



Short communication

Determination of IgG levels in bulk ewe's milk

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ABSTRACT

Colostrum addition to milk results in a reduction of the yield during cheese manufacturing. Determination of IgG levels is accepted as an evidence of this troublesome habit due to the high level of this protein in colostrum. This study shows for the first time the IgG levels in bulk ewe's milk samples from Spain throughout the year. IgG quantification was carried out by a sandwich ELISA that recognizes specifically ovine IgG. The mean value of IgG concentration obtained from 481 samples was 0.271 ± 0.253 mg/ml. In general, this value is within the range considered acceptable for mature milk according to quality payment systems. Surprisingly, a high mean concentration was obtained in samples collected in winter, 0.742 ± 0.378 mg/ml, suggesting that births were grouped in that period. These results point up that standardization of ewe's milk production has not been achieved yet. For this reason, determination of immunoglobulin levels periodically would be a good parameter to control milk quality.

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1. Introduction

Colostrum is secreted by mammary glands during the first days after parturition and its composition differs from milk mainly in its higher levels of total solids, total proteins, fat and ash, but lower levels of casein and lactose (Hadjipanayiotou, 1995). The presence of high levels of proteins such as α -lactalbumin, β -lactoglobulin and especially immunoglobulins has been described in the first milking (Perez et al., 1990; Levieux and Ollier, 1999; Mainer et al., 2000). Most studies were done mainly in cow's and goat's milk where a high concentration of immunoglobulin G (IgG) was found in the first milking, about 40–50 mg/ml

and 48 mg/ml respectively. However, levels of IgG decrease sharply during the first week post-partum (Mainer et al., 2000; Levieux et al., 2002). Ruminants have an epithelio-chorial placenta which implies that neonates do not receive antibodies during pregnancy. For this reason colostrum intake from the mother is essential until individual immunity response is developed in newborns. In lambs passive immunoglobulin absorption occurs during the first 24 h after birth, and thereafter immunoglobulins are not able to be transferred through intestinal epithelium (Dominguez et al., 2001).

Addition of colostrum to milk can disrupted several technological processes applied in the dairy industry. High levels of whey proteins decrease heat stability of milk producing fouling on pasteurization equipments and therefore, leading to less effective heating treatments (Jeurnink, 1995; Bansal and Chen, 2006). These proteins do not only precipitate on the surface of equipment, but also confer off-flavors to pasteurized milk, and alter renneting times

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and curd firmness (Feagan, 1979; Raynal-Ljutovac et al., 2005). In addition, this practice affects the yield during cheese manufacturing due to a reduction of casein concentration in milk (Raynal-Ljutovac et al., 2008). Measurement of IgG levels in milk has been established a reliable parameter to control the colostrum addition in milk (Levieux, 1999; Mata et al., 2001; Conesa et al., 2005; Raynal-Ljutovac et al., 2005). As consequence of an intramammary infection, IgG levels can be also raised (Guidry et al., 1980a). Determination of IgG levels can be done mainly by radial immunodiffusion (Fleenor and Stott, 1980), nephelometry (Collin et al., 2002) or enzyme-linked immunosorbent assay (Kummer et al., 1992). Although there is no consensus on the limit of IgG level to consider adulteration of milk by adding colostrum, some studies have reported information about the average IgG levels in milk and colostrum of cow (Levieux and Ollier, 1999; Mainer et al., 2000; Conesa et al., 2005) and goat species (Mata et al., 2001; Levieux et al., 2002; Raynal-Ljutovac et al., 2005). However, no comprehensive study has been performed in ewe's milk.

Milk from ewes is mainly used in Mediterranean countries to manufacture cheeses such as Manchego, Feta, Pecorino and Roquefort, among others. Without considering other countries, Spanish ewe's milk production was 552.517 metric tons (mt) in 2012 (MAGRAMA, 2013b), of which nearly 92.5% was delivered to dairy industry for cheese production (Martínez et al., 2011). Thus, to standardize sheep's cheese manufacturing conditions and to improve the yield; the establishment of a control on the IgG content in milk seems to be necessary. Similar quality controls were already established for cow's and goat's milk. The purpose of this study was to determine the levels of IgG in ewe bulk milk from different flocks along the year.

2. Materials and methods

2.1. Collection of milk samples

Samples of raw bulk ewe milk were collected from different flocks in Castilla y Leon. Each sample was obtained from one tank that contains milk from several producers. Each month, from January to December, between 22 and 53 samples were received frozen and were kept at -20°C until analysis.

2.2. Quantification of immunoglobulins by sandwich ELISA

IgGs in ewe's milk were analyzed with the test Calokit-Sheep (ZEULAB, Zaragoza Spain), a sandwich enzyme-linked immunoabsorbent assay (ELISA) that allows determining the concentration of specific ovine IgG in ewe's milk. IgG purified from milk were used as standards. In order to provide an IgG concentration range between 0 and $1\text{ }\mu\text{g/ml}$, standards were diluted in distilled water 1:100. Samples were diluted in water 1:1000 or 1:2000 to make sure that IgG levels were inside the working range. ELISA was performed following manufacture instructions. Absorbance was read at 450 nm in a Multiskan microplate reader (Labsystems, Helsinki, Finland). IgG concentration of each sample was directly determined with a

polynomial calibration curve obtained with the absorbance values obtained from the standards.

2.3. Assay validation

Assay validation was conducted following the guidelines of Eurachem and AOAC (EURACHEM, 1998; AOAC, 2012). To determine the limit of detection (LOD) and quantification (LOQ), goat and cow milk samples were used as blanks because it is not possible to obtain ewe's milk lacking in IgG at any time during lactation. In our work, repeatability was obtained by analyzing 3 different samples (10 replicates of each) at various concentrations across the working range in the same assay. Intra-laboratory reproducibility was evaluated by analyzing 4 different samples at various concentrations across the working range in 3 independent assays. Mean, intra-assay relative standard deviation (CV), and inter-assay relative standard deviation (RSD) values were determined. Specificity was tested against the most abundant proteins in milk (casein, β -lactoglobulin, α -lactalbumin and serum albumin). Proteins were obtained from SIGMA-Aldrich.

2.4. Statistical methods

Statistical analysis was carried out using a one-way ANOVA statistical package of Excel 2007 for Windows. The data were subjected to analysis of variance with IgG concentration as an independent variant. Significant differences between IgG values in each season were calculated from *T*-test for two samples assuming unequal variances. Significant differences were considered at *P* level <0.05 .

3. Results

3.1. Validation results

Several commercial tests are available to measure immunoglobulins in milk, but none of them are specific for ewe's milk. This study presents an ELISA sandwich based on the use of antibodies that detect specifically ovine IgG. The assay was validated to verify the test fulfills all requirements for a reliable quantification of IgGs in milk. The validation results are summarized in Table 1. LOD and LOQ values were calculated using goat's milk as blank samples, values of 0.0014 and 0.0051 mg/ml were found, respectively. Similar LOQ and LOD values were obtained using cow's milk as blank (data not shown). These results not only showed a high sensitivity of the test, but also good specificity for ovine IgG. Neither cow's nor goat's IgG were detected. According to the validation results, the sandwich ELISA was able to quantify IgG levels from 0.0051 mg/ml up to 2 mg/ml when samples were diluted 1:2000. Precision of the ELISA assay was evaluated by measuring the standard deviation of several samples at concentrations within the working range in repeatability and intra-assay reproducibility conditions. The CV and RSD for IgG quantification in ewe's milk samples are shown in Table 1.

Finally, specificity of the assay showed that the anti-ovine IgG antibodies used in the test did not recognize other milk proteins such as casein, β -lactoglobulin,

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