

Evaluation of passive transfer in goat kids with Brix refractometer and comparison with other semiquantitative tests

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Received: 13.05.2019 • Accepted/Published Online: 11.09.2019 • Final Version: 02.10.2019

Abstract: The aim of this study was to evaluate the passive transfer (PT) status of goat kids by Brix refractometry and compare the results with other semiquantitative tests (total protein - TP, glutaraldehyde coagulation test - GCT, and gamma-glutamyltransferase - GGT) and immunoglobulin G (IgG). The study was conducted on 75 goat kids born from 47 Saanen goats. The blood samples were collected from the kids on day 0 (presuckling) and on the 1st, 2nd, and 3rd days after birth. The Brix% and TP concentrations were measured with refractometers, and GGT activity was measured using a dry chemistry system. The duration of the GCT was determined in the first 60 min. The serum IgG concentration was measured by goat IgG ELISA kit. On the 1st and 2nd days, serum Brix% in the kids was measured as $9.33 \pm 0.17\%$ and $9.17 \pm 0.14\%$, respectively. In the 1st and 2nd day serum samples of the kids, IgG was 817.76 ± 37.34 mg/dL and 1173.29 ± 47.81 mg/dL, respectively, and GGT was 1298.07 ± 133.29 IU/L and 692.26 ± 79.86 IU/L, respectively. The Brix refractometer was found to be more sensitive for detection of PT status in kids on the first and second days after birth, such as TP and GCT, whereas GGT, as an early indicator of PT, was useful only on the first day after birth. We conclude that the Brix refractometer could be used to determine the PT status in goat kids and Brix measurements lower than 8.6%, 9.2%, and 9.3% indicate failure of PT in 1-, 2-, and 3-day-old kids, respectively.

Key words: Goat kid, passive transfer, Brix refractometer, semiquantitative tests, immunoglobulin G

1. Introduction

Goat kids are born hypogammaglobulinemic due to the epitheliochorial placenta [1,2]; therefore, the passive transfer (PT) of immunity from the colostrum they consume is very important [3,4]. The main factors affecting PT are birth weight, number of offspring, sex of the newborn, the amount and quality of colostrum ingested, and the time of first feeding [1,5]. In addition, factors such as nutrient intake of dam during pregnancy and dystocia could affect PT by delaying the standing up and first feeding in newborn [4].

Failure of passive transfer of immunity (FPT) is reported to increase morbidity and mortality rates in the first 6–7 weeks of life and the risk of mortality is greatly increased in the first 4 days [6]. Although there are sources indicating that a goat kid should consume colostrum corresponding to approximately 10%–20% of their body weight after birth in order to reach an adequate level of PT [7,8], it has also been reported that the serum immunoglobulin G (IgG) threshold needs to be at or above 1200 mg/dL in order to minimize morbidity and mortality rates during

the neonatal period [9]. However, in other studies the IgG threshold was reported to be 800 mg/dL in the first 24 h [7,10]. For these reasons, PT is very important in newborn goat kids, and it is necessary to achieve adequate levels. The concentration of IgG in the serum of newborn ruminants is an important indicator for determining passive transfer failure [11,12]. One of the criteria for high concentrations of serum IgG in newborn goat kids is the amount of IgG that is contained in the colostrum they are consuming [2,13,14]. The concentration of colostrum and serum IgG can be determined using RID and ELISA [2,15,16]. These tests are associated with high costs and specific laboratory requirements. Tests such as serum total protein (TP), gamma-glutamyltransferase (GGT) activity, zinc-sulfate turbidity tests, and glutaraldehyde coagulation tests (GCTs) are economical and practical applications to determine the PT status in newborn ruminants [9,12,17,18].

In recent years, there have been many studies that have evaluated the use of Brix refractometers for determining PT level in calves [19–22]. Brix refractometry is not as widely studied in small ruminants as in cattle. Torres-

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Rovira et al. [23] studied Brix refractometry in sheep. Recent studies with Brix refractometry conducted in goat kids [24] and goat colostrum [25] indicated that Brix refractometry could potentially be used as an on-farm tool for estimating PT of immunity and colostrum quality status of goats. On the other hand, along with evaluation of usefulness of Brix refractometry for determination of PT status in goat kids, the aim of the present study was also to use other semiquantitative tests to evaluate PT in newborn kids based on serum IgG. We achieved this by (1) collecting serum samples at different times (presuckling, 1st, 2nd, and 3rd day after birth) in order to determine the best timing for using Brix for the evaluation of PT and (2) using other semiquantitative tests (TP, GCT, and GGT) to compare the results with Brix measurements.

2. Materials and methods

Seventy-five kids born to 47 Saanen goats were used in this study. Thirteen of the kids were singletons, 50 were twins, and 12 were triplets, born between March and May on the same farm. Colostrum (5 mL) was collected from the dams within 6 h after parturition for the evaluation of colostrum quality. The mean lactation number of the goats was 1.74 (range: 1–7 lactations). Kids were housed with their dams and were allowed to suckle freely [1,16]. Blood samples were collected from the kids at latest 30 min after birth before colostrum intake (presuckling), and 1, 2, and 3 days after birth. Blood samples collected from the goat kids were centrifuged at 3000 rpm for 5 min to obtain sera. Serum samples were analyzed using a digital Brix refractometer (Milwaukee MA882, Hungary), total protein was analyzed with an optical refractometer (Atago Sur-Ne Clinical, Japan), GGT concentrations were measured with a dry system chemical analyzer (Ref: 10745081; Reflotron Plus, Roche Diagnostics GmbH, Germany), and coagulation times were measured with 10% glutaraldehyde solution (G6257, Merck, USA). For the coagulation test, 0.05 mL of 10% glutaraldehyde solution was added to 0.5 mL of serum, and the coagulation time was determined within 60 min [17,18]. Similarly, the duration for the noncoagulated samples was presumed to be over 60 min. Serum samples were stored at -20°C until IgG analysis. The IgG concentrations were determined by a commercial ELISA kit (Goat IgG ELISA Quantitation Set, Cat No: E50-104, Bethyl Laboratories, USA).

Based on the results of previous studies the IgG threshold for FPT in our study was set as 800 mg/dL [7,10]. Kids with serum IgG levels lower than 800 mg/dL were considered to be suffering from FPT.

The first milking colostrum samples were centrifuged at 4500 rpm for 30 min to obtain the colostrum serum. Colostrum IgG levels were determined from colostrum sera using an ELISA kit (Goat IgG ELISA Quantitation Set, Cat No: E50-104, Bethyl Laboratories, USA).

Sigma Plot 12 software was used for the statistical analyses in this study. The normality of the data was determined by Shapiro–Wilk test. Spearman's correlation test was applied to calculate the correlation between data obtained from serum samples of the kids. The one-way repeated measures ANOVA test was used to determine whether there was a difference between the values of the same parameters on different days. A receiver operating characteristic (ROC) curve analysis was performed using MedCalc 14 software. Cut-off values were determined using the Youden index, and the sensitivity, specificity, positive and negative predictive values, and accuracy were determined using 2×2 tables according to the cut-off values. For all analyses, $P < 0.05$ was determined to be significant.

3. Results

Mean IgG concentration of first milking colostrum samples was measured as 4757.53 ± 121.61 mg/dL.

FPT was detected in 51.47%, 16.00%, and 2.70% of goat kids on the 1st, 2nd, and 3rd days, respectively, when the cut-off value of IgG was set as <800 mg/dL. Table 1 shows the IgG, Brix%, TP, GGT, and GCT levels in blood serums of kids at 4 different sampling times.

Kids were hypogammaglobulinemic according to serum IgG levels before colostrum intake (Table 1). IgG levels increased significantly on days 1, 2, and 3 ($P < 0.01$). Brix% and TP levels in the first three days after birth significantly increased when compared to those before suckling ($P < 0.01$). The GCT duration was also shorter during the first 3 days after suckling compared to the duration before suckling ($P < 0.01$). GGT level also increased significantly on the 1st day after suckling and then decreased on the 2nd and 3rd days ($P < 0.01$).

The correlations between IgG and the semiquantitative tests for Brix%, TP, GCT, and GGT levels at 1, 2, and 3 days after birth are shown in Table 2. Brix% and TP were only positively correlated with IgG at 1 ($P < 0.001$) and 2 days ($P < 0.05$) after suckling. GCT was negatively correlated during the same period. GGT was only correlated with IgG on the 1st day after suckling ($P < 0.001$). The correlation coefficients of IgG with other tests were found to be moderate ($r = 0.38$ – 0.54) on day 1 and low ($r = 0.23$ – 0.26) on day 2.

The characteristics of the semiquantitative tests were calculated based on the premise that FPT in kids occurs at a level of IgG of <800 mg/dL by using ROC curves and the Youden index (Figures 1a–1d). Table 3 presents the test characteristics and the cut-off values of Brix%, TP, GCT, and GGT levels on days 1, 2, and 3.

The sensitivity and negative predictive value (NPV) of Brix were found to be highest on day 2, and specificity,

Table 1. Serum IgG, Brix%, TP, GCT, and GGT levels in goat kids on the different days of the study.

Parameter	Presuckling n = 52*	Day 1, n = 75**	Day 2, n = 75**	Day 3, n = 75**
IgG (mg/dL)	173.65 ± 17.90 ^a	817.76 ± 37.34 ^b	1173.29 ± 47.81 ^c	1131.85 ± 39.30 ^c
Brix%	6.86 ± 0.07 ^a	9.33 ± 0.17 ^b	9.17 ± 0.14 ^b	9.05 ± 0.15 ^b
TP (g/dL)	3.81 ± 0.06 ^a	6.00 ± 0.15 ^b	5.87 ± 0.13 ^b	5.71 ± 0.12 ^b
GCT (min)	60.00 ± 0.00 ^a	15.24 ± 2.84 ^b	11.98 ± 2.41 ^b	10.89 ± 2.34 ^b
GGT (U/L)	149.70 ± 38.12 ^a	1298.07 ± 133.29 ^b	692.26 ± 79.86 ^c	449.22 ± 40.13 ^d

*GGT n =35, **GGT n = 42.

Significant difference (P < 0.01) between parameters with different letters in the same row.

Table 2. Correlation coefficients (r) between IgG, Brix%, TP, GCT, and GGT on days 1, 2, and 3 after birth.

	Day	Brix%	TP	GCT	GGT
IgG	1	0.43***	0.44***	-0.38***	0.54***
	2	0.25*	0.23*	-0.26*	0.14
	3	0.00	0.02	-0.04	0.23

*P < 0.05, ***P < 0.001.

positive predictive value (PPV), and accuracy on day 1. Cut-off values for TP did not differ significantly between the different days. The sensitivity, specificity, and accuracy values of TP were calculated to be close to each other between days 1, 2, and 3, and the highest calculated accuracy level was detected as 72.97% on the 3rd day. The highest sensitivity, specificity, and accuracy values of GCT were detected on day 2. Because the highest level of serum GGT was seen on day 1 (as shown in Table 1), the cut-off specificity and PPV were calculated as being highest on day 1. However, the highest accuracy for GGT was detected on day 3.

4. Discussion

In the present study, the highest rate of FPT (51.47%) was detected on the 1st day, based on serum IgG concentrations from goat kids. Lower FPT rates seen on the second (16.00%) and third (2.70%) days indicate that immunoglobulins in the colostrum continue to be absorbed by the intestines after 24 h. A similar study conducted in goat kids had similar results [18], where serum IgG concentrations were double on day 4 when compared to day 1 after birth. Furthermore, the serum immunoglobulin levels in calves were reported to be highest at 36 h [11], and even higher immunoglobulin levels have been reported on the 2nd and 3rd days after birth [26]. Colostrum quality of goats

in the present study was determined by measurement of IgG and was determined as 4757.53 ± 121.61 mg/dL. This concentration was close to the IgG levels reported (4900.1 ± 260.4 mg/dL) in the colostrum from the first milking in goats by ELISA [27], higher than IgG levels reported in other studies [2,20,28], and lower than in another study conducted on goats [25]. The different results obtained in these studies may be attributed to the breed of animals used, physiological characteristics of the animals, or even laboratories.

In the present study, Brix% results of goats kids on day one are similar to the results of Oman et al. [24]. Furthermore, in the present study it was observed that, similar to a study conducted in calves [22], Brix% and TP levels were higher in the first 3 days when compared to presuckling levels. The higher Brix% and TP levels compared to IgG on the first day could be related to the absorption of solids other than immunoglobulin from the colostrum as colostrum is reported to contain 7%–13% fat, 4%–10% nonimmunoglobulin proteins, and 2%–5% lactose [4]. Due to the increased immunoglobulin concentration, GCT coagulation time was shorter in the first 3 days when compared to the presuckling levels. This result is consistent with the results of another study [18] conducted in goat kids, in which the shortest coagulation time was measured on day 4 after birth. GGT enzyme activity also reached the highest level on day 1 and decreased between days 2 and 3 (P < 0.01). The rapid decrease in GGT after the first day is similar to the results from other studies of goat kids [18], lambs [12], and calves [22,29]. Thus, we can suggest that among the semiquantitative tests, GGT is an early indicator for determining FPT in goat kids.

A positive correlation was detected between Brix% and IgG on day 1 (P < 0.001) and on day 2 (P < 0.05), as reported for calves [19–21]. Brix% can be used to evaluate PT status in goat kids, and the best timing for this evaluation is on the 1st and 2nd days after birth. TP was also found to be correlated with IgG in the same manner as Brix%. Although the correlation between GCT and

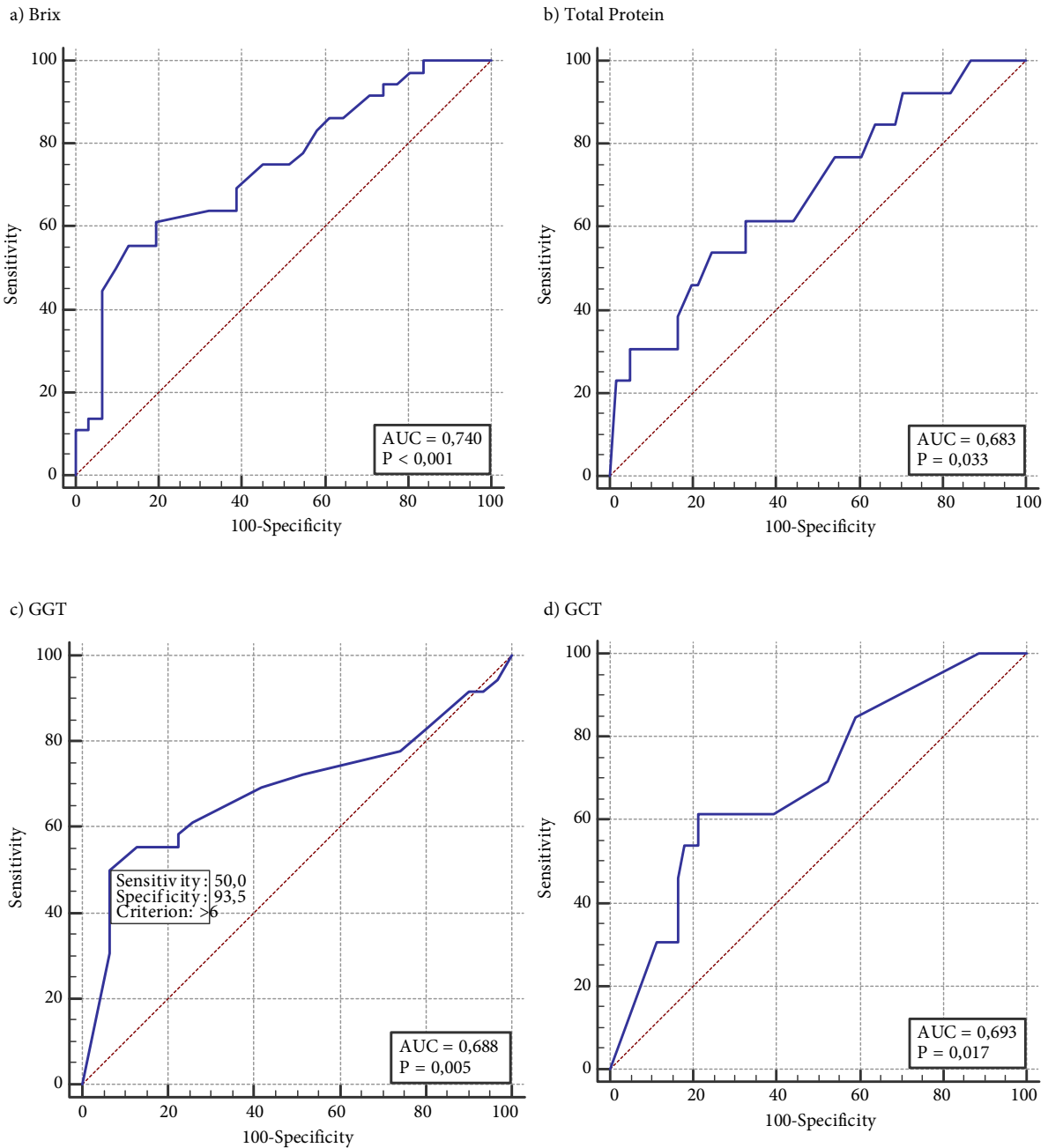


Figure 1. ROC curves on day one for a) Brix (cut-off 8.6%), b) TP (cut-off 5.3 g/dL), c) GGT (cut-off 890 IU/L), and d) GCT (cut-off 2 min).

IgG was not significant, it was found to be higher than in another study [18]. GGT was positively correlated with IgG on day 1 ($r = 0.54, P < 0.001$) and this result is similar to results obtained from lambs ($r = 0.63, P = 0.001$) [12] and higher than the correlation between the 1st day GGT and IgG obtained ($r = 0.24, P < 0.05$) in a different study that was also conducted on goat kids [18]. Thus, it could be stated that GGT is a useful tool for determining the PT status in 1-day-old goat kids.

In test characteristics, the cut-off value of Brix% was determined to be 8.6%, 9.2%, and 9.3% on day 1, 2, and 3, respectively. The cut-off value for Brix% was calculated as 8.5%–8.6% [20] in one study conducted in calves, and 8.6% on the 1st and 9.0% on the 3rd day in another study [22]. Thus, Brix% cut-off values in goat kids were close to the cut-off values calculated for calves. In the present study, the highest sensitivity rate (84.62%) of Brix% was detected on day 2, and the highest specificity (87.50%)

Table 3. Test characteristics of Brix%, TP, GGT, and GCT on different days after birth.

	Days after birth	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Brix (%)	1	8.6	37.25	87.50	90.5	30.4	70.15
	2	9.2	84.62	52.46	27.5	94.1	59.46
	3	9.3	68.75	46.55	26.2	84.4	50.00
Total Protein (g/dL)	1	5.3	35.29	87.50	90.0	29.8	69.70
	2	5.2	53.85	75.41	31.8	88.5	68.92
	3	5.1	43.75	74.14	31.8	82.7	72.97
GGT (IU/L)	1	890	53.33	81.82	88.9	39.1	63.41
	2	448	71.43	64.10	26.3	92.6	65.22
	3	206	33.33	93.94	60.0	83.8	85.71
GCT (min)	1	2	50.98	75.00	86.7	32.4	69.70
	2	5	61.54	78.69	38.1	90.6	74.32
	3	0.5	31.25	84.48	35.7	81.7	21.26

and accuracy (70.15%) were detected on day 1. Although the sensitivity, specificity, and accuracy (%) were the highest on the 3rd day in a different study that evaluated calves on the 1st and 3rd days [22], the results from the first day of that study were similar (78.33%) to the results in the present study. According to these results, a Brix refractometer can be used on the 1st and 2nd days after birth to determine the PT status of goat kids. The cut-off value for TP in the first 3 days was calculated to be between 5.1 and 5.3 g/dL. Similarly, the cut-off value with the highest specificity for calves was reported as 5.2 g/dL [30]. As shown in Table 3, the sensitivity and specificity values of TP were different on different days of the study, and the accuracy (%) values were very similar on the different days of this study (69.70%, 68.92%, and 72.97%, respectively).

The cut-off value of the GCT in the first 3 days measured between 0.5 and 5 min (Table 3). Although the cut-off value was very low on day 3, the sensitivity and accuracy rates were also low. However, the values on days 1 and 2 were higher and accuracy was 69.70% and 74.32%, respectively. The cut-off value of GGT was calculated as 890, 448, and 206 IU/L on day 1, 2, and 3, respectively. Although the accuracy (%) was highest (85.71%) on the

3rd day, the sensitivity was lowest (33.33%). However, sensitivity, specificity, and accuracy were more compatible with each other on days 1 and 2. Maden et al. [12] detected a PPV of 100% for GGT in lambs. Similarly, PPV for GGT was found to be 88.9% in our study. According to this, GGT, which may be a significant early indicator for determining the PT status in calves [20] in the first 2 days, can be recommended to evaluate GGT on the 3rd day, when the accuracy value is high. Additionally, it has also been reported that GGT may be useful in the first 3 days after birth for determining PT status in lambs [12].

In conclusion, the digital Brix refractometer could be used to assess the PT status in goat kids. Brix refractometry was found to be more reliable on days 1 and 2 as an indicator of FPT, similar to total protein and GCT. However, GGT is valuable as an early indicator on day 1. Brix measurements lower than 8.6%, 9.2%, and 9.3% indicate FPT in 1-, 2-, and 3-day-old kids, respectively.

Acknowledgments

This study was financially supported by the Research Fund of Bursa Uludağ University (Grant No: HDP (V)-2017-26). This study was approved by the Bursa Uludağ University Animal Research Local Ethics Committee (2017-04/01).

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