed with a Brix refractometer, and reported that colostrum quality was higher in twin-bearing multiparous goats compared with single offspring. These differences may be related to the goat breed used in the studies and accordingly to the milk yield and varied milk contents.

Conclusion

Digital Brix refractometers can be used to determine colostrum quality in goats. The Brix value, TP, and TPw were more significant in the first milking colostrum, but GCT and GGT were significant in the first two days after parturition.

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Authors' contributions

HB, ZM, YK and OT conceived the research, helped in interpreting the data and participated in manuscript drafting. OT and YK collected samples and analysed the samples. ZM and YK performed the statistical analyses. All authors read and approved the final manuscript.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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