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Comparison of IgG and semiquantitative tests for evaluation of passive transfer immunity in calves

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Abstract: Serum immunoglobulin (IgG) and semiquantitative tests are used for the evaluation of passive transfer immunity (PTI) in calves. We aimed to evaluate PTI in calves by using a Brix refractometer, total protein (TP), gamma-glutamyl transferase (GGT), and glutaraldehyde coagulation test (GCT) on days 1, 3, and 7 after birth; to compare the results with serum IgG; and to evaluate which day these tests are given on will give the best results. The blood samples were collected from 60 Holstein dairy calves on day 0, just after birth, and on the 1st, 3rd, and 7th days after birth. The serum IgG concentration was measured by ELISA, the Brix % and TP concentrations with refractometers, and GGT activity using a dry chemistry system. The duration of the GCT was determined in the first 60 min. The IgG, TP concentration, and Brix % all peaked on the 3rd day of the study. GGT showed a significant decline after 24 h. Evaluating the Brix %, IgG, TP, and GCT levels more than 24 h after birth gives better results. However, GGT activity was observed as an early indicator of failure of passive transfer, as the GGT levels were highest 24 h after birth.

Key words: Calf, immunity, Brix refractometer, total protein, gamma-glutamyl transferase, glutaraldehyde coagulation test

1. Introduction

Calves are born hypogammaglobulinemic because cows have an epitheliochorial placenta. Therefore, to prevent infectious diseases, calves should receive colostrum with high immunoglobulin (Ig) concentrations and low bacterial contamination as soon as possible after birth. A failure of passive transfer (FPT) develops in calves that do not drink enough high-quality colostrum after birth. The risk of infectious diseases is very high in calves with FPT (1,2). Therefore, evaluating the passive transfer status of calves is crucial for their proper management; 24–48 h after birth is the most suitable timeframe for assessing the passive transfer status in calves (3).

The gold standard method for assessing the status of passive transfer in calves is to measure immunoglobulin in the serum. The serum IgG levels are generally evaluated because it is the most abundant fraction of Ig (4). IgG is determined by RID, TIA, or ELISA assays. However, these methods are expensive and require laboratory analysis, and are thus not widely used in practice (3,4). Hence, more practical tests are widely used to evaluate the passive transfer status in calves (5). Among these tests, total protein (TP) (5) and the glutaraldehyde coagulation test (GCT) (6,7) are easily applicable tests in field conditions. In contrast, measuring the GGT activity in calf serum and the $ZnSO_4$ turbidity test can be performed more simply and rapidly in laboratory conditions (3,8,9).

The GCT is a method used for semiquantitative determination of serum fibrinogen and immunoglobulin concentrations (10). It is based on determination of time in which gel formation occurs related to the reaction between blood fibrinogen, immunoglobulin, and aldehyde groups of the reagent (10). In adult cattle, testing is done by mixing equal amounts of 1.25% glutaraldehyde solution with blood collected in tubes containing EDTA (10,11). In calves, the test procedure is to mix 10% glutaraldehyde solution with a 10 times greater volume of serum (6,7). Several studies have investigated the association between GCT and IgG, and other semiquantitative tests have been used for evaluation of FPT (12,13). Significant association between IgG and GCT in calves of 1–8 days old has been reported (1,13); however, in the same study, it was concluded that relevance between the two parameters is negligible (13).

The Brix refractometer has been used as a practical device to evaluate passive transfer status in calves in recent years (4,14–16). Both the passive transfer status of the calf and the colostrum quality in the mean immunoglobulin

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levels can be determined by using simple and economical devices in field conditions (17–20). Studies that used Brix refractometers to evaluate passive transfer status in calf serum IgG and total protein results were compared (4,14). In this study, we aimed to evaluate the practical semiquantitative tests by using a Brix refractometer, TP, gamma-glutamyl transferase (GGT), and the GCT on days 1, 3, and 7 after birth; to compare the results with serum IgG; and to evaluate on what day these tests give the best results.

2. Materials and methods

The material for this study consisted of 60 calves (with two twin sets) born between January and December from 58 Holstein cows aged 2.5–7 years on a farm. The mean birth weight of 27 male and 33 female calves was 39.41 ± 0.62 kg. According to the farm management program, 2–2.5 L of colostrum was offered to calves by the employees within 3 h after birth. They received 2.5 L of freshly milked colostrum from their dams twice a day in the first three days of life. Quality of colostrum was measured by using a Brix refractometer in 27 cows.

Blood samples were collected before the calves received colostrum (at birth) and on the 1st (24 ± 4 h after birth), 3rd, and 7th days. The blood samples were centrifuged at 5000 rpm for 5 min within 4 h of sample collection.

Serum samples were analyzed using a digital Brix refractometer (Milwaukee MA882, Hungary), TP with an optical refractometer (Atago Sur-Ne Clinical, Japan), GGT concentrations with a dry system chemical analyzer (Ref: 10745081; Reflotron Plus, Roche Diagnostics GmbH, Germany), and coagulation time with 10% glutaraldehyde solution (Merck, Germany). For the coagulation test of glutaraldehyde, 0.05 mL of 10% glutaraldehyde solution was added to 0.5 mL of serum, and the coagulation times were determined within 60 min (6,7). Similarly, the duration of the noncoagulated samples was calculated over 60 min. Other amounts of separated serum samples were stored at –20 °C in a deep-freezer. The IgG concentrations of the obtained serum samples were determined by ELISA (Bio-X Diagnostics, ELISA Kit for Bovine Immunoglobulin Assays, Belgium).

The IgG threshold for the FPT in our study was determined as IgG of >10 g/L, which is similar to the thresholds of previous studies (4,14,15,21).

The IBM SPSS Statistic 22 program was used for the statistical data obtained in the 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 Friedman test was used to determine whether there was a difference between the values of the same parameter on different days. Receiver operating characteristic (ROC) curve analysis was performed using MedCalc. Cut-off values were determined with 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 analysis, $P < 0.05$ was determined to be significant.

3. Results

In this study, FPT was detected in 33.33%, 30.00%, and 36.66% of the calves on days 1, 3, and 7, respectively. The average Brix of 27 colostrum samples was 28.43 ± 1.16%. The serum IgG, TP, and GGT levels as well as the GCT time and Brix measurements of 60 calves on days 0, 1, 3, and 7 of the study are given in Table 1.

Along with IgG, all the other tests indicate FPT in samples collected before colostrum feeding (Table 1).

Table 1. Immunoglobulin G (IgG), total protein (TP), gamma-glutamyl transferase (GGT), glutaraldehyde coagulation test (GCT), and Brix measurements of calves in the first 7 days of the study.

Parameter	n	Day 0	Day 1	Day 3	Day 7
IgG (g/L)	51	1.26 ± 0.66^{a}	12.45 ± 0.88^{bcd}	13.44 ± 0.87^{cb}	12.35 ± 0.95^{db}
	Min–max	0.92–3.49	1.20–45.76	2.00–36.50	1.88–47.27
Total protein (g/dL)	60	4.14 ± 0.06^{a}	5.86 ± 9.60^{bde}	6.21 ± 0.12^{c}	5.99 ± 0.11^{db}
	Min–max	3.20–5.30	3.80–9.60	4.00–9.00	4.40–8.80
GGT (IU/L)	60	12.13 ± 2.28^{a}	2242.07 ± 183.38^{b}	1016.65 ± 87.01^{c}	505.04 ± 43.00^{d}
	Min–max	5.00–87.00	7.49–6240.00	53.90–3430.00	37.60–1540.00
GCT (min)	60	59.41 ± 0.50^{af}	9.91 ± 2.20^{b}	5.87 ± 1.45^{cde}	5.15 ± 1.08^{dce}
	Min–max	35.00–60.00	0.58–60.00	0.85–60.00	0.83–45.50
Brix (%)	60	7.34 ± 0.08^{a}	9.20 ± 0.15^{be}	9.61 ± 0.16^{cd}	9.55 ± 0.14^{dc}
	Min–max	5.50–9.00	6.70–13.50	7.00–12.90	7.50–14.00

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

IgG and TP levels peaked on day 3 of the study (Table 1). Parallel to IgG and TP, the GCT time was shorter on day 3 when compared to day 1. Similarly, the Brix % measurements were highest on day 3. In contrast, the GGT levels peaked on day 1. The mean GCT time was 9.91 ± 2.2 min on day 1, and it ranged from 5.15 ± 1.08 min to 5.87 ± 1.45 min between days.

3.1. Correlations

Significant correlations were detected between Brix and the other parameters, and between each of the other parameters (Table 2). On day 1 of the study, all parameters were positively correlated with each other and negatively correlated with GCT (Table 2). Similar correlations were detected between the parameters on days 3 and 7. On day 0, a positive correlation was detected between Brix and TP ($r = 0.71$, $P < 0.001$) and between IgG and GGT ($r = 0.36$, $P = 0.009$); a negative correlation between IgG and GCT ($r = –0.302$, $P = 0.031$) was detected on day 0.

As shown in Table 2, on day 1, the correlations were positive, with correlation coefficients between Brix and IgG of 0.67 ($P < 0.001$), between Brix and GGT of 0.56 ($P < 0.001$), and between Brix and TP of 0.79 ($P < 0.001$). In contrast, the correlation between Brix and GCT was negative ($r = –0.73$, $P < 0.001$) on the same day (Table 2). IgG was positively correlated with TP and GGT on day 1 (Table 2), but the highest correlation ($r = –0.86$, $P < 0.001$) on day 1 was detected between TP and GCT. The correlations between Brix and IgG and Brix and TP were more pronounced on day 3 compared to day 1.

On the seventh day of our study, a higher correlation was observed between Brix and IgG compared to day 1. The correlation between Brix and GGT was positive ($r = 0.60$, $P < 0.001$) and that between Brix and GCT was negative ($r = –0.75$, $P < 0.001$) on day 3 (Table 2). The means of all parameters except GGT were highest on day 3. Similarly, the correlations among IgG, Brix, and TP were more significant on day 3 compared to day 1, which indicated that measuring these parameters on day 3 is more reliable for detecting FPT. The correlations between Brix and GGT and Brix and GCT were similar on days 1 and 3. The correlations between Brix and IgG and TP and GCT were similar on days 3 and 7; however, the correlation between Brix and GGT was lower on day 7. This could have been related to a significant decrease in GGT activity after day 1. At the same time, the correlations between GGT and IgG and TP and GCT were lower on day 7.

3.2. Test characteristics

Test characteristics were calculated based on IgG of <10 g/L indicating FPT. In Table 3, the test characteristics of Brix % calculated according to the different cut-off values are presented. The cut-off values for Brix on days 1, 3, and 7 were calculated as 8.6%, 9.0%, and 9.4%, respectively.

The cut-off values for serum TP on days 1, 3, and 7 were calculated as 5.2 g/dL, 6.1 g/dL, and 5.8 g/dL, respectively (Table 4). Test characteristics of GGT and GCT on different days were demonstrated for the first time in the present study. The sensitivity, specificity, and

Table 2. Correlations between Brix, immunoglobulin G (IgG), total protein (TP), gamma-glutamyl transferase (GGT), and glutaraldehyde coagulation test (GCT) on days 1, 3, and 7 after birth.

	Day	TP	GGT	GCT	Brix %
IgG	1	0.79**	0.65**	–0.77**	0.67**
	3	0.83**	0.63**	–0.78**	0.81**
	7	0.83**	0.48**	–0.67**	0.79**
TP	1		0.70**	–0.86**	0.79**
	3		0.57**	–0.81**	0.89**
	7		0.47	–0.82	0.94**
GGT	1			–0.63**	0.56**
	3			–0.55**	0.60**
	7			–0.41	0.48**
GCT	1				–0.73**
	3				–0.75**
	7				–0.78**

**Correlation is significant at the 0.01 level.

Table 3. Test characteristics of Brix % calculated according to the cut-off values determined based on previous studies.

Day	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
1	8.0	95.00	35.00	74.5	77.8	73.33
	8.3	87.50	45.00	76.1	64.3	75.00
	8.6	85.00	70.00	85.0	70.0	78.33
	9.0	67.50	80.00	87.1	55.2	70.00
	9.4	47.50	80.00	82.6	43.2	61.66
3	8.0	97.50	25.00	72.2	83.3	70.00
	8.3	97.50	45.00	78.0	90.0	76.66
	8.6	97.50	55.00	81.2	91.7	81.66
	9.0	95.00	80.00	90.5	88.9	86.66
	9.4	75.00	90.00	93.7	64.3	83.33
7	8.1	100.0	21.74	67.3	100.0	66.66
	8.3	100.0	30.43	69.8	100.0	71.66
	8.6	97.30	47.83	75.0	91.7	75.00
	9.0	83.78	73.91	83.8	73.9	78.33
	9.4	78.38	86.96	90.6	71.4	80.00

Table 4. Test characteristics of Brix, total protein (TP), gamma-glutamyl transferase (GGT), and glutaraldehyde coagulation test (GCT) on different days after birth.

	Days after birth	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Brix %	1	8.60	85.00	70.00	85.00	70.00	78.33
	3	9.00	95.00	80.00	90.50	88.90	86.66
	7	9.40	78.40	87.00	90.60	71.40	80.00
Total protein (g/dL)	1	5.20	87.50	65.00	83.30	72.20	78.33
	3	6.10	82.50	90.00	94.30	72.00	83.33
	7	5.80	81.10	82.60	88.20	73.10	78.33
GGT (IU/L)	1	1810.00	75.00	80.00	88.20	61.50	75.00
	3	516.00	95.00	65.00	84.40	86.70	83.33
	7	333.00	75.70	69.60	80.00	64.00	71.66
GCT (min)	1	2.41	60.00	90.00	92.30	52.90	66.66
	3	4.25	92.50	75.00	88.10	83.30	85.00
	7	2.83	83.80	73.90	83.80	73.90	76.66

accuracy of Brix at the cut-off value of 8.6% on day 1 were calculated as 85%, 70%, and 78.3%, respectively (Table 4). In addition, sensitivity, specificity, and accuracy of Brix at the cut-off value of 9% on day 3 were calculated as 80%, 90.5%, and 86.6%, respectively (Table 4).

The most appropriate cut-off value for TP on the first day was 5.2 g/dL; sensitivity, specificity, and accuracy of TP at this cut-off value were calculated as 87.50%, 65.00%, and 78.33%, respectively. On day 3 of the study, the cut-off value for TP was calculated as 6.1 g/dL, with a sensitivity of 82.50%, specificity of 90.00%, and accuracy of 83.33%.

When the optimal cut-off value of GGT activity was determined as 1810 IU/L on day 1 and 516 IU/L on day 3, the sensitivity was calculated as 75% and 95%, and the specificity was calculated as 80% and 65%, respectively. Cut-off values and ROC curves on the days with the highest accuracy—for Brix, TP, and GCT on day 3, and for GGT on day 1—are given in Figures 1A–1D.

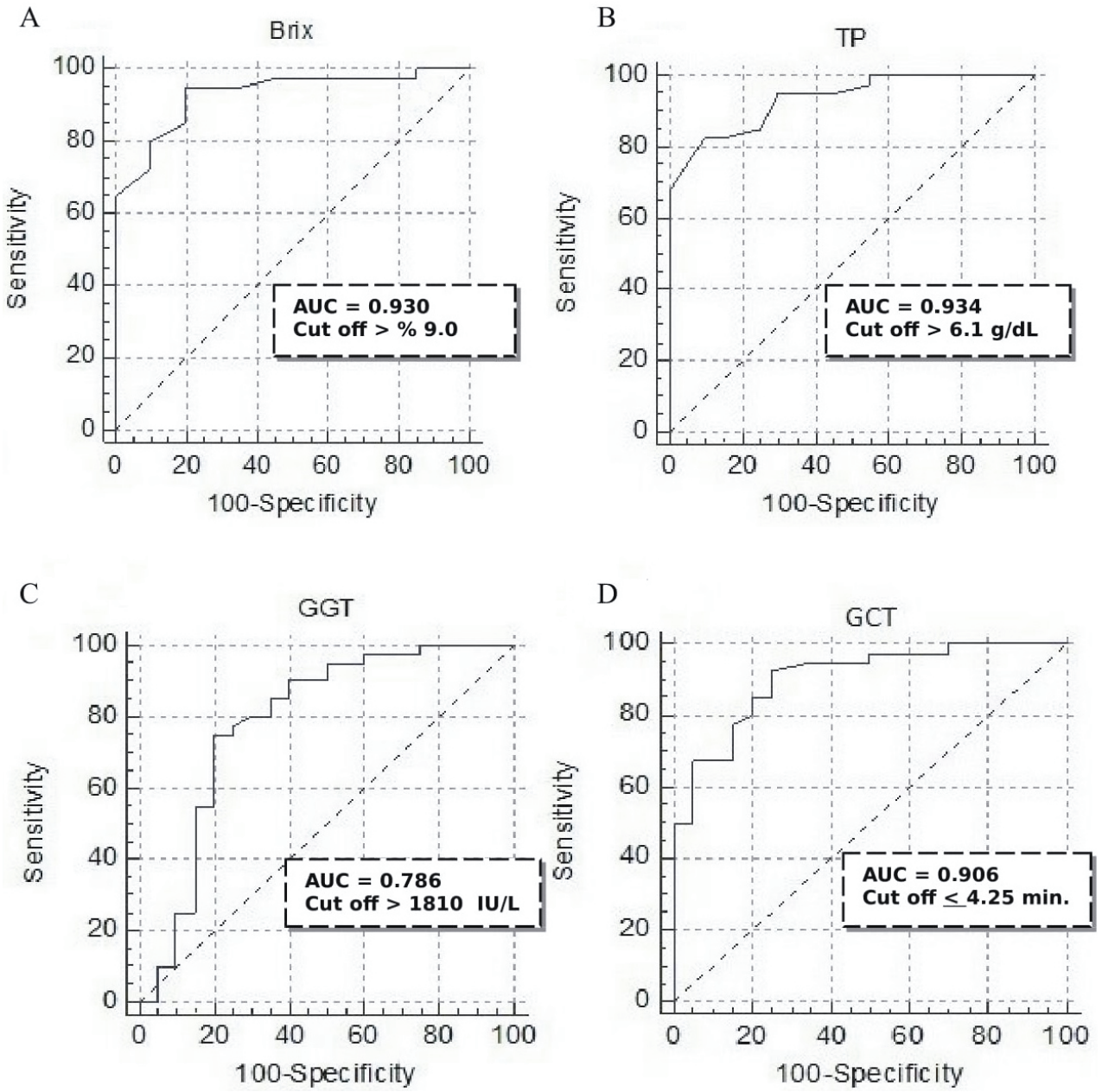

Figure 1. Cut-off values and ROC curves on the days with the highest accuracy: for Brix on day 3 (A), TP on day 3 (B), GGT on day 1 (C), and GCT on day 3 (D).

4. Discussion

In this study, FPT was detected in 30.00%–36.6% of the calves on days 1–7. The FPT levels reported in previous studies were as low as 1.3% (4), 4.75% (14), and 17.4% (21). However, FPT in 27.5% (15) and 47.9% (16) of examined calves was recorded. The FPT levels in our study are within the results of these studies. In contrast, the IgG levels of 5 calves on day 1 of the study were detected to be higher than 9 g/L. In our study, calves were fed 2–2.5 L of colostrum twice a day; however, in another study, calves were fed 3 L of colostrum twice a day (14). The serum IgG levels in our study were detected by using ELISA, in contrast to previous studies, in which the IgG levels were detected by using RID (14,15) and TIA (4). ELISA has also been used to detect the serum IgG levels in some previous studies (22). In such a manner, the IgG levels detected by ELISA in our study are within the ranges reported in previous studies (14,15).

IgG, TP, and Brix % levels peaked on day 3 of the study. The GCT time was shorter on day 3 when compared to day 1 (Table 1). Thus, 24–48 h after the first colostrum intake is the ideal timeframe for evaluating FPT in calves, with the peak IgG levels detected at approximately 36 h after the first colostrum intake (3). It has been stated that IgG and TP reached the maximum concentration at 2–3 days of age (23). Although the FPT status was not evaluated 48 h after birth, all measurements except GGT were more pronounced at 72 h than at 24 h. GGT came into prominence as the earliest indicator of FPT, as the serum GGT levels were 54% and 77% lower on days 3 and 7, respectively, than they were on day 1. This decrease is in accordance with the results of a previous study (24); however, the GGT levels detected in our study are much higher than in that study. Conversely, the mean GGT levels on day 1 in our study (2242.07 IU/L) are similar to those of a study in which mean GGT levels of 2286 IU/L were detected on day 1 (25). It has also been reported that along with TP, the GGT levels 24 h after birth can be used to evaluate FPT in calves (22). In a study conducted in beef calves to investigate the effectiveness of GGT in evaluating FPT, it was concluded that GGT levels should be used for FPT evaluation only in calves younger than 8 days old (26).

The correlations between Brix and IgG and Brix and TP were more pronounced on day 3 compared to day 1. Higher correlation between Brix and TP (r = 1.00) and Brix and IgG (r = 0.93) has been previously reported; however, the samples in this study were collected from 3- to 6-day-old calves (14). A similar high correlation between Brix and IgG (r = 0.79) and between Brix and TP (r = 0.91) was also reported (4).

On the seventh day of our study, a higher correlation was observed between Brix and IgG compared to day 1. In other studies, correlations of 0.87 (27) and 0.79 (15) between Brix % and IgG were determined. In our study, there was a correlation between Brix and TP of 0.79 to 0.94. Similar results, with a correlation of 0.74 between Brix and TP in 1- to 11-day-old calves and correlations of 0.91 and 0.97 in two separate experiments, have been previously reported (4,15).

The Brix % measurements, the test characteristics, and correlation coefficients for TP and IgG reached the highest values on day 3 of the study. The cut-off value for TP was calculated as 6.1 g/dL, with a sensitivity of 82.50%, specificity of 90.00%, and accuracy of 83.33%. Similarly, the highest sensitivity (94.5%) for TP was detected at the cut-off value of 5.2 g/dL and highest specificity (100%) at the cut-off value of 5.7 g/dL (28). The results of a previous study (15) accord with our results of a TP cut-off at 5.5 g/dL for 1- to 11-day-old calves, with a sensitivity of 80%, a specificity of 80.7%, and an accuracy of 80.5%.

Sensitivity of 100% and 83.3% and specificity of 89.2% and 86.2%, at Brix cut-off values of 8.5% and 8.6%, respectively, were detected in a previous study (4). The 1% difference between the cut-off values is due to the decrease in sensitivity with an increasing FPT ratio. It is reported that the PPV % increased at the hypothetical prevalence of 25% FPT (4). Therefore, the lower values of the characteristics in our study may be associated with a higher FPT rate. In one study (21), the Brix refractometer was reported to be useful for evaluating colostrum quality but not FPT status in calves. However, the results of our study accord with other previous reports that indicated that the Brix refractometer can be used to evaluate FPT status in calves (4,14,15).

The optimal cut-off value of GGT activity was determined as 1810 IU/L on day 1 and 516 IU/L on day 3, the sensitivity was calculated as 75% and 95%, and the specificity was calculated as 80% and 65%, respectively. A sensitivity of 80% and specificity of 97% are reported with the GGT cut-off level of 200 IU/L (29). Despite the very high GGT cut-off values in our study, the sensitivity and specificity were also quite high.

Although it is well known that GGT can be used to determine FPT status in calves, our study demonstrates that the day of GGT evaluation is also very critical for FPT evaluation (22,24,29). Calves with adequate PT must have serum GGT enzyme activities higher than 1000 IU/L and even 1500 IU/L, on day 1, >500 IU/L on day 3, and >300 IU/L on day 7 after birth. Similarly, coagulation times shorter than 5 min in the GCT are sufficient to reveal PT status in calves. In fact, the highest sensitivity, specificity, and accuracy on day 3 were detected at a cut-off of 4.25 min. The effectiveness of the GCT for determination of PT status in calves was evaluated, and results of that study indicated that although specificity was high, the sensitivity of the test was low (30). In the same study, tests were conducted in calves of 1–8 days old by mixing whole blood with a commercial GCT solution; thus, coagulation time in this study was not only determined by serum Ig concentration but also by blood fibrinogen levels. However, in the present study, the GCT was conducted with serum with the aim of surpassing the effects of fibrinogen on GCT time, as described in previous studies (6,7).

In conclusion, along with those of IgG, the Brix, TP, and GCT results determined on the third day after birth give the best estimate for PT status in calves. Cut-off values of 9.0%, 6.10 g/dL, and 4.25 min for Brix, TP, and the GCT, respectively, can be used for evaluation of PT status in 3-day-old calves. On the other hand, the GGT is more suitable for evaluation of PT status in calves at 24 h of life. GGT levels higher than 1810 IU/L in 1-day-old calves and GGT levels higher than 516 IU/L in 3-day-old calves indicate sufficient PT. In accordance with previous

studies, we have demonstrated the following: the Brix refractometer is a sensitive and practical method for the determination of FPT in calves; the best timing for Brix evaluation is 24 h after the first colostrum intake; the same time period is suitable for evaluating FPT by using IgG, TP, and the GCT; and the GGT is an early indicator of PT in calves because levels higher than 1000 IU/L 24 h after the colostrum intake indicate PT.

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