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# Evaluation of the capillary electrophoresis method for measurement of immunoglobulin concentration in ewe colostrum

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## ABSTRACT

Capillary electrophoresis (CE) is a technique routinely used in clinical laboratories that allows the separation and quantification of blood serum proteins in a rapid, precise, accurate, and inexpensive manner. Recently, CE has been proposed to separate and measure colostral proteins, but an evaluation of the agreement between CE and radial immunodiffusion (RID) method, currently used to quantify IgG in colostrum, is still lacking. The purpose of this study was to test the ability of a CE instrument, normally used in blood serum protein analysis, to realize the correct quantification of total Ig concentration in ewe colostrum, using RID assay as reference. Colostrum samples (n = 68) were collected from 35 multiparous Sarda ewes at first milking (n = 33) and at 24 h postpartum (n = 35). The mean ± standard deviation of IgG concentration measured by RID and whey colostrum total Ig concentration measured by CE were 54.76 ± 41.82 g/L and 54.70 ± 41.43 g/L, respectively. Lin's concordance correlation coefficient (r = 0.993; 95% confidence interval = 0.989 to 0.996) and linear regression analysis results (RID = 1.0022CE − 0.063; $R^2$ = 0.986) showed an excellent agreement between these 2 methods. Bland-Altman analysis confirmed that CE method can be a suitable alternative to RID: the mean of the differences between CE and RID was −0.055 ± 4.95 g/L (95% confidence interval = −1.25 to 1.14 g/L) and the agreement limits were −9.75 to 9.60 g/L (low limit 95% confidence interval = −11.82 to −7.68 g/L; high limit 95% confidence interval = 7.57 to 11.72 g/L). In conclusion, the current study indicates that CE method may be a reliable tool for the quantification of the total Ig concentration in ewe colostrum.

**Key words:** colostrum, immunoglobulin, capillary electrophoresis, radial immunodiffusion

## INTRODUCTION

In the last years, the importance of sheep dairy farms has increased worldwide, especially in developing countries (Lérias et al., 2014). To increase milk production, lambs are reared by artificial feeding systems providing them colostrum and milk replacer (Demirören et al., 1995). The early intake of colostrum in ruminant newborns is fundamental to transfer them an adequate quantity of IgG that provides passive immunity and to increase their survival rate (Constant et al., 1994; Stelwagen et al., 2009). Some authors proposed the bovine colostrum as alternative source to feed lambs (Quigley et al., 2002; Moretti et al., 2010), but lambs fed with this colostrum source run the risk of developing anemia (Winter and Clarkson, 1992; Winter, 2011; Ruby et al., 2012). It is preferable to feed lambs with colostrum by creating a high-quality ewe colostrum bank.

To date, the radial immunodiffusion (**RID**) method is the gold standard method for measurement of IgG concentration in blood serum. Moreover, RID assay is currently used to determine IgG concentration also in milk and colostrum even if it is not a reference method for colostral IgG determination. Nevertheless, RID is expensive, laborious for analysis of a large number of samples, and highly time consuming (18 to 24 h to determine the results), so it is not a method eligible for the routine analysis of IgG concentration. Due to the high resolving power and unique selectivity in separation and characterization of proteins (Zhu et al., 1990), capillary electrophoresis (**CE**) is routinely used in the clinical laboratories to separate and quantify blood serum proteins. In the past years, CE has been successfully used to analyze milk whey proteins and has been demonstrated to be rapid and easy to use (Cifuentes et al., 1993; Cartoni et al., 1999) and requires a very small volume of sample. Recently, Ceniti et al. (2016) employed a CE instrument to separate ewe whey colostrum proteins and successively identified on an electrophoretogram the fraction corresponding to total Ig. The authors conclude by proposing the use of CE to measure total Ig concentration in ewe colostrum.

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Therefore, before accepting CE method as alternative to others currently employed in ovine colostral Ig quantification, it is necessary to demonstrate its interchangeability. At this end, the aim of the present study was to assess the level of agreement between CE and RID method in quantifying the Ig concentration in ewe colostrum.

On the basis that IgG represent the major class of colostral Ig in ruminants, reaching 94% of the total Ig content in the ovine colostrum (Lérias et al., 2014), Bland-Altman analysis was performed to evaluate the agreement between the 2 methods.

## MATERIALS AND METHODS

### Colostrum Sample Collection

Between April and July 2014, colostrum samples from 35 multiparous Sarda ewes were collected at first milking within 2 h after lambing (first milking, n = 33) and at 24 h after parturition (n = 35). The farm employee collected a volume of 50 mL of colostrum from each animal by hand-milking into plastic test tubes. Samples were inverted 8 to 10 times to thoroughly mix the colostrum for an accurate homogenization, and an aliquot of each colostrum sample (approximately 5 mL) was transferred into another plastic test tube for measurements of IgG content by RID. Samples were labeled with animal identification number and date of collection and frozen at −20°C. Samples were then transferred to the Magna Græcia University of Catanzaro, where they were stored at −80°C until analysis.

### Radial Immunodiffusion Analysis

Colostrum samples were thawed at 4°C and then warmed for 15 min in a water bath at 40°C and gently mixed to ensure good homogeneity. The IgG concentration was assessed by RID technique using the Sheep and Goat IgG IDRing Test (IDBiotech, ImmunoDiffusion Biotechnologies SARL, Issoire, France).

Briefly, 50 μL of colostrum were diluted in 4,950 μL of physiological solution (dilution 1) and then 50 μL of dilution 1 were diluted in 450 μL of SRID buffer 1X delivered by the manufacturer. The plate wells were filled with 15 μL of sample, reserving 4 wells to the corresponding standards delivered by the manufacturer. A total of 12 plates was used. Plates were placed in a humid box and incubated for 18 to 22 h at 37°C. Diffusion was stopped by adding 5 mL of freshly prepared 2% acid acetic solution (code no. 401424, CARLO ERBA Reagents S.r.l., Cornaredo, Milano, Italy) and leaving the plates for 1 min at room temperature. Plates were rinsed twice with deionized water, and after adding 5 mL of deionized water, they were incubated for 15 min at room temperature. The resulting ring diameters were measured using the IDRing Viewer system (IDBiotech, ImmunoDiffusion Biotechnologies SARL). The standard curve for the single plate was established by plotting the square root concentration on the abscissa against the diameters for each standard on the ordinate ($R^2$ of each plate was between 0.98 and 0.99), and IgG concentration was calculated by linear regression.

### Whey Colostrum Preparation and Total Whey Protein Determination

Colostrum samples were thawed at room temperature and gently mixed to ensure a good homogeneity. Then, rennet solution (with 100% chymosin; 200 international milk clotting units/mL, water, sodium chloride, sodium benzoate, and chymosin; Hansen Standard CHY-MAX Plus 200, CHR Hansen, Hoersholm, Denmark) was added and samples were incubated for 30 min at 37°C. After 30 min from rennet addition, clot was manually cut with 2 orthogonal vertical cuts using a stainless steel spatula. Once each clot was cut, samples were centrifuged at 2,500 × *g* for 30 min at 4°C and whey was collected and filtered using a 0.45-μm syringe filter (Minisart, Sartorius Stedim Biotech GmbH, Göttingen, Germany).

Total whey proteins (**TWP**) were quantified by Biuret assay using commercial reagents on a Dimension EXL analyzer (Siemens Healthcare Diagnostics s.r.l., Milan, Italy) according to the manufacturer's instructions (www.siemens.com/diagnostics).

### Capillary Electrophoresis Analysis

The CE analysis was performed using a Minicap capillary electrophoresis system (Sebia, Lisses, France). The fully automated instrument performs whey colostrum proteins separation using a 17 cm (16 cm to detection point) × 25 μm i.d. coated fused-silica capillary with an applied voltage of 7.8 kV at 35.5°C. The instrument requires a dead volume of sample approximately to 200 μL, but the sample injected is about 1 nL. The injection is carried out at the anode by using a pressure (hydrodynamic injection) of 2,000 Pa for 1 s. The electrophoresis buffer is a 150 m*M* borate buffer pH 9.9 ± 0.5 (Sebia). Time migration is 238 s and the system performs 2 tests simultaneously using 2 capillaries that work at the same time. The protein detection at the cathode extremity takes place by reading the absorbance at 200 nm.

After manual selection of the total Ig fraction area (Ceniti et al., 2016) on the electrophoretogram, the instrument software calculates the percentage of total