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Research article

BRIX REFRACTOMETRY OF COLOSTRUM FROM PRIMIPAROUS DAIRY COWS AND NEW-BORN CALF BLOOD SERUM IN THE EVALUATION OF FAILURE OF PASSIVE TRANSFER

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Failure of passive transfer (FPT) of immunoglobulins (IgG) is associated with increased morbidity and mortality of calves. In this study we evaluated the digital Brix refractometer usefulness for the assessment of FPT. A number of 16 colostrum samples from the first milking (2-3h post-partum) of Holstein-Friesian dairy heifers and 29 blood sera of 3-6 days old calves were analyzed with a digital Brix refractometer. Total proteins were determined by the biuret reaction. Colostral IgG were determined by radial immunodiffusion (RID), and colostral whey and blood serum γ globulin (composed almost entirely of IgG) were determined by agarose protein gel electrophoresis (APE) and densitometry. Colostral % Brix score was 25.5±3.4%. Concentrations of colostrum IgG and colostral whey γ globulin were 130±33 g/L and 100±24 g/L respectively. The concentration of total proteins in colostral whey was 134±30 g/L. The correlations between Brix values and the concentrations of IgG determined with RID and the concentrations of γ globulin determined with APE were positive and highly significant ($P<0.001$ and $P<0.01$). The concentration of serum proteins of new-born calves was 57.75±11.8 g/L, the concentration of γ globulin was 14.4±7.8 g/L, and the Brix score was 8.6±1.0%. FPT (serum γ globulin<10 g/L) was detected in 34.5% (10/29) calves. Brix score correlated with the concentration of blood serum γ globulins in all examined calves. The results have confirmed that digital Brix refractometry allows the producers to use this technique in order to estimate colostral and calf serum IgG, thereby monitoring both colostrum quality and success of passive transfer.

Key words: agarose gel protein electrophoresis, brix refractometer, colostrum IgG, failure of passive transfer, new-born calf sera IgG, radial immunodiffusion.

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INTRODUCTION

Colostrum is an important source of immunity and nutrition for the neonate. It contains immunoglobulins and functional proteins, lipids, carbohydrates, growth factors, minerals and vitamins. Adequate intake of high quality colostrum immediately after birth is the most important factor which determines calf health, survival and future production [1].

Newborn calves are agammaglobulinemic, or might have IgG in traces [2,3]. They derive passive immunity by absorbing immunoglobulins and leukocytes from the colostrum [2,4-6]. Protection from infectious diseases of newborn calves is completely dependent on the success of passive transfer of maternal IgG via the colostrum [2]. Because of that, failure of passive transfer (FPT) of colostral immunoglobulins, composed mainly of IgG [2], is associated with increased morbidity and mortality [2-11]. FPT of immunoglobulins is defined as a circulating IgG concentration less than 10 g/L in newborn calves between 24 and 48 hours of age [12,13].

A variety of methods is available to evaluate colostrum quality. Immunochemical techniques (immune-nephelometry, radial immunodiffusion; RID, turbidimetric immunoassay; TIA, enzyme-linked immunosorbent assay; ELISA) are considered as the most accurate methods for analysis of IgG in the colostrum and milk [14,15]. Among them the most accurate method for analysis of IgG in the colostrum is radial immunodiffusion (RID) assay that directly measures the IgG concentration and represents the gold standard for the determination of IgG concentration in the bovine colostrum or serum. The RID assay is an expensive laboratory procedure, demands special equipment and educated technicians. Besides, it is time consuming (requires 18 to 72 h to determine the results), and as such, it is not suitable for on-farm use [16,17].

Alternative to the immunochemical assay, electrophoretic techniques (gel electrophoresis, capillary electrophoresis) and high-performance chromatographic techniques have been used for the determination of bovine colostral whey proteins [14]. The agarose gel serum protein electrophoresis (APE) is the mostly used electrophoretic technique for diagnostics in both human and veterinary medicine allowing thus the identification and quantification of protein fractions in different body fluids [18]. Electrophoretic determination of IgG concentration is reliable, more rapid and less expensive then RID, but it also demands special equipment, technical knowledge, and it is also time consuming and not suitable for on-farm use.

Physical techniques, including colostrometry and refractometry are also used for the assessment of colostrum quality [1]. The most common methods for assessing colostrum quality are colostrometry (hydrometry) and visual inspection. The colostrometer was introduced as a practical field tool for measuring IgG concentration in bovine colostrum based on the linear relationship between colostral specific gravity and IgG concentration [19]. Although colostrometry allows the producers a fast assessment of colostrum quality, it is often inaccurate [20] because it depends on the colostrum temperature [21,22], cow breed, month of calving and parity [20].

Consequently an alternative, reliable/accurate, rapid, inexpensive and easy to use method for on-farm monitoring of colostrum management as a predictor of calf health is needed. Refractometers, digital or optical, are used to measure total protein in the colostrum and calf serum [1,23-25]. The digital Brix refractometer measures the index of refraction. Proteins in body fluids refract light and refractometry is an indirect measure of the concentration of total proteins [26]. The major protein fraction in the colostrum is IgG, thus, measurement of total protein in the colostrum may provide a value that is highly correlated with IgG concentration [15,27]. Refractometry using a Brix refractometer has advantages over other methods for estimating the concentration of colostral IgG. It is inexpensive, readily available, less fragile and not sensitive to the temperature of the colostrum at the time of analysis, season of the year and other factors [24].

Brix refractometer is not used in our country. This is the first study where we evaluated its usefulness for the measurement of colostrum IgG concentration on our farms. We determined the correlation between the refractometry values obtained by Brix and the IgG concentration determined with RID (gold standard) and γ globulins determined with APE. Besides, we analyzed if Brix refractometry of colostrum and new-born calf serum can be used for the assessment of the success of passive transfer of maternal immunoglobulins.

MATERIAL AND METHODS

Animals

Holstein-Friesian pregnant heifers (n=16) and 3-6 days old Holstein-Friesian calves (n=29) were from a farm owned by PKB Corporation (Padinska Skela, Belgrade, Serbia). The use of animals was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade in accordance with the National Regulation on Animal Welfare. All heifers and calves were in good general health condition without any obvious clinical signs of disease. Gestation length of the dams of the calves was in the range of 275–284 days. Parturition was normal and without complications. Calves were removed from the dams within 1 hour after parturition. They did not suck the dams; instead they were hand-fed a known volume of colostrum using a nipple bottle. Calves received 2L of first colostrum within 2 h after birth and additional 2L within 12 h, by nipple feeding. In the postcolostral feeding period calves were provided with 2 L of whole milk twice a day.

Collection of colostrum and isolation of colostral whey

Two samples (No 1 and 2 in a volume of 200 mL) of colostrum were collected from the first milking 2 - 3 h after calving into a sanitized milk bucket and were placed into plastic vials labelled with the cow identification number. Sample No 1 was analyzed within 1 h of collection with the digital Brix refractometer (Atago Co. Ltd., Tokyo,

Japan). After the measurements, it was frozen and stored at −20°C until used for the determination of colostral IgG concentration by RID (RID kit, The Binding Site group Ltd, Birmingham, UK). Sample No 2 was frozen and stored at −20°C until used for the analysis of colostral whey proteins by electrophoresis. Frozen colostrum samples were thawed in a warm water bath (37°C), brought up to room temperature (20°C) and analyzed with RID within 4 h of thawing.

For isolation of colostral whey thawed colostrum was centrifuged (2000 rpm, 20 min) and the fatty layer was carefully removed by vacuum aspiration. Casein was precipitated by adding seven drops (approximately 175 μL) of rennet to 10 ml of centrifuged colostrum supernatant. After 30 min of incubation at 37°C, samples were centrifuged for 15 minutes at 3000 x g and colostral whey (supernatant) was aliquoted and stored at -20°C until use.

Calves blood samples collection and isolation of blood serum

Whole blood was collected from 3-6 days old Holstein calves (n=29) by jugular venipuncture into sterile, plastic, vacutainer tubes without anticoagulant. Samples (6 mL) were stored on ice and transported to the Faculty of Veterinary Medicine, University of Belgrade. Within 4-6 h of collection the serum was separated by centrifugation at 2000 x g for 15 min.

Brix refractometry

The Brix refractometer was calibrated with distilled water before analysis of every sample. Then, colostrum or blood serum were homogenized by inverting 10 to 15 times, approximately 500 μL of the sample was placed on the prism of the Brix refractometer, and %Brix value was read. The digital Brix refractometer has a range from 0 to 85% Brix.

Radial immunodiffusion (RID) assay

The concentration of colostrum IgG was performed using a commercially available bovine IgG RID test plate (The Binding Site group Ltd, Birmingham, UK) according to the manufacturer. Colostrum samples were diluted 100-150 times with PBS (0.8 % NaCl, 10 mM sodium-phosphate, pH 7.2-7.4) to ensure that sample ring diameters were within the range of the standards. Plates were incubated for 72 hours at 20 to 25°C. The diameter of precipitation rings were measured using the ImageMaster TotalLab TL 120 software (GE Health Care Life Science, NJ, USA). IgG concentrations of colostrum samples were determined by comparing the diameter of the precipitation ring with a standard curve generated by assaying the internal standards of each kit. Concentrations of IgG standards were 2.5, 1.25, 0.62 and 0.31 g/L.

Agarose gel serum protein electrophoresis (APE)

APE of the colostral whey and serum proteins was performed according to the procedure of Johansson [28]. The eight samples (in volume of 3 μL of each) were simultaneously separated in 0.1 mm tick, 1% agarose gel buffered with 46 mM sodium-barbital buffer, pH 8.6, poured on a glass plate. Electrophoresis was carried out in Multiphor II Electrophoresis System (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) cooled with tap water. Sodium-barbital buffer (92 mM, pH 8.6) was used as the electrophoresis buffer. The proteins were separated for 50 min at constant current (6 mA per sample). After that the gel was dried at 65°C, fixed and stained in 0.25% Coomassie blue R-250 in 10% acetic acid/45% methyl alcohol aqueous solution and destained in acetic acid/methyl alcohol aqueous solution. The relative content of γ globulins (percentage) was quantified by densitometry using ImageMaster Total- Lab v1.11 software (Amersham Pharmacia Biotech, Uppsala Sweden). The concentration of γ globulins was calculated based on total protein concentration determined by the biuret method as described by Doumas et al. [29].

Statistical analysis

The statistical significance of differences between concentration of colostral IgG estimated by RID and APE was determined by the two-tailed T test, using Microsoft Office Excel software. Differences with p-values of <0.05 were considered significant. The correlation between results of % Brix and concentrations of colostral IgG and γ globulin (RID and APE) and the correlation between % Brix and concentrations of new-born serum γ globulin (APE) were determined by linear correlation using OriginPro 8 software. Differences with p-values of <0.05 were considered significant.

RESULTS

Sixteen (n=16) first milking composite colostrum samples were collected from Holstein-Friesian heifers. Their quality was assessed by Brix refractometry, and concentration of IgG and γ globulin (containing mainly IgG) were determined with RID and APE (Table 1, Figures 1 and 2). The mean ± SD % Brix score was 25.5±3.4 %. The concentration of total proteins in colostral whey was 134±30 g/L. In the agarose gel, proteins of colostrum whey were separated into fractions whose electrophoretic mobility corresponded to albumin, α, β and γ globulins (Figure 1A). The relative concentration of the γ globulin fraction (mostly IgG1) [33] was 75±4 %. The absolute concentration (g/L) of γ globulin was 100±24 g/L. When IgG was measured by RID (Figure 2) the concentration was 130±33 g/L.

The correlation between results obtained with Brix refractometer and concentrations of total colostral whey proteins, γ globulins (i.e. IgG) measured with APE and IgG measured with RID was determined by linear correlation analysis (Figure 3). Correlation between Brix values and the concentrations of IgG determined with both methods

and also the correlation between Brix values and concentration of total colostral whey proteins was positive and statistically highly significant ($P<0.001$ and $P<0.01$). We also compared the concentration of colostral IgG obtained by APE (colostral whey γ globulins) and RID (whole colostrum IgG) and found that there was a statistically significant positive correlation ($P<0.001$) between these two techniques (Figure 4). However, concentrations of γ globulins obtained by APE were significantly lower than concentrations of IgG obtained by RID (21±16 %). The correlation between colostral whey protein and colostral IgG was positive and statistically significant, independently of the method used for the determination of IgG concentration (Figure 5).

Table 1. Assessing the colostrum quality by Brix measurement and by determination of the colostral γ globulin with agarose gel serum protein electrophoresis (APE) and colostral IgG with radial immunodiffusion (RID).

Samples n=16	Brix[1] (%)	Total col. whey proteins[2] (g/l)	γ globulin by APE		IgG by RID[5] (g/l)
			(%)[3]	(g/l)[4]	
1	21,5	106	74	78	70
2	20,7	91	77	70	97
3	26,2	147	78	115	129
4	29,0	168	77	129	159
5	28,2	171	73	124	165
6	27,3	146	76	111	140
7	18,0	84	74	62	65
8	26,5	136	72	98	155
9	28,1	137	74	102	142
10	30,8	163	71	115	143
11	24,6	114	76	87	85
12	28,4	181	76	138	165
13	26,2	116	79	92	160
14	25,8	114	71	81	135
15	25,0	104	67	70	123
16	22,4	161	84	135	154
mean	25,5	134	75	100	130
SD	3,4	30	4	24	33
mediana	26,2	137	75	100	141
min	18,0	84	67	62	65
max	30,8	181	84	138	165
CV (%)	13	23	5	24	26

[1]Colostral Brix readings; [2]Total proteins in colostral whey determined by Biuret reaction; [3]Relative content of γ globulins (%) determined by APE; [4]Concentration of colostral γ globulins determined by APE and densitometry.; [5]Concentration of colostral IgG determined by RID

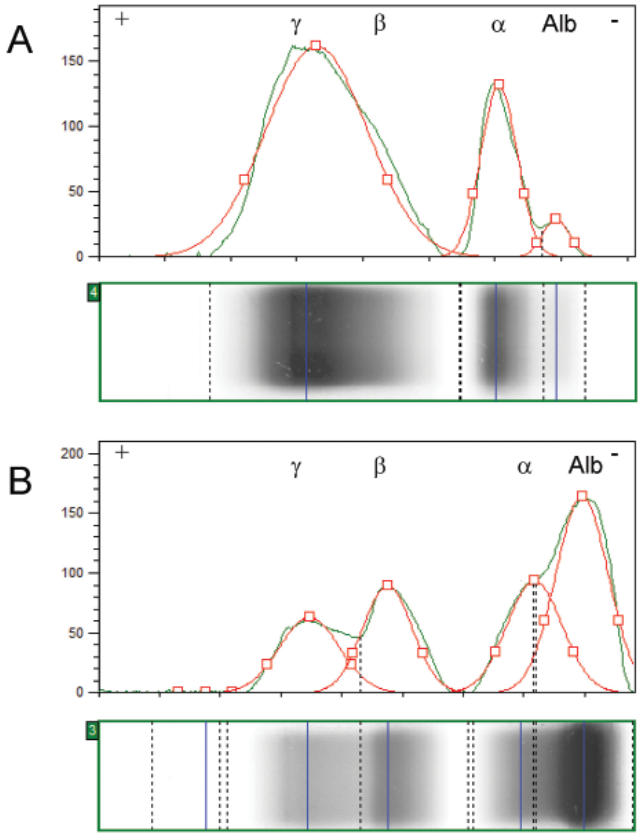

Figure 1. Agarose gel protein electrophoretic (APE) pattern in heifer's colostral whey **(A)** and new-born calf serum **(B)**. α – α globulin, β – β globulin, γ – γ globulin; Dark line: Densitometry recorded color intensity; Red line: Deconvolution of the densitometric profile.

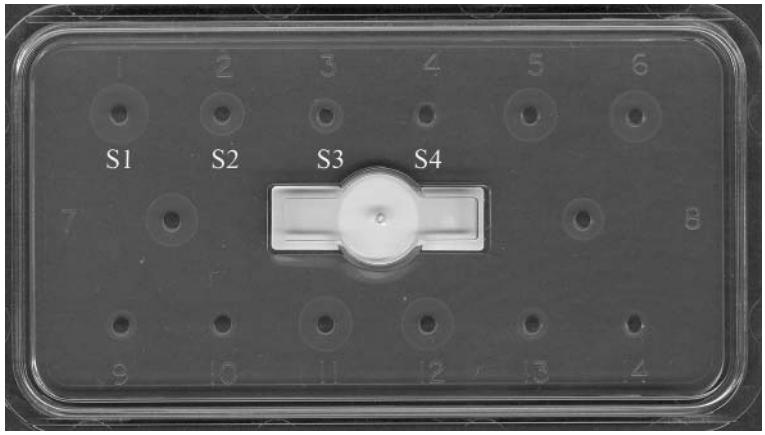

Figure 2. Determining the concentration of colostral IgG with radial immunodiffusion (RID) assay. Wells 1-4, S1-S4: Standard IgG samples containing 2.5, 1.25, 0.62, and 0.31 g/L IgG respectively. Wells 5-14: Diluted colostrum samples.

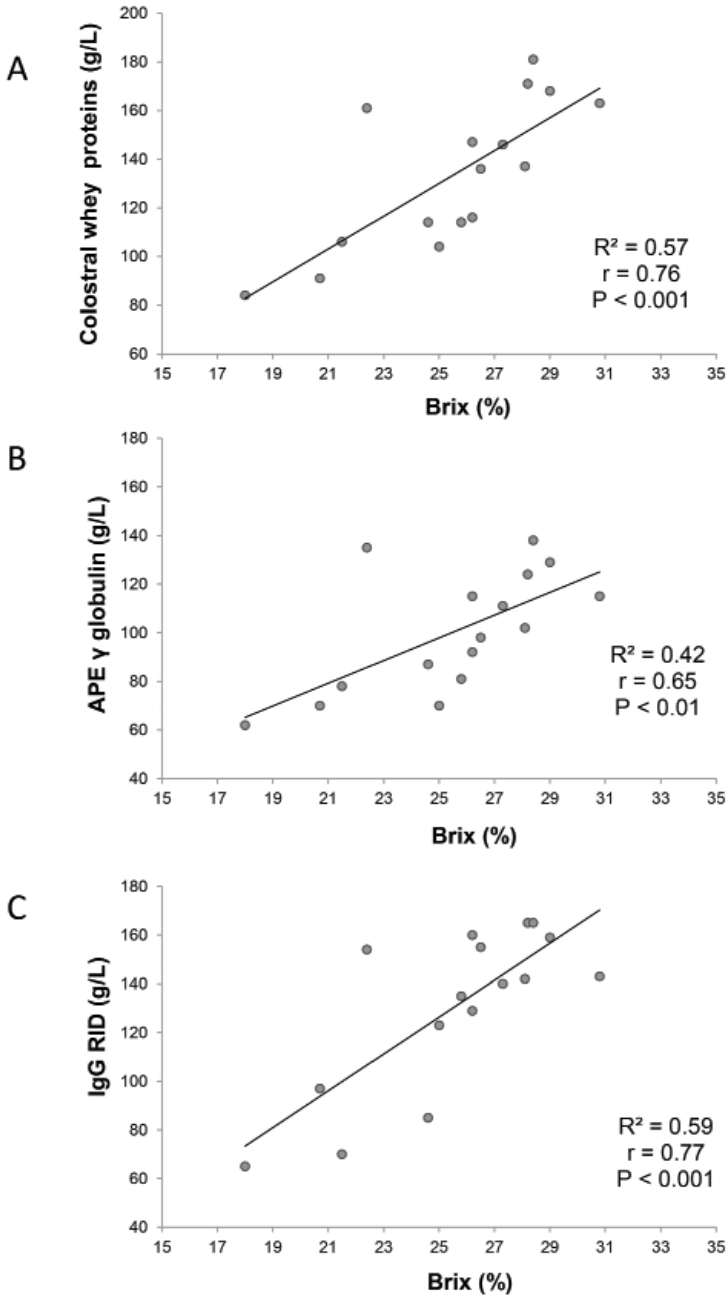

Figure 3. Correlation between Brix scores (%) and total colostral proteins **(A),** APE determined concentration of γ globulins **(B)** and RID determined concentration of colostral IgG **(C)**. (●) – Experimental data; (──) – Linear regression analysis.

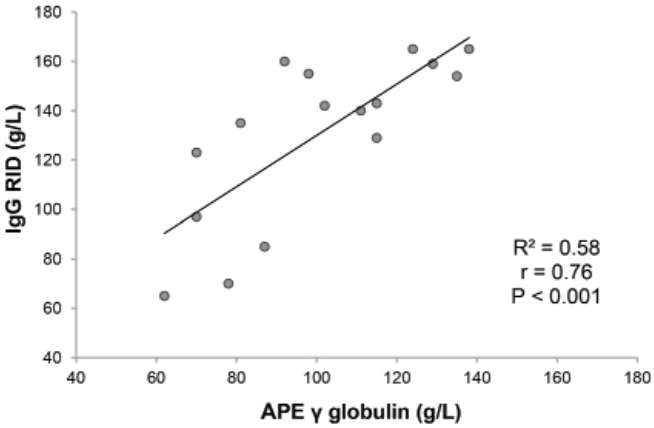

Figure 4. Correlation between APE determined concentration of γ globulins and RID determined concentration of colostral IgG. (●) – Experimental data; (——) – Linear regression analysis.

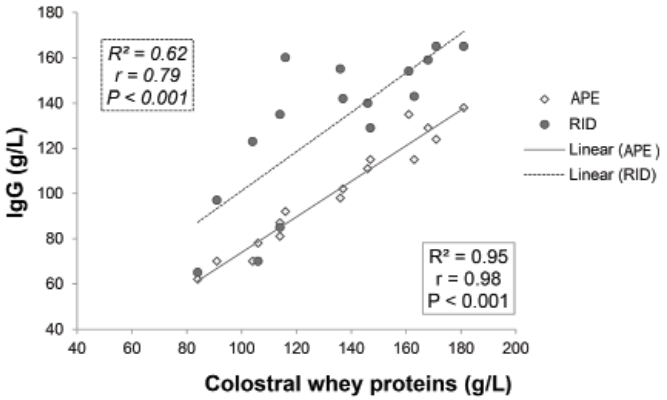

Figure 5. Correlation between APE determined concentration of γ globulins (IgG) and RID determined concentration of colostral IgG (♦) and concentration of total colostral proteins. (●) – Experimental data; (——) – Linear regression analysis for APE; (.....) – Linear regression analysis for RID. APE: R^2, r, and P given in regular letter. RID: R^2, r, and P given in italic.

Further on we evaluated FPT by analyzing the 3-6 days old calves' blood sera with Brix refractometry, the concentration of total blood serum proteins and the concentration of γ globulins (IgG) determined by APE (Table 2, Figure 1B). The mean total serum protein concentration was 57.75±11.8 g/L, γ globulins concentration was 14.4±7.8 g/L and the Brix score was 8.6±1.0 %. 10 samples had γ globulins concentrations lower than 10 g/L, consistent with inadequate passive transfer. The percentage of calves with FPT (IgG<10 g/L) was 34.5 % (10/29 calves). Brix score significantly correlated with the concentration of blood serum γ globulins in all examined calves (Figure 6). Thus, a value of % Brix score lower than 8.6 (mean+2SD), which reflects a concentration of serum γ globulin of <10 g/L, most accurately predicted FPT. Unlike γ globulins, total serum proteins positively correlated with Brix scores only in sera

having more than 10 g/L γ globulins. In accordance with this finding was the result showing that the concentration of total serum proteins positively correlated with the level of γ globulins only if their concentration exceeded 10 g/L (Figure 7).

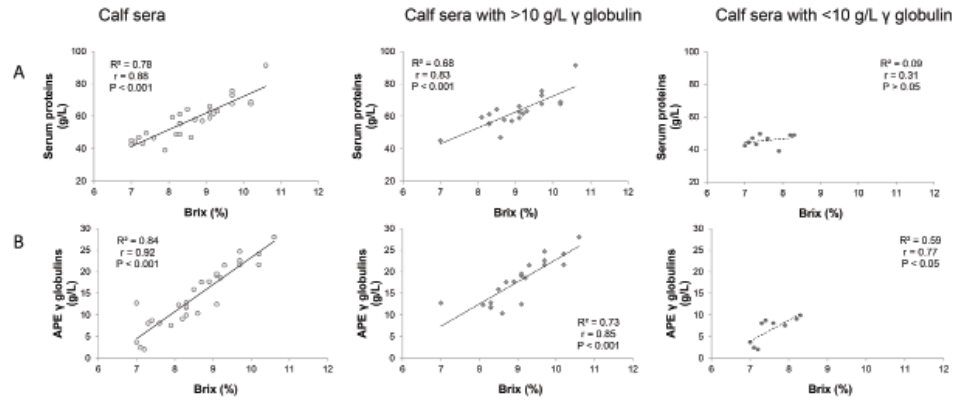

Figure 6. Correlation between Brix scores (%) and total new-born serum proteins **(A)**, APE determined concentration γ globulins **(B)**. (o,♦,●,) – Experimental data for total sera, sera having less 10 g/L and sera more than 10 g/L of γ globulins; (——) – Linear regression analysis.

Table 2. Quality of new-born calves' sera assessed by Brix refractometer and by agarose gel serum protein electrophoresis (APE)

	Brix[1] (%)	**Total serum roteins[2] (g/l)**	**γ globulin by APE**	
			(%)[3]	**(g/l)[4]**
All sera (n=29)				
mean ± SD	8.6 ± 1.0	57.5 ± 11.8	23.3 ± 8.3	14.4 ± 7.8
median	8.5	57.9	23.2	12.8
(min-max)	(7.0-10.6)	(39.0-91.4)	(4.3-35.7)	(2.0-28.0)
CV (%)	11.9	20.6	34.9	48.4
> 10 g/L γ globulin (n=19)				
mean ± SD	9.0 ± 0.7	63.9 ± 9.4	28.0 ± 5.1	18.1 ± 5.1
median	9.1	62.7	30.4	18.6
(min-max)	(8.1-10.6)	(46.9-91.4)	(19.4-35.7)	(10.4-28.0)
CV (%)	9.5	14.8	18.4	28.4
< 10 g/L γ globulin (n=10)				
mean ± SD	7.6 ± 0.5	45.5 ± 3.5	14.5 ± 6.4	6.6 ± 3.0
median	7.4	46.6	17.6	8.1
(min-max)	(7.0-8.3)	(39.0-49.6)	(4.3-20.3)	(2.0-9.9)
CV (%)	6.3	7.8	44.1	45.7

[1]Serum Brix readings; [2]Total proteins in serum by Biuret reaction; [3]Relative content of γ globulins (%) determined by APE; [4]Concentration of serum γ globulin determined by APE and densitometry.

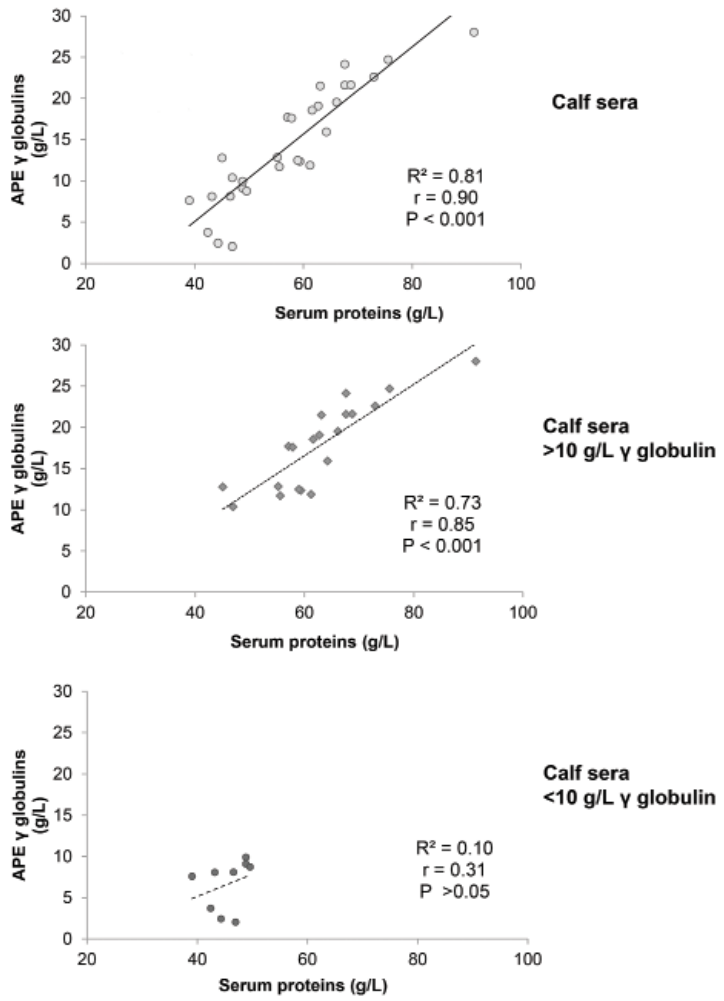

Figure 7. Correlation between the APE determined concentration of γ globulins and the concentration of total new-born serum proteins. (o,♦,●,) – Experimental data for total sera, sera having less 10 g/L and sera more than 10 g/L of γ globulins; (—) – Linear regression analysis.

DISCUSSION

The concentration of IgG in the colostrum has traditionally been considered the most important component for evaluating colostrum quality as high-quality colostrum is considered the one which has more than 50 g/L IgG [10]. The current recommendation is to assess the quality of collected colostrum before it is fed to calves [1], but this is not applied on our farms. On the farms owned by PKB Corporation (Belgrade, Serbia) colostrum quality is estimated by colostrometry and visual inspection. However, several studies [20,31,32] reported a low correlation between the concentration of colostral

IgG measured by RID and colostral specific gravity. Refractometry with the Brix refractometer has been proposed as a way to estimate IgG concentration in bovine colostrum [1]. Both the optical and digital Brix refractometers showed considerable potential in determining colostrum quality [1]. The same authors reported that Brix refractometry gave accurate results independently whether colostrum samples were fresh or frozen.

Brix refractometers are widely used in dairy farms in developed countries. On our dairy farms it is not used at all, even though its price is not an obstacle. In the current study we undoubtedly showed the importance of applying refractometry thus recommending it for testing the colostrum quality. In accordance with the results of Bartens et al. [33] our results also showed that the Brix refractometer has provided a rapid and accurate tool for the measurement of colostral IgG and determination of colostrum quality. In our study we showed that Brix values were highly correlated with colostral IgG analyzed by RID. This finding is similar to the results of other research groups [1,15,34,35], who also found a moderate to high correlation between % Brix and IgG measured by RID. Besides we found that there was a significant, but somewhat lower, correlation between the results obtained with the Brix refractometer and the concentration of colostral whey IgG measured with APE. We have not expected that the two methods provide identical results because in the fraction of γ globulins, beside IgG representing the predominant class of immunoglobulins, there are small amounts of IgM and IgA in colostral whey and calf serum after colostrum ingestion [36]. However, the recorded lower concentration of colostral whey IgG is not unexpected. It is known that bovine IgG might form complexes with lipids [37] and its removal by centrifugation of colostrum can be one of the reasons for a lower concentration of IgG in colostral whey. Besides, any purification process (such as removal of colostrum lipids by centrifugation followed by casein precipitation) results in a decreased in yield of purified molecules. Independently of the lower yield of colostral whey γ globulins, their concentration determined by APE significantly correlated with the concentration of colostral IgG determined by RID. This result pointed that APE can be used as an accurate method for laboratory determination of IgG in bovine colostrum and it can be used for the assessment of colostrum quality when RID is not available.

Data on APE analysis of bovine colostral whey proteins are rare. We found that a relative concentration of colostral whey γ globulins (IgG) was 75±4 %. Lopez et al. [38], using cellulose acetate electrophoresis, reported a relative concentration of colostral whey γ globulin of 62.4 % within 12 hours of delivery. Besides, we found that the significant correlation between colostral whey IgG and total colostral whey proteins can be also confirmed by APE. According to our knowledge APE is not used by other research groups for determining this correlation. However, Chen et al. [39] and Quiles et al. [40] applying APE, confirmed the significant correlation coefficient between colostral *γ* globulin and total protein in goats during the first 5 days after parturition. Such a high correlation gives us the possibility to use the biuret method for rapid screening of colostrum quality in laboratory work. This correlation is due to

the fact that the IgG is the predominant immunoglobulin and predominant protein in the colostrum [14].

Our previous studies estimated FPT and IgG concentration in calves serum using RID as the gold standard. Knowing that the Brix refractometer can be used to measure IgG concentrations in the calf serum [41] we have investigated further options for its application on our farms. Its advantages were previously described by Wallace et al. [42] who reported that the results of refractometry from centrifuge- and noncentrifuge-harvested sources of serum were highly correlated so producers can conduct this test on-farm without the need of a centrifuge. Similar to results of Deelen et al. [41], our results showed that % Brix highly correlated with the concentration of serum IgG, when all calves where considered, independently of the serum IgG concentration. This result undoubtedly showed that Brix refractometry can be used for assessing FPT. Also, similar to Deelen et al. [41] we found a correlation between % Brix and total serum proteins when all sera were included in the calculation. However, when we analyzed sera of calves with FPT (<10 g/L IgG) we did not find a significant correlation between % Brix and the concentration of total serum proteins. Besides, we did not find correlations between total serum proteins and serum IgG. It is known that different proteins do not have the same refractive indexes. We think that in the sera of calves with FPT, decrease of total serum proteins concentration and the differences in relative concentrations (percentages) in the major protein fractions (*data not shown*) probably results in an absence of correlation between the analyzed parameters.

CONCLUSION

The result of this study have confirmed that Brix refractometer scores were highly correlated with the concentration of both measured colostral IgG and new-born calves serum IgG which means that digital Brix refractometry allows producers to use this technique to estimate colostral and calf serum IgG, thereby monitoring both colostrum quality and success of passive transfer.

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Authors' contributions

SM performed all experiment, analysis data and wrote the manuscript. FN designed and oversaw th research, analysed data and wrote the manuscript. IV helped in extablishing analytical methods, analysed data and assisted with preparation of the manuscript.

KM and SO performed experiment and analysed data. GD, ĐR nad VO critically read the manuscript and assisted with preparation of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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BRIX REFRAKTOMETRIJA KOLOSTRUMA PRVOTELKI MLEČNIH KRAVA I KRVNOG SERUMA NOVOROĐENE TELADI U PROCENI NEUSPEŠNOG PASIVNOG TRANSFERA

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Neuspešan pasivni transfer (FPT) imunoglobulina (IgG) u vezi je sa povećanim morbiditetom i mortalitetom kod teladi. U ovoj studiji vršili smo procenu upotrebe Brix refraktometra za utvrđivanje FPT. Digitalnim Brix refraktometrom analizirano je ukupno 16 uzoraka kolostruma iz prve muže (2-3h posle teljenja) od junica holštajn frizijske rase i 29 uzoraka krvnog seruma teladi iste rase, starosti 3-6 dana. Ukupni proteini određeni su biuretskom metodom. Kolostralni IgG su određivani radijalnom imunodifuzijom (RID), a γ globulini (u čijem sastavu su pregomintno IgG) kolostralnog i krvnog seruma elektroforezom u gelu agaroze (APE) i denzitometrijom. Dobijene vrednosti za % Brix-a u kolostrumu iznosile su 25,5±3,4 %. Koncentracije IgG u kolostrumu i γ globulina u kolostralnom serumu bile su 130±33 g/l i 100±24 g/l pojedinačno. Koncentracija ukupnih proteina u kolostralnom serumu iznosila je 134 ± 30 g/l. Korelacija između vrednosti Brix-a i koncentracije IgG određenih RID i γ globulina određenih APE metodom bila je pozitivna i visoko statistički značajna ($P<0,001$ i $P<0,01$). Koncentracija serumskih proteina novorođene teladi bila je 57,75±11,8 g/l, koncentracija γ globulina 14,4±7,8 g/l a vrednosti % Brix-a 8,6±1,0%. Procenat teladi sa FPT (IgG<10g/L) bio je 34,5% (10/29 teladi). Vrednosti dobijene Brix-om značajno koreliraju sa koncentracijom γ globulina (IgG) krvnog seruma kod svih ispitivanih teladi. Rezultati ove studije pokazuju da upotreba digitalnog Brix refraktometra omogućava proizvođačima upotrebu ove tehnike za procenu IgG kolostruma i seruma teladi, što znači da se može istovremeno koristiti za praćenje kvaliteta kolostruma kao i uspešnog pasivnog transfera.