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Original article

# Evaluation of usefulness of a commercial agarose gel electrophoresis kit (QuickGel SP) for bovine serum protein electrophoresis

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## Abstract

The aim of this study was to show the usefulness of a commercial agarose gel electrophoresis (AGE) kit (QuickGel SP) for separating bovine serum protein fractions in comparison with conventional cellulose acetate electrophoresis (CAE). Serum protein bands were verified using five reference reagents corresponding to albumin and  $\alpha_1$ -,  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins. AGE clearly revealed six separated fractions of albumin and  $\alpha_1$ -,  $\alpha_2$ -,  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulin fractions in 100% and 77.8% in serum samples of dairy cows from the healthy ( $n=27$ ) and diseased groups ( $n=27$ ), respectively. The  $\alpha_1$ - and  $\alpha_2$ -globulins were not separated by CAE in 14.8% and 96.3% of the samples from the healthy and diseased groups, respectively, whereas  $\beta_2$ - and  $\gamma$ -globulin were not separated by CAE in 96.3% and 100% of the samples from the healthy and diseased groups, respectively. More than 94% of the points for the  $\alpha$ -globulin fractions ( $\alpha_1$ - and  $\alpha_2$ -globulins), the  $\beta$ - $\gamma$ -globulin fractions ( $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins), and the albumin/globulin ratio between AGE and CAE were within agreement on the Bland-Altman plots. However, the mean biases were not near zero in the albumin and  $\beta$ - $\gamma$ -globulin fractions. These results suggest that the high-resolution commercial AGE kit can be utilized to separate bovine serum protein fractions.

**Key words:** agarose gel electrophoresis, bovine serum protein, cellulose acetate electrophoresis, QuickGel SP

## Introduction

In bovine practice, total serum protein concentrations, the albumin-to-globulin (A/G) ratio and serum protein electrophoresis (SPE) results should be evaluated in cases of dysproteinemia (Morris and Johnston 2009). SPE is a technique used to separate serum

proteins by size and electrical charge, allowing identification and quantification of the protein fractions (Tappin et al. 2011). The protein bands separated by SPE are usually divided into four main fractions, including albumin and  $\alpha$ -,  $\beta$ -, and  $\gamma$ -globulins. The  $\alpha$ - and  $\beta$ -globulins are further divided into  $\alpha_1$ - and  $\alpha_2$ - or  $\beta_1$ - and  $\beta_2$ -globulins, respectively (Alberghina et al.

2011, Tóthová et al. 2014). The number of serum protein fractions varies with the type of supporting medium (Alberghina et al. 2011). Cellulose acetate is the most popular supporting medium for performing routine SPE, because of its simplicity, reproducibility, reliable quantification of the protein fractions by densitometry and relatively low cost (Keren 2003a). Agarose gel is now beginning to replace cellulose acetate in veterinary and human medicine, as it provides superior reproducibility of results and greater electrophoretic pattern clarity (Alberghina et al. 2011, Tóthová et al. 2014). In general, the resolution on cellulose acetate electrophoresis (CAE) is inferior to that obtained by most agarose gel electrophoresis (AGE) (Keren 2003a, Alberghina et al. 2011, Tóthová et al. 2014).

A commercial AGE kit (QuickGel SP; J711, Helena Laboratories Japan, Saitama, Japan) was recently developed for human SPE; however, the precision and utility of the serum protein fractions determined by using this AGE kit in bovine practice have not been reported. In addition, few reports have considered the electrophoretic patterns of bovine serum protein fractions obtained from AGE compared with those obtained from CAE, although several studies have reported normal serum protein reference values in cattle using AGE (Alberghina et al. 2011, Tóthová et al. 2014).

The aim of the present study was to evaluate the precision of bovine serum protein fractions obtained using this commercial AGE kit and apply it to reveal the serum protein electrophoretic patterns in dairy cows using this kit.

## Materials and Methods

The study protocol and experimental design were approved by the Iwate University Laboratory Animal Care and Use Committee (#201330).

### Reagents to verify the serum protein fractions

To verify each protein fraction, bovine serum albumin (albumin from bovine serum; Sigma-Aldrich, St. Louis, MO, USA), human  $\alpha_1$ -antitrypsin ( $\alpha_1$ -antitrypsin from human; Sigma-Aldrich), bovine transferrin (transferrin, bovine, Holo Form; Life Technologies, Grand Island, NY, USA), bovine fibrinogen (fibrinogen from bovine plasma; Sigma-Aldrich), and bovine  $\gamma$ -globulin ( $\gamma$ -globulin from bovine, fraction II; Tokyo Chemical Industry, Tokyo, Japan) were used as references corresponding to the protein fractions of albumin and  $\alpha_1$ -,  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins, respectively

(Keren 2003b, O'Connell et al. 2005, Dawson et al. 2011). Each reagent was prepared alone for electrophoresis and as a mixture (bovine serum albumin: 33 g/L, human  $\alpha_1$ -antitrypsin: 10 g/L, bovine transferrin: 10 g/L, bovine fibrinogen: 10 g/L, bovine  $\gamma$ -globulin: 33 g/L).

### Serum samples

A total of 54 serum samples obtained from 27 clinically healthy Holstein dairy cows (healthy group) and 27 other Holstein dairy cows with various diseases (diseased group) were used. All cows in the healthy group were lactating (2.2-9.6 years) and came from a single commercial dairy farm. Cows in the diseased group (2.1-12.0 years) were also lactating but were from different dairy farms. The diseased cows were clinically diagnosed with chronic nephritis (n=1), ketosis (n=1), left abomasal displacement (n=1), mastitis (n=15), metritis (n = 5), pneumonia (n=1), ruminal acidosis (n=1), or theileriosis (n = 2). Blood was withdrawn from the jugular or coccygeal veins of the animals and the sera were separated and frozen at -50°C until analyses.

### Agarose gel electrophoresis (AGE)

AGE was performed using the QuickGel SP agarose gel kit according to the manufacturer's instructions (Helena Laboratories). Briefly, 30  $\mu$ L of each sample was applied to numbered sample wells of the sample plate (J637, Helena Laboratories). Each plate accommodated up to 20 samples. The samples were applied on the QuickGel SP using an applicator (J653, Helena Laboratories). The gels were electrophoresed at 240 V for 8 min at 20°C using the QuickGel Chamber P (J678, Helena Laboratories). Then, the gels were dried and stained in Ponceau S (J845, Helena Laboratories) for 5 min. After destaining in 5% acetic acid solution and drying completely, the gels were scanned on a QuickScan densitometer (J121, Helena Laboratories). QuickScan computer software was used to identify the lanes, subtract background, and obtain the densitometric tracing (electrophoretogram) for each sample. Each electrophoretogram was examined visually, and the protein fractions were identified and labelled using the software. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/L) were calculated using the total serum protein concentration.

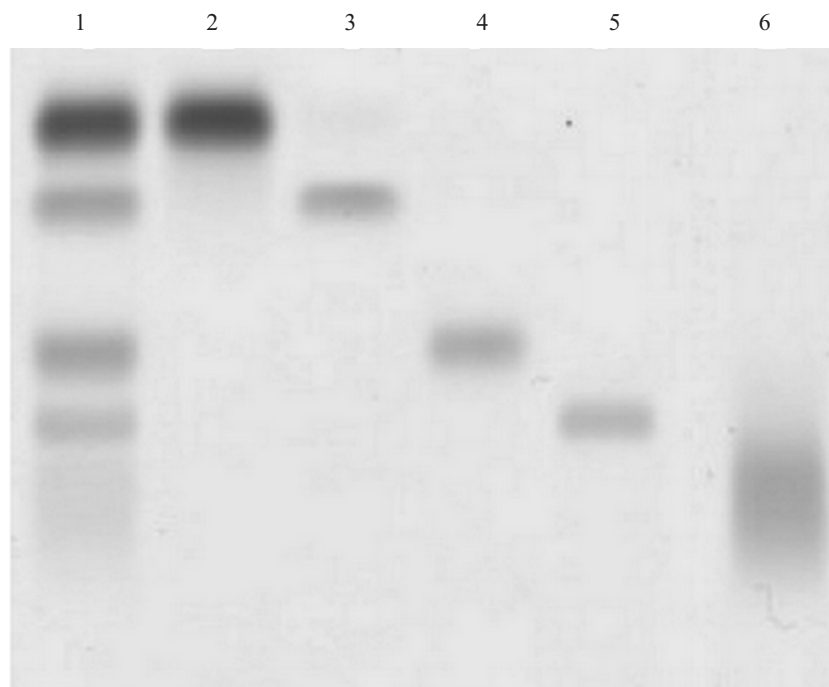


Fig. 1. Photograph of agarose gel electrophoresis (AGE) using the reference reagents. Lane 1: mixture of bovine serum albumin (33 g/L), human  $\alpha_1$ -antitrypsin (10 g/L), bovine transferrin (10 g/L), bovine plasma fibrinogen (10 g/L), and bovine  $\gamma$ -globulin (33 g/L). Lane 2: bovine serum albumin (33 g/L) as an albumin reference fraction. Lane 3: human  $\alpha_1$ -antitrypsin (10 g/L) as a reference for the  $\alpha_1$ -globulin fraction. Lane 4: bovine transferrin (10 g/L) as a reference for the  $\beta_1$ -globulin fraction. Lane 5: bovine plasma fibrinogen (10 g/L) as a reference for the  $\beta_2$ -globulin fraction. Lane 6: bovine  $\gamma$ -globulin (33 g/L) as a reference for the  $\gamma$ -globulin fraction. Lane 7: serum sample from a 4.7-year-old cow with mastitis in the diseased group (total protein concentration: 78 g/L).

### Cellulose acetate electrophoresis (CAE)

CAE was performed manually using an electrophoresis chamber (Model EPC105AA; Advantec, Tokyo, Japan) with SEPARAX cellulose acetate strips (Jokoh, Tokyo, Japan) in 0.06 mol/L sodium barbital buffer, pH 8.6 (Wako, Osaka, Japan). The samples (0.8  $\mu$ L) were applied to the strips using a micropipette and electrophoresed at 0.8 mA/strip for 40 min at room temperature. The strips were stained with Ponceau 3R (Wako), destained in 5% acetic acid solution, dried completely, and then diaphanized in decahydronaphthalene (Wako). The diaphanized strips were scanned on the QuickScan densitometer and evaluated in the same manner as for AGE.

### Biochemical determination of serum total protein and albumin concentrations

Serum concentrations of total protein (TP) and albumin were determined by the Biuret and bromocresol purple (BCP) methods, respectively, using an automated clinical chemistry analyzer (ACCUTE TBA-40FR autoanalyzer; Toshiba Medical Systems, Tochigi, Japan).

### Statistical analysis

All numerical data from the serum electrophoretic and biochemical analyses of the 54 dairy cows (healthy and diseased groups) were subjected to the following statistical analyses. The chi-square ( $\chi^2$ ) test was used to examine the associations between the electrophoretic method and the separation of the globulin fractions because the  $\alpha_1$  and  $\alpha_2$ -globulin and  $\beta_1$ -,  $\beta_2$ - and  $\gamma$ -globulin fractions were not separated on the electrophoretograms of some samples. Serum albumin concentrations obtained from AGE were compared with those obtained from CAE or BCP according to a standard recommendation for comparative analytical techniques based on Deming regression and Bland-Altman analyses (Bland and Altman, 1986; Kotake and Sato 2009). Similarly, comparisons of the serum concentrations of the  $\alpha$ -globulin fraction (i.e., total fraction of  $\alpha_1$  and  $\alpha_2$ -globulins) or the  $\beta$ - $\gamma$ -globulin fraction (i.e., a total fraction of  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins) and the A/G ratio obtained from AGE with those obtained from CAE were also analyzed using Deming regression and Bland-Altman analyses. Medians and interquartile ranges (IQRs) were calculated as concentrations for each protein fraction and age of cows in the healthy group using descriptive

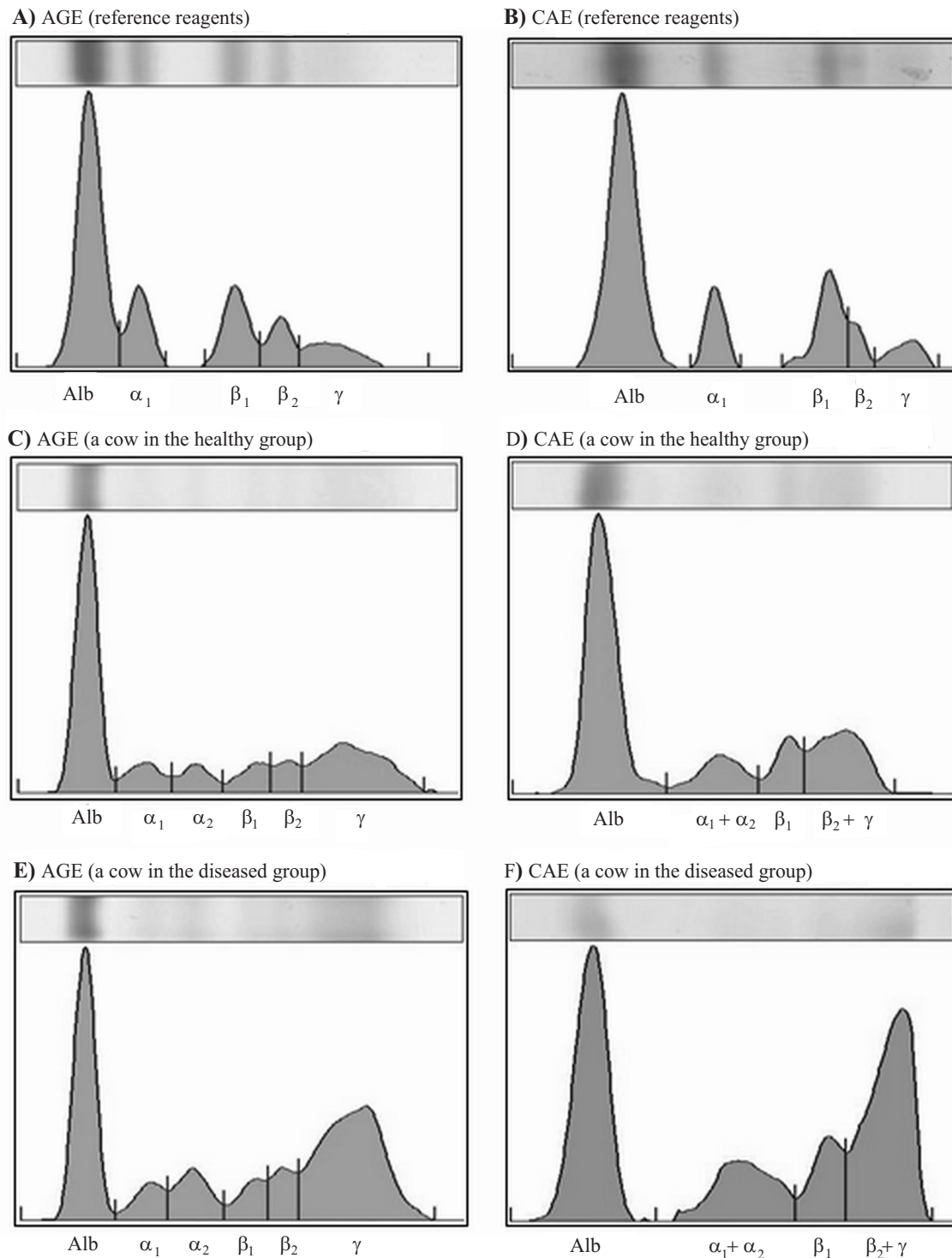


Fig. 2. Agarose gel electrophoresis (AGE) and cellulose acetate electrophoresis (CAE) densitometer trace pattern images of the mixture of reference reagents and serum samples from two cows in the healthy and diseased groups. AGE (A) and CAE (B) electrophoretograms of the mixture of reference reagents in Fig. 1 (lane 1). AGE (C) and CAE (D) electrophoretograms of serum sample from a 3.1-year-old cow in the healthy group (total protein concentration: 79 g/L). AGE (E) and CAE (F) electrophoretograms of serum sample from a 7.5-year-old cow with mastitis in the diseased group (total protein concentration: 83 g/L). Alb, albumin fraction corresponding to bovine serum albumin.  $\alpha_1$ ,  $\alpha_1$ -globulin fraction corresponding to human  $\alpha_1$ -anti-trypsin.  $\beta_1$ ,  $\beta_1$ -globulin fraction corresponding to bovine transferrin.  $\beta_2$ ,  $\beta_2$ -globulin fraction corresponding to bovine plasma fibrinogen.  $\gamma$ ,  $\gamma$ -globulin fraction corresponding to bovine  $\gamma$ -globulin.  $\alpha_1 + \alpha_2$ : unseparated fraction of  $\alpha_1$  and  $\alpha_2$ -globulins.  $\beta_2 + \gamma$ : unseparated fraction of  $\beta_2$  and  $\gamma$ -globulins.

Table 1. Chi-square test<sup>1</sup> to verify the association between electrophoretic method and separation of the globulin fractions.

Fraction	Group	Separation	Agarose gel electrophoresis	Cellulose acetate electrophoresis	$\chi^2$	P value
$\alpha$ -globulins fraction ( $\alpha_1$ - and $\alpha_2$ -globulins)	All cows (n=54)	Separation	54	24	38.82	< 0.0001
		Non-separation	0	30		
	Healthy group (n=27)	Separation	27	23	2.43	0.119
		Non-separation	0	4		
	Diseased group (n=27)	Separation	27	1	46.36	< 0.0001
		Non-separation	0	26		
$\beta$ to $\gamma$ -globulins fraction ( $\beta_1$ -, $\beta_2$ - and $\gamma$ -globulins)	All cows (n=54)	Separation	48	1	79.05	<0.0001
		Non-separation	6	53		
	Healthy group (n=27)	Separation	27	1	46.36	< 0.0001
		Non-separation	0	26		
	Diseased group (n=27)	Separation	21	0	31.17	< 0.0001
		Non-separation	6	27		

<sup>1</sup> Chi-square test with Yate's correction was used.

Table 2. Deming regression analysis to compare agarose gel electrophoresis (AGE) with cellulose acetate electrophoresis (CAE) or the bromocresol purple (BCP) method in bovine serum samples.

Comparison	Protein fraction	n	Slope		y-Intercept		P value
			mean	95% CI <sup>1</sup>	mean	95% CI <sup>1</sup>	
AGE vs. CAE	Albumin	54	1.090	(0.921-1.259)	0.006	(-0.579-0.591)	< 0.0001
	$\alpha$ -globulins fraction ( $\alpha_1$ - and $\alpha_2$ -globulins)	54	0.927	(0.698-1.163)	0.020	(-0.236-0.277)	< 0.0001
	$\beta$ to $\gamma$ -globulins fraction ( $\beta_1$ -, $\beta_2$ - and $\gamma$ -globulins)	54	1.008	(0.951-1.066)	-0.272	(-0.458-0.086)	< 0.0001
	A/G ratio	54	1.232	(1.002-1.462)	-0.149	(-0.376-0.078)	< 0.0001
AGE vs. BCP	Albumin	54	0.926	(0.786-1.067)	0.317	(-0.169-0.803)	< 0.0001
CAE vs. BCP	Albumin	54	0.844	(0.693-0.996.)	0.333	(-0.239-0.905)	< 0.0001

<sup>1</sup> CI: confidence interval.

statistical procedures. All statistical analyses were performed with Prism ver. 6 for Windows software (GraphPad Software Inc., La Jolla, CA, USA). A p<0.05 was considered significant.

## Results

Serum concentrations of TP in cows of the healthy and diseased groups were 65-83 g/L and 49-120 g/L, respectively.

Fig. 1 shows an agarose gel image after AGE of the reference reagents of albumin;  $\alpha_1$ -,  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins. Each reference reagent (lanes 2-6) migrated to a different position, and the mixture of reagents revealed markedly separated fractions corresponding to individual reagents (lane 1).

Fig. 2 shows representative electrophoretograms of the mixture of reference reagents and two serum samples obtained from AGE and CAE. As for the

serum samples, the AGE electrophoretogram (Fig. 2C) of a 3.1-year-old cow in the healthy group (TP: 79 g/L) showed albumin: 37.4 g/L (47.3%),  $\alpha_1$ -globulin: 6.4 g/L (8.1%),  $\alpha_2$ -globulin: 5.2 g/L (6.6%),  $\beta_1$ -globulin: 5.3 g/L (6.7%),  $\beta_2$ -globulin: 4.7 g/L (5.9%) and  $\gamma$ -globulin: 20.1 g/L (25.4%), whereas the CAE electrophoretogram in this cow (Fig. 2D) revealed albumin: 45.3 g/L (57.4%), unseparated fraction of  $\alpha_1$ - and  $\alpha_2$ -globulins: 9.2 g/L (11.8%),  $\beta_1$ -globulin: 7.8 g/L (9.9%), unseparated fraction of  $\beta_2$ - and  $\gamma$ -globulins: 16.7 g/L (21.1%). The 7.5-year-old cow with mastitis in the diseased group (TP: 83 g/L) showed the AGE electrophoretogram (Fig. 2E) with albumin: 26.7 g/L (32.2%),  $\alpha_1$ -globulin: 5.0 g/L (6.0%),  $\alpha_2$ -globulin: 7.7 g/L (9.3%),  $\beta_1$ -globulin: 5.1 g/L (6.2%),  $\beta_2$ -globulin: 5.5 g/L (6.6%) and  $\gamma$ -globulin: 33 g/L (39.7%) and the CAE electrophoretogram (Fig. 2F) with albumin: 31.0 g/L (37.4%), unseparated fraction of  $\alpha_1$ - and  $\alpha_2$ -globulins: 12.9 g/L (15.5%),  $\beta_1$ -globulin: 8.6 g/L (10.4%), unseparated fraction of  $\beta_2$ - and  $\gamma$ -globulins: 30.5 g/L (36.7%).



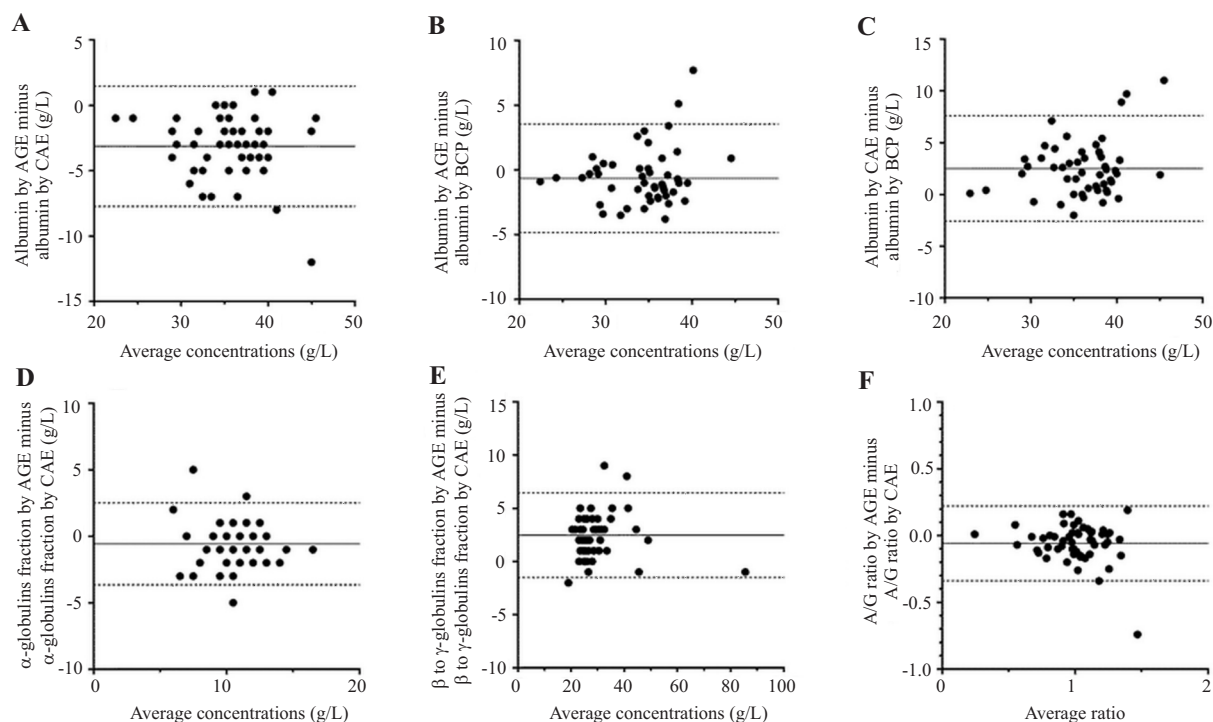


Fig. 3. Bland-Altman plots showing differences in the concentrations of each fraction or the albumin-to-globulin (A/G) ratio estimated by agarose gel electrophoresis (AGE), cellulose acetate electrophoresis (CAE), and the bromocresol purple method (BCP) using serum samples from healthy and diseased cattle. (A) Differences in the albumin concentrations between AGE and CAE ( $n=54$ ). Mean bias (solid line): -3.1. The 95% confidence limits (dotted line) for the agreement plots are -7.7 to 1.4. (B) Differences in the albumin concentrations between AGE and BCP ( $n=54$ ). Mean bias (solid line): -0.6. The 95% confidence limits (dotted line) for the agreement plots are -4.8 to 3.5. (C) Differences in the albumin concentrations between CAE and BCP ( $n=54$ ). Mean bias (solid line): 2.5. The 95% confidence limits (dotted line) for the agreement plots are -2.6 to 7.6. (D) Differences in the  $\alpha$ -globulin fraction concentrations ( $\alpha_1$ - and  $\alpha_2$ -globulins) between AGE and CAE ( $n=54$ ). The  $\alpha_1$ - and  $\alpha_2$ -globulin fractions were not separated by CAE in 30 samples (55.6%) (Table 1). Mean bias (solid line): -0.6. The 95% confidence limits (dotted line) for the agreement plots are -3.6 to 2.5. (E) Differences in the total  $\beta$ - $\gamma$ -globulin fraction concentrations ( $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins) between AGE and CAE ( $n=54$ ). The  $\beta_1$ - and  $\beta_2$ -globulin fractions were not separated by AGE in six samples (11.1%), and the  $\beta_2$ - and  $\gamma$ -globulin fractions were not separated by CAE in 53 samples (98.1%) (Table 1). Mean bias (solid line): 2.5. The 95% confidence limits (dotted line) for the agreement plots are -1.5 to 6.5. (F) Differences in the A/G ratio between AGE and CAE ( $n=54$ ). Mean bias (solid line): -0.06. The 95% confidence limits (dotted line) for the agreement plots are -0.34 to 0.22.

The AGE electrophoretogram clearly revealed six separated fractions of albumin and the  $\alpha_1$ -,  $\alpha_2$ -,  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins in all 27 samples (100%) from the healthy group (Table 1). However, it was difficult to separate the  $\beta_1$ - and  $\beta_2$ -globulin fractions in six samples (22.2%), including four cows with mastitis (2.8, 4.1, 4.5, and 8.0 years of age; TP: 72-83 g/L), one cow with ruminal acidosis (4.1 years; TP: 73 g/L), and one cow with left abomasal displacement (2.1 years; TP: 106 g/L). The  $\alpha_1$ - and  $\alpha_2$ -globulin fractions were not separated on CAE in four and 26 samples from the healthy (14.8%; TP: 74-83 g/L) and diseased (96.3%; TP: 49-120 g/L) groups, respectively, whereas the  $\beta_2$ - and  $\gamma$ -globulin fractions were not separated in 26 and all other samples from the healthy (96.3%; TP: 65-83 g/L) and diseased (100%; TP: 49-120 g/L) groups, respectively.

Table 1 shows the chi-square results to verify the association between the electrophoretic method and

the separation of the globulin fractions. With the exception of the  $\alpha_1$ - and  $\alpha_2$ -globulin fractions in the serum samples from the healthy group ( $p=0.119$ ), AGE revealed significantly more serum samples that were separated into the  $\alpha_1$ - and  $\alpha_2$ -globulin fractions and the  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins compared with CAE ( $p<0.0001$ ).

Table 2 shows the Deming regression analysis results comparing the serum concentrations of the albumin,  $\alpha$ -globulin, and  $\beta$ - $\gamma$ -globulin fractions as well as the A/G ratio obtained from AGE with those obtained from the other methods (CAE or BCP). The serum concentrations of each parameter obtained from AGE were strongly correlated ( $p<0.0001$ ) with those from the other methods, yielding slopes near 1 and intercepts around zero for all estimated values.

Fig. 3 shows the Bland-Altman plots compares the serum concentrations of the albumin,  $\alpha$ -globulins, and  $\beta$ - $\gamma$ -globulin fractions as well as the A/G ratio

obtained from AGE with those obtained using the other methods (CAE or BCP). More than 94% of the points (51/54) were within the agreement plots for each parameter, although two or three points deviated from their plots. The mean biases were close to zero for the difference in albumin between AGE and BCP (-0.6) as well as for the  $\alpha$ -globulin fractions (-0.6) and the A/G ratio (-0.06) between AGE and CAE, whereas the biases in the other parameters were not near zero (-3.1 or 2.5).

## Discussion

Electrophoresis is the standard of reference for fractionating serum proteins in clinical biochemistry (Kaneko 1980). Identifying and quantifying protein fractions enables the identification of animals with altered serum protein patterns, which may reflect responses to changes in homeostasis or disease (Alberghina et al. 2011). Protein electrophoresis of bovine serum has been studied using cellulose acetate and agarose gel as the supporting matrix. Kaneko (1980) reported four fractions in bovine serum separated by CAE. In contrast, recent studies have reported the separation of healthy cow and calf serum proteins into five to six fractions by AGE (Alberghina et al. 2011, Tóthová et al. 2013, 2014).

In the present study, the serum protein fractions obtained from AGE and CAE were verified using five reference reagents corresponding to albumin and  $\alpha_1$ -,  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins. However, human  $\alpha_1$ -antitrypsin was used as a reference corresponding to the  $\alpha_1$ -fraction because of difficulty in purchasing the bovine origins. Although human  $\alpha_1$ -globulin fraction protein characteristics in the electric field may differ from those of bovine, this band seemed to migrate to the predictable location of  $\alpha_1$ -globulin fraction on the AGE and CAE electrophoretograms. Bovine fibrinogen, which is a soluble plasma protein, seems to migrate on the border between  $\beta$ - and  $\gamma$ -globulin class; therefore, it may not be the best candidate to determine the location of  $\beta_2$ -globulin fraction of serum proteins. On the other hand, one investigation has reported that plasma fibrinogen migrates in the  $\beta_2$ -globulin fraction as a distinct separate band between the  $\beta_1$ - and  $\gamma$ -globulin peaks on the AGE electrophoretogram (Dawson et al. 2011). The CAE electrophoretogram revealed five protein fractions (albumin,  $\alpha_1$ -,  $\alpha_2$ -, and  $\beta_1$ -globulin and a fused  $\beta_2$ - and  $\gamma$ -globulin fraction) in the vast majority of healthy samples, whereas nearly all samples from the diseased group separated into four fractions (albumin, a fused  $\alpha_1$ - and  $\alpha_2$ -globulin fraction,  $\beta_1$ -globulin, and a fused  $\beta_2$ - and  $\gamma$ -globulin fraction). The AGE electrophoretogram revealed six protein fractions (albumin and  $\alpha_1$ -

$\alpha_2$ -,  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins) in all cows of the healthy group and in 77.8% (21/27) of the diseased cows, suggesting that the resolution of the AGE kit was superior to that of the conventional CAE system under our laboratory conditions.

In AGE, the  $\beta_1$ - and  $\beta_2$ -globulin fractions did not separate in six samples obtained from the diseased group. The clinical diagnoses in these cows were mastitis (n=4), ruminal acidosis (n=1), and left abomasal displacement (n=1). Globulins are a group of proteins containing antibodies, inflammatory molecules and lipid carriers, vitamins, and hormones (Kaneko 1980). In particular,  $\alpha_1$ - and  $\alpha_2$ -globulins include  $\alpha_1$ -antitrypsin,  $\alpha_1$ -acid glycoprotein,  $\alpha_2$ -macroglobulin, ceruloplasmin, and haptoglobin. The  $\beta_1$ - and  $\beta_2$ -globulin fraction contains C<sub>3</sub> and C<sub>4</sub> complement and plasminogen. These proteins often increase concurrently in acute inflammatory diseases (Kaneko 1980), and the others are acute-phase proteins that undergo substantial changes in concentration following the development of infectious, inflammatory, or traumatic conditions, such as mastitis, ruminal acidosis, arthritis, and hoof diseases by cows (Eckersall et al. 2001, Nielsen et al. 2004, Gozho et al. 2005, Jawor et al. 2008, Kujala et al. 2010). Therefore, the increase in acute-phase proteins made it difficult to separate the  $\beta$ -globulin fraction in six diseased cows.

The Deming regression analysis revealed that all AGE parameters were significantly correlated with those obtained by the other methods. The Bland-Altman analysis showed that more than 94% of the points for each parameter were within the limits of the agreement plots. However, the mean biases were not close to zero for albumin between CAE and AGE or BCP and the  $\beta$ - $\gamma$ -globulin fractions between AGE and CAE, suggesting that the levels of these parameters need to be adjusted when comparing the results obtained from the two methods.

In summary, our results suggest that the high-resolution commercial AGE kit can be utilized to separate bovine serum protein fractions. This electrophoretic method can be executed more easily and rapidly than the conventionally performed CAE technique, which requires several pieces of rare equipment (e.g., sample plates, applicator, chamber, and densitometer). Finally, our data also provide the electrophoretic pattern and reference intervals for the serum protein fractions in clinically healthy dairy cows using the AGE system, which are important background data for bovine medicine. As the electrophoretic patterns of serum proteins differ with age, breed, and physiological condition (e.g., colostrum intake, pregnancy, and lactation) (Pierce 1955a,b, Larson and Kendall 1957, Tumbleson et al. 1973, Kaneko 1980), it is essential that the "normal" elec-

trophoretic patterns and the reference intervals in cattle in various conditions are determined. Further study is necessary to examine the electrophoretic patterns of serum proteins specific to several disease conditions in cattle to develop a useful diagnostic aid for veterinary clinicians.

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