The assessment of serum biochemical indicators in neonatal beef calves with different passive immunity statuses

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Abstract

While failure of transfer of passive immunity (FTPI) is associated with high morbidity and mortality in beef calves, adequate transfer of passive immunity (ATPI) positively affects calf health, survival and performance. This study was aimed at the assessment of alterations in the serum biochemical indicators of beef calves with different passive immunity statuses. Blood samples were collected from 1- to 7-day-old, healthy beef calves (n = 51) by jugular venipuncture. Serum immunoglobulin G (IgG) concentrations were measured with the radial immunodiffusion (RID) assay. IgG concentrations of < 10 g/l were described as indicating FTPI, 10–24 g/l were described as indicating moderate transfer of passive immunity (TPI), and ≥ 24 g/l were described as indicating ATPI. Based on their serum IgG concentrations, calves were assigned to one of the passive immunity groups (FTPI, n = 15; moderate TPI, n = 10; ATPI, n = 26). Differences among the passive immunity groups for the biochemical indicators were determined by one way analysis of variance (ANOVA) or Kruskal Wallis test according to normality assumption of parameters. The gamma-glutamyl-transferase (GGT) activity, and serum total protein (STP), calcium (Ca), phosphorus (P) and cholesterol concentrations of the beef calves with ATPI were significantly higher than those with FTPI. Serum magnesium concentrations of the beef calves with ATPI were greater than those with FTPI. Serum aspartate aminotransferase activity, urea, albumin, β-hydroxybutyric acid, and non-esterified fatty acid concentrations did not differ between the groups. In conclusion, serum concentrations of biochemical indices such as STP, GGT, cholesterol, Ca, and P were altered in the beef calves with FTPI.

Bovine, colostrum, IgG, reproduction, total protein

The structure of the ruminant placenta does not allow the transplacental transfer of maternal immunoglobulins (Igs), thus, calves are born immunologically naïve. The transfer of maternal antibodies to neonatal calves requires the consumption of an adequate amount of good quality colostrum by the newborn shortly after birth, and the absorption of Igs from the ingested colostrum. Calves which do not absorb an adequate amount of Igs develop failure of transfer of passive immunity (FTPI) (Godden et al. 2019; McGee and Earley 2019). Colostrum provides newborn calves with not only antibodies, but also nutrients that support their postpartum growth and development, as well as leukocytes, growth factors, antimicrobial molecules, cytokines and hormones (Godden et al. 2019; Fischer-Tlustos et al. 2021). Thus, the health and future performance of neonatal calves

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is closely related to their passive immunity status (Godden et al. 2019; McGee and Earley 2019). The high morbidity and mortality rates and low growth performance associated with FTPI in calves leads to major economic losses in the beef and dairy industries (Raboisson et al. 2016).

Generally, the serum IgG concentration threshold used for the diagnosis of FTPI in dairy calves is < 10 g/l (Godden et al. 2019). However, no consensus has been reached on the threshold to be used for the diagnosis of FTPI in beef calves. Based on the assessment of serum IgG concentrations of < 16g/l, < 24 g/l and < 27 g/l for mortality and morbidity in beef calves (Wittum and Perino 1995; Dewell et al. 2006; Waldner and Rosengren 2009), it was suggested to use a serum IgG concentration threshold of ≥ 24 g/l for adequate transfer of passive immunity (ATPI) (Waldner and Rosengren 2009).

The reference method for the determination of IgG concentrations is the radial immunodiffusion (RID) assay (Hogan et al. 2015). However, being expensive and time-consuming, this method has not found common use in clinical practice. This has led to the investigation of the accuracy of inexpensive and practical tests that could be used as an alternative to the RID assay. Reports have been published on the effects of colostrum consumption on some biochemical indicators, independent from the passive immunity status of calves (Herosimczyk et al. 2011; Rocha et al. 2012; Nagyová et al. 2017; Aydoğdu and Güzelbetaş 2018; Meyer et al. 2018; Probo et al. 2019; Larson-Piene et al. 2022). The present study was aimed at the assessment of the alterations of certain biochemical indicators, namely, serum total protein (STP), gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST) activities, and urea, calcium (Ca), magnesium (Mg), phosphorus (P), cholesterol, albumin, β-hydroxybutyric acid (BHBA), and non-esterified fatty acid (NEFA) levels with passive transfer of colostral immunity in beef calves, and to determine the diagnostic accuracy of potential biochemical indicators to detect FTPI and ATPI in beef calves.

Materials and Methods

Animals and blood collection

This study was conducted pursuant to the approval of the Local Ethics Board for Animal Experiments of Harran University (Decision number: 2018 004, 01-03). The study material comprised of 51 apparently healthy, not visibly dehydrated (based on visual examination) 1- to 7-day-old Aberdeen Angus calves reared on a commercial beef farm located in Şanlıurfa province of Türkiye. Based on their serum IgG concentrations, the calves were assigned to three groups. According to the serum IgG thresholds, TPI in each was assessed under three categories (< 10 g/l; 10–24 g/l; and ≥ 24 g/l). In this study, beef calves with serum IgG concentrations of < 10 g/l, 10–24 g/l, and ≥ 24 g/l were defined as FTPI, moderate TPI, and ATPI, respectively.

Approximately 5,000 beef cattle were imported to this farm from Uruguay in 2017. Breeding bulls were introduced into breeding herds and stayed approximately 2 months in the herd. At the end of this period, the breeding bulls were removed from the herd and pregnancy diagnosis was performed approximately 35 days later. Pregnant cattle were allocated to separate herds. After the reproductive examination, the open animals were taken into breeding again. Breeding was continued throughout the year. The parturitions of the cattle were carried out in the calving pastures. According to the management practice of the farm, cows ready to calve were placed on a pasture, where they could be easily viewed and checked often. All cattle were fed at a feed alley next to the pasture, on a total mixed ration (TMR) twice a day. Their labour was monitored by experienced employees.

The calves suckled the colostrum of their dams and were allowed to suckle continuously. There was no controlled feeding regimen in place until the time of sampling. The dams' colostrum quality was not assessed. If calves did not suckle their dams, they were given stored colostrum obtained from Holstein cows on the same farm. These calves were marked and treated, and were not included in the study. The health of the calves was checked daily by the same farm veterinarian. Calves with symptoms such as drooping ears, dull eyes, reluctance to move, softening of faecal consistency, coughing or decreased sucking were considered sick and treated. Calves not showing these symptoms were considered to be healthy looking. Throughout the study period, the calves included in the study did not suffer from diarrhoea or any other disease. No inclusion or exclusion criteria were applied other than the apparent health status of the calves.

Ten-ml jugular blood samples were collected from the calves into plain sterile vacutainers (HemaTube, Ankara, Türkiye). The blood samples were centrifuged on the farm within 4 h after collection. The collected samples were stored at room temperature until centrifugation. Sera were extracted by centrifuging the blood samples at 3000 × *g*

for 10 min. The sera were transferred into 2-ml microcentrifuge tubes and stored at −20 °C until being used for laboratory analyses.

Biochemical analyses

Serum IgG concentrations were measured with the aid of a commercial RID assay (Triple J Farms, Bellingham, USA). The test procedure was applied in accordance with the manufacturer's instructions. Frozen serum samples were thawed at room temperature. The RID assay kits, which were stored in a polystyrene box in the refrigerator, were transferred to room temperature 30 min before the analyses. Three reference sera with known IgG concentrations and the serum samples were transferred into the wells of the RID plates using 5-µl automatic pipettes and were left for incubation at room temperature. After 24 h, the diameter of the precipitation rings around each well were measured using a magnifier (Peak loupe × 10) précised to 0.1 mm. The IgG concentrations of the serum samples were determined according to the reference table provided with the test kit. The serum samples with a precipitation ring diameter greater than the highest value indicated in the reference table were diluted 1:1 with sterile sodium chloride (NaCl) solution and re-analysed. GGT and AST activities, and Ca, P, Mg, urea, cholesterol, STP, albumin, BHBA and NEFA concentrations were measured using Randox assay reagents with the aid of an auto-analyser (Randox Imola, Randox Laboratories, Crumlin, United Kingdom).

Statistical analyses

Assessment of the normality of data was based on Shapiro-Wilk test. Descriptive statistics were calculated for the biochemical indicators of each group. Results were presented as means ± standard deviations and median ± interquartile range (IQR). Correlation coefficients were calculated to determine the correlation between the biochemical parameters and RID IgG concentrations. Significance levels of the differences among different passive immunity categories were determined with one-way analysis of variance (ANOVA) for normally distributed serum biochemical parameters and Kruskal-Wallis test for non-normally distributed serum biochemical parameters. Pairwise comparisons of biochemical indicators showing significant differences between different passive immunity categories were performed using Tukey test for parameters showing normal distribution and Dunn's multiple comparison test for parameters not showing normal distribution. Statistical significance was set at $P \leq 0.05$.

A receiver operating characteristics (ROC) curve was constructed for the assessment of the diagnostic accuracy of the potential biochemical indicators of FTPI and ATPI in beef calves. Sensitivity referred to the proportion of calves, accurately determined with the biochemical indices as having IgG concentrations of < 10, and ≥ 24 g/l (number of true-positive results/ [number of true-positive results + number of false-negative results]), whilst specificity referred to the proportion of calves accurately determined with the biochemical indices as having IgG concentrations of ≥ 10, and < 24 g/l (number of true-negative results/[number of true negative results plus number of false-positive results]). The positive predictive value (PPV) referred to the probability of calves, classified with biochemical indices as having serum IgG concentrations of < 10, and ≥ 24 g/l, truly having IgG concentrations of < 10, and ≥ 24 g/l (number of true-positive results/[number of true-positive results plus number of false-positive results]). The negative predictive value (NPV) referred to the probability of calves, classified with biochemical indices as having IgG concentrations of ≥ 10, and < 24 g/l, truly having IgG concentrations of ≥ 10, and < 24 g/l (number of true-negative results/[number of true negative results + number of false-negative results]). The optimal thresholds of the biochemical indices to detect FTPI and ATPI were selected based on Youden's *J*-index calculated following equation: J = Se + Sp – 1. The discriminant ability of the biochemical indices to calves with serum IgG concentrations of < 10, and ≥ 24 g/l and ≥ 10, and < 24 g/l was assessed using the area under the ROC curves (AUC). Accuracy was defined as excellent, high, moderate or poor, when AUC values were of 1.0, > 0.9, 0.7 to 0.9 or 0.5 to 0.7, respectively. Statistical analyses were performed using the SPSS version 24 statistical software package and MedCalc Version 20.218 statistical software package.

Results

The descriptive statistics of the serum biochemical indicators are presented in Table 1. Concentrations of STP, P, and NEFA were normally distributed whereas other biochemical indicators were non-normally distributed. STP concentrations were significantly higher in the moderate TPI group than in the FTPI group, in the ATPI group than in the moderate TPI group, and in the ATPI group than in the FTPI group ($P < 0.001$). While Ca concentrations did not differ between FTPI and moderate TPI groups, it was significantly higher in the ATPI group than in the moderate TPI group ($P = 0.007$) and the FTPI group ($P < 0.001$). While P concentrations did not differ between FTPI and moderate TPI groups, and between moderate TPI and ATPI groups; it was significantly higher in the ATPI group than in the FTPI group ($P = 0.003$). Cholesterol concentrations were significantly higher in the moderate TPI group than in the FTPI group ($P = 0.013$), and in the ATPI group than in the FTPI group ($P < 0.001$). Mg concentrations were significantly higher in the

ATPI group than in the FTPI group ($P = 0.04$). The other biochemical indicators did not display any difference among passive immunity categories (Table 2). A very high correlation coefficient was calculated between the serum IgG concentrations with STP concentrations, GGT activity, and Ca concentrations ($r = 0.876$, $r = 0.763$, $r = 0.718$, respectively), whilst a moderate correlation coefficient was calculated between the serum IgG concentrations with P concentrations ($r = 0.508$). The correlations determined between the biochemical parameters are presented in Table 3. The sensitivity, specificity, PPV, NPV and AUC are presented for the STP concentrations and GGT activity, which are used to detect FTPI and ATPI in beef calves (Table 4). STP and cholesterol concentrations and GGT activity showed high discriminatory ability to detect beef calves with FTPI and ATPI at the optimal thresholds.

Table 1. Descriptive statistics of serum biochemical indicators of the 1- to 7-day-old beef calves (n = 51).

	Reference interval	N (%) < reference interval	N (%) > reference interval	Mean ± SD	Median	IQR	Minimum	Maximum
IgG (g/l)				23.22 ± 18.12	24.88	29.69	0.0	70.06
STP (g/dl)	5.9–9.1[a]	21 (41.2)	6 (11.8)	6.66 ± 1.9	6.83	3.41	3.89	11.42
GGT (U/l)	32–1,037[b]	5 (9.8)	8 (15.7)	413.08 ± 426.86	215.00	581.00	18.00	1280.00
Ca (mg/dl)	8.7–10.1[a]	23 (45.1)	25 (49.0	8.75 ± 4.57	9.91	8.99	1.52	16.35
P (mg/dl)	4.4–6.9[a]	–	49 (96.1)	11.20 ± 2.82	10.83	4.46	6.10	16.58
Mg (mg/dl)	1.5–2.6[a]	–	8 (15.7)	2.24 ± 0.37	2.19	0.53	1.50	3.20
Urea (mg/dl)	2–33[a]	–	2 (3.9)	16.83 ± 11.39	13.37	7.06	5.99	69.16
AST (U/l)	38–92[a]	13 (25.5)	2 (3.9)	50.00 ± 17.74	50.00	27.00	21.00	107.00
Cholesterol (mg/dl)	34.8–139.21[b]	11 (21.6)	2 (3.9)	65.75 ± 42.11	55.00	56.00	10.00	182.00
Albumin (g/dl)	3.4–4.1[a]	42 (82.4)	–	3.04 ± 0.36	2.98	0.40	2.44	3.89
BHBA (mmol/l)	0–0.59[b]	–	–	0.14 ± 0.11	0.10	0.06	0.06	0.53
NEFA (mmol/l)	0,09–0,72[c]	–	4 (7.8)	0.44 ± 0.18	0.41	0.23	0.14	0.93

IgG: Immunoglobulin G; STP: serum total protein; GGT: gamma-glutamyl transferase; Ca: calcium; P: phosphorus; Mg: magnesium; AST: aspartate aminotransferase; NEFA: non-esterified fatty acid; BHBA: β-hydroxybutyric acid; IQR: interquartile range

[a,b,c] Reference ranges were adapted from [a] Larson-Peine et al. (2022), [b] Roadknight et al. (2020) and [c] Yu et al. (2019).

Table 2. Biochemical indicators of the beef calves with different statuses of passive immunity.

Indicator	Statistic	Passive immunity categories			*P*
		IgG < 10 g/l	IgG 10–24 g/l	IgG ≥ 24 g/l	
STP (g/dl)	Mean	4.47[c]	6.37[b]	8.03[a]	< 0.001
	SE	0.11	0.37	0.26	
	Median	4.39	6.06	8.12	
	SD	0.42	1.15	1.33	
	Minimum	3.89	5.19	5.02	
	Maximum	5.24	8.69	11.42	
	IQR	0.66	1.87	1.73	
GGT (U/l)	Mean	91.27[c]	329.60[b]	630.85[a]	< 0.001
	SE	35.59	124.72	84.02	
	Median	53.00	140.00	557.00	
	SD	137.84	394.40	428.41	
	Minimum	18.00	36.00	45.00	
	Maximum	576.00	1280.00	1280.00	
	IQR	54.00	495.25	817.00	
Ca (mg/dl)	Mean	5.16[b]	7.03[b]	11.48[a]	< 0.001
	SE	0.88	1.38	0.67	
	Median	3.76	6.01	12.73	
	SD	3.40	4.37	3.42	
	Minimum	2.02	1.52	4.06	
	Maximum	11.74	16.35	15.35	
	IQR	6.33	6.54	3.15	

Table 2. Biochemical indicators of the beef calves with different statuses of passive immunity.

Indicator	Statistic	Passive immunity categories			*P*
		IgG < 10 g/l	IgG 10–24 g/l	IgG ≥ 24 g/l	
P (mg/dl)	Mean	9.59[b]	10.37[a,b]	12.46[a]	0.003
	SE	0.45	0.91	0.54	
	Median	9.22	10.12	13.06	
	SD	1.76	2.88	2.77	
	Minimum	6.94	6.10	6.41	
	Maximum	12.72	15.26	16.58	
	IQR	2.86	5.53	4.95	
Mg (mg/dl)	Mean	2.12	2.18	2.34	NS
	SE	0.10	0.09	0.07	
	Median	2.08	2.13	2.37	
	SD	0.38	0.30	0.38	
	Minimum	1.50	1.72	1.68	
	Maximum	3.20	2.67	3.11	
	IQR	0.37	0.44	0.54	
Urea (mg/dl)	Mean	18.40	13.70	17.13	NS
	SE	4.27	0.77	1.95	
	Median	11.37	13.37	14.06	
	SD	16.53	2.43	9.95	
	Minimum	5.99	9.81	8.49	
	Maximum	69.16	17.64	58.31	
	IQR	17.12	3.13	7.25	
AST (U/l)	Mean	48.47	47.50	51.85	NS
	SE	5.17	5.99	3.21	
	Median	43.00	45.50	54.50	
	SD	20.04	18.94	16.37	
	Minimum	27.00	26.00	21.00	
	Maximum	107.00	94.00	85.00	
	IQR	22.00	17.75	26.25	
Cholesterol (mg/dl)	Mean	34.20[b]	83.90[a]	76.96[a]	< 0.001
	SE	5.21	19.41	6.51	
	Median	30.00	61.00	67.00	
	SD	20.17	61.38	33.20	
	Minimum	10.00	20.00	31.00	
	Maximum	91.00	182.00	133.00	
	IQR	24.00	113.00	61.50	
Albumin (g/dl)	Mean	3.04	3.08	3.02	NS
	SE	0.05	0.13	0.08	
	Median	2.99	3.01	2.92	
	SD	0.19	0.40	0.42	
	Minimum	2.76	2.48	2.44	
	Maximum	3.52	3.78	3.89	
	IQR	0.18	0.54	0.57	
BHBA (mmol/l)	Mean	0.16	0.16	0.12	NS
	SE	0.04	0.04	0.01	
	Median	0.09	0.10	0.10	
	SD	0.15	0.13	0.06	
	Minimum	0.07	0.07	0.06	
	Maximum	0.53	0.48	0.27	
	IQR	0.08	0.12	0.05	
NEFA (mmol/l)	Mean	0.40	0.45	0.46	NS
	SE	0.04	0.06	0.04	
	Median	0.38	0.47	0.42	
	SD	0.15	0.20	0.18	
	Minimum	0.14	0.14	0.15	
	Maximum	0.64	0.75	0.93	
	IQR	0.23	0.27	0.24	

IgG: Immunoglobulin G; STP: serum total protein; GGT: gamma-glutamyl transferase; Ca: calcium; P: phosphorus; Mg: magnesium; AST: aspartate aminotransferase; NEFA: non-esterified fatty acid; BHBA: β-hydroxybutyric acid; IQR: interquartile range; SD: standard deviation; SE: standard error

Table 3. Pearson correlation coefficients between the STP, P and NEFA concentrations, and Spearman's rho correlation coefficients between the other biochemical indicators of the beef calves.

	IgG	STP	GGT	Ca	P	Mg	Urea	AST	Cholesterol	Albumin	BHBA	NEFA
IgG	1	0.876**	0.763**	0.718**	0.508**	0.337*	0.104	0.239	0.470**	-0.25	-0.14	-0.03
STP		1	0.770**	0.810**	0.537**	0.412**	0.207	0.214	0.554**	-0.023	-0.08	0.143
GGT			1	0.637**	0.402**	0.344*	-0.010	0.361**	0.278*	-0.416	0.035	0.103
Ca				1	0.457**	0.570**	0.012	0.27	0.430**	0.041	-0.26	0.031
P					1	0.094	0.123	0.249	0.428**	0.098	-0.18	0.008
Mg						1	0.161	0.152	0.127	0.035	-0.12	0.199
Urea							1	-0.01	0.095	0.189	0.489**	0.321*
AST								1	-0.02	0.023	0	0.189
Cholesterol									1	0.352*	-0.27	-0.03
Albumin										1	-0.09	0.238
BHBA											1	0.518**
NEFA												1

IgG: Immunoglobulin G; STP: serum total protein; GGT: gamma-glutamyl transferase; Ca: calcium; P: phosphorus; Mg: magnesium; AST: aspartate aminotransferase; NEFA: non-esterified fatty acid; BHBA: β-hydroxybutyric acid; NS: non-significant.

Table 4. Diagnostic test characteristics at the optimal thresholds of serum total protein, gamma-glutamyl transferase and cholesterol for detecting failure of transfer of passive immunity and adequate transfer of passive immunity in beef calves.

	IgG threshold	Optimal thresholds	Prevalence (%)	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)	PPV (95% CI) (%)	NPV (95% CI) (%)	AUC (95% CI)
STP (g/dl)	<10 g/l	≤5.24	29.4	100 (78.2–100.0)	91.67 (77.5–98.2)	83.3 (62.9–93.7)	100	0.99 (0.911–1.000)
	≥24 g/l	>6.28	50.1	92.31 (74.9–99.1)	84 (63.9–95.5)	85.7 (70.8–93.7)	91.3 (73.3–97.6)	0.928 (0.819–0.981)
GGT (U/l)	<10 g/l	≤134	29.4	93.33 (68.1–99.8)	86.11 (70.5–95.3)	73.7 (55.1–86.5)	96.9 (82.3–99.5)	0.911 (0.798–0.973)
	≥24 g/l	>141	50.1	96.15 (80.4–99.9)	80 (59.3–93.2)	83.3 (69.5–91.7)	95.2 (74.3–99.3)	0.864 (0.739–0.944)
Cholesterol (mg/dl)	<10 g/l	≤44	29.4	86.67 (59.5–98.3)	75 (57.8–87.9)	59.1 (44.2–72.5)	93.1 (78.6–98.0)	0.858 (0.732–0.940)
	≥24 g/l	>55	50.1	73.08 (52.2–88.4)	76 (54.9–90.6)	76 (60.3–86.9)	73.1 (58.1–84.1)	0.737 (0.595–0.850)

STP: serum total protein; GGT: gamma-glutamyl transferase; PPV: positive predictive value; NPV: negative predictive value; AUC: area under the receiver operating characteristics (ROC) curve; CI: confidence interval.

Discussion

In neonatal calves, colostrum consumption is known to significantly affect the STP concentration and the relative and absolute concentrations of major protein electrophoretic fractions (Nagyová et al. 2017). The effect of colostrum consumption on the STP concentration is mainly related to the ingestion of immunoglobulins via colostrum (Quigley et al. 2002). Therefore, the measurement of the STP concentration is used to determine the level of passive immunity transfer (Godden et al. 2019). On the other hand, colostrum also provides calves with macro and trace elements, enzymes and bioactive molecules, including growth factors. Recently, studies have focused on the use of various biochemical indicators obtained with colostrum consumption for the diagnosis of FTPI. While the correlation of passive immunity status with biochemical indicators has undergone extensive research in dairy calves (Parish et al. 1997; Pekcan et al. 2013; Šlosárková et al. 2014; Mondal et al. 2020), there is a paucity of information for beef calves due to the very few number of studies available (Wilson et al. 1999; Larson-Peine et al. 2022; Meyer and Rathert-Williams 2024).

The GGT enzyme is produced in the cells lining the excretory ducts of mammary glands (Weaver et al. 2000). Therefore, serum GGT activity is observed in parallel with the passage of IgG into the blood serum following colostrum consumption (Parish et al. 1997; Wilson et al. 1999). The mean serum GGT activity determined in our study was either higher than the activities reported in a previously conducted study (Wilson et al. 1999) or similar to those reported in some other research (Pekcan et al. 2013; Šlosárková et al. 2014; Larson-Peine et al. 2022). The serum GGT activity determined for beef calves with ATPI in our study was consistent with the activity previously reported by Pekcan et al. (2013) for dairy calves. Owing to the short half-life of GGT, serum GGT levels are highest during the first and second days of life, and show a rapidly progressive decrease over the following days (Braun et al. 1982; Ježek et al. 2006; Šlosárková et al. 2014). Herein, the exact age (day of life) of the neonatal calves during days 1–7 of the study was not known. Nonetheless, the significant difference observed among different passive immunity categories suggested that, as is the case in dairy calves, GGT activity could be used to detect FTPI in beef calves. The sensitivity and specificity of a GGT activity of ≤ 134 IU/l, identified as the optimal threshold for the detection of FTPI in beef calves, was similar (Mugnier et al. 2020) or slightly lower (Hogan et al. 2015) than values reported in previous studies.

Previous research has demonstrated that the STP concentrations measured by digital or optical refractometers and the biuret method can be used to determine the passive immunity status or to diagnose FTPI in neonatal animals (Santiago et al. 2020; Gamsjäger et al. 2021; Akköse et al. 2022; Akköse et al. 2023a). It was ascertained that serum IgG concentrations were very highly correlated with STP concentrations (r = 0.876). The STP threshold determined in the present study for the detection of calves with serum IgG concentrations of <10 g/l was similar to thresholds reported in previous studies (Gamsjäger et al. 2021; Akköse et al. 2023b). The STP threshold determined in the present study for the detection of serum IgG concentrations of ≥ 24 g/l was higher than the thresholds reported by Gamsjäger et al. (2021) and Kreuder et al. (2023), and similar to the threshold value reported by Akköse et al. (2023b). The sensitivity and specificity of the optimal STP thresholds were comparable to the values described in previous studies on the estimation of serum IgG concentrations (Vandeputte et al. 2011; Gamsjäger et al. 2021; Kreuder et al. 2023; Akköse et al. 2023b). Note that while previous studies determined STP thresholds that predicted serum IgG concentrations of < 24 ml/l, in our study the STP threshold was determined that predicted serum IgG concentrations of ≥ 24 ml/l. As a result, sensitivity vs specificity and PPV vs NPV values will be exactly the opposite.

The macro-mineral content of colostrum is significantly higher than that of milk (Godden et al. 2019; Valldecabres and Silva-del-Río 2022). While the serum Ca and Mg concentrations determined in our study were similar to those reported in previous research (Piccione et al. 2010; Rocha et al. 2012; Aydoğdu and Güzelbektaş 2018; Larson-Peine et al. 2022), the P concentration was slightly higher than the values reported in previous studies (Ježek et al. 2006; Piccione et al. 2010; Rocha et al. 2012). Interestingly, we observed that the beef calves with different passive immunity statuses significantly differed for serum Ca and P concentrations. Calves with higher passive immunity had higher serum Ca concentrations, which is in agreement with the study by Meyer and Rathert-Williams (2024).

When compared to the results of a recent study conducted by Larson-Peine et al. (2022) in beef calves, the mean serum AST activity and urea, NEFA and albumin concentrations determined in the present study were similar. Even though serum AST activity, urea, and NEFA concentrations increase in the postnatal period (Larson-Peine et al. 2022), these values do not appear to be affected by colostrum consumption. Foetuses have the capacity to deaminate amino acids and produce urea, and in the postnatal period, there is a strong correlation between the serum urea nitrogen concentrations of the foetus and the dam (Meyer et al. 2018). The urea concentrations determined in the present study were higher than those reported by Pekcan et al. (2013) in dairy calves but lower than those reported by Mondal et al. (2020) in Jersey calves. In agreement with the study of Mondal et al. (2020), we also observed no difference between the urea concentrations of the calves with different passive immunity statuses. Cholesterol is used as a marker of calf mortality risk during the first 3 weeks after birth (Renaud et al. 2018). It has been reported that, similar to IgG, higher cholesterol concentrations are associated with lower mortality risk during the first 21 days after birth in calves raised at veal farms (Renaud et al. 2018). Goetz et al. (2021) also indicated that higher cholesterol and IgG levels were associated with a lower mortality risk during the first 78 days after birth at veal farms. In a report by Marcato et al. (2024), calves with pasty and loose faeces during the two-week-period following their transport to veal farms, were indicated to have lower cholesterol concentrations during the first week after birth. Compared to milk, cholesterol concentration is significantly higher in colostrum. Thus, cholesterol could be used as a marker of colostrum intake (Marcato et al. 2018). Serum cholesterol concentrations have been demonstrated to increase after colostrum consumption (Johnson et al. 2007; Aydoğdu and Güzelbektaş 2018). Our findings revealed that calves with moderate TPI and ATPI had higher cholesterol concentrations than calves with FTPI. The cholesterol concentrations of calves could be affected by different factors (Santos et al. 2023; Marcato et al. 2024). Therefore, a better understanding of the mechanisms by which cholesterol influences calf passive immunity, health, and growth is needed.

None of the values measured for the selected serum biochemical indicators were all within the reference ranges. While the serum total protein, albumin, cholesterol and calcium concentrations and AST activity measured in more than 10% of the samples were below the lower limit of the reference range, the STP, Ca, Mg and P concentrations of another set of samples exceeded the upper limit of the reference range by more than 10%. Similarly, Larson-Peine et al. (2022) reported that most of the values they measured for biochemical indicators fell outside the reference ranges. Likewise, Santos et al. (2023) reported serum albumin concentrations below the lower limit of the reference range in samples from dairy calves. As the animals included in the present study were healthy-appearing beef calves, clinical disease was not considered a possible cause of the measurements falling outside the reference ranges. Thus, these results clearly show that bovine reference ranges, established on the basis of data from adult animals, do not apply to calves. Furthermore, the majority of the P measurements exceeding

the upper limit of the reference range was attributed to the occurrence of phosphorus leakage from erythrocytes during the storage period of the blood samples until the time of serum extraction (Humann-Ziehank and Ganter 2012).

Limitations of the study

Age-dependent (Herosimczyk et al. 2011; Probo et al. 2019; Larson-Peine et al. 2022) variations have been reported for some biochemical metabolites. Despite no data having been published for beef calves, it has been reported that serum IgG concentration decreased by 0.7 g/l within a day in dairy calves (Wilm et al. 2018). In the present study, the age of the calves was not known. This may have led to the beef calves with serum IgG concentrations close to the serum IgG thresholds (10, 24 g/l) having been misclassified for their passive immunity status, and thus, may have affected our analysis results. We consider there to be only a minimum risk for the passive immunity statuses of the beef calves with serum IgG concentrations close to the thresholds having been misclassified and the study data being indefinite.

Moreover, biochemical indicators were not measured in precolostral blood serum samples. While some studies have tested precolostral serum samples (Herosimczyk et al. 2011; Mondal et al. 2020), others have investigated alterations in biochemical indicators with age, without testing precolostral blood serum samples (Parish et al. 1997; Wilson et al. 1999; Piccione et al. 2010; Pekcan et al. 2013; Šlosárková et al. 2014). In the present study, we monitored the changes in the biochemical indicators of only calves with different passive immunity categories. Therefore, we performed a single sampling within days 1–7, which is the period during which the level of passive immunity transfer is evaluated in calves. There is need for further comprehensive research on time-dependent changes that occur in the biochemical indicators of beef calves as these could serve as valuable indicators for the monitoring of population health.

In conclusion, when compared to those diagnosed with FTPI, beef calves with ATPI had significantly higher STP concentrations, serum GGT activity, and Ca, P and cholesterol concentrations. While STP concentration and GGT activity are already used as indicators of passive immunity transfer status in calves, the serum cholesterol, Ca, and P concentrations could serve as some additional indicators for the diagnosis of FTPI or ATPI. Further, more comprehensive research is needed to confirm the diagnostic value of these analyses in the context of FTPI in beef calves.

Conflict of interest

No potential conflict of interest was reported by the authors.

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