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Short communication

Assessment of sheep colostrum *via* laser induced fluorescence and chemometrics

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ABSTRACT

In the present work sheep colostrum and milk at different milking times postpartum were qualitatively evaluated using laser-induced fluorescence (LIF). First and second milking samples showed higher fluorescence intensity compared with the subsequent milking samples. Such increase in the fluorescence intensity in first and second milking samples can be interpreted in view of the presence of high levels of immunoglobulins (IgG) and lactoferrin in the colostrum. The LIF results have been confirmed by the quantitative evaluation of the IgG in the same samples adopting the enzyme-linked immunosorbent assay (ELISA) as validation technique and a very good agreement has been obtained. Principal component analysis (PCA) of the obtained data led to the conclusion that the fluorescence band from 475 nm to 560 nm is strongly connected to changes during milking time from colostrum to milk. The fluorescence band is linked to changes in the concentration of proteins (IgG, lactoferrin) from colostrum to milk. According to the presented results, LIF spectroscopy can be used as a reliable, accurate, and fast method for real time and *in situ* evaluation of sheep colostrum. LIF coupled with the chemometric analysis of the data can be utilized in designing feeding strategies for newly born lambs.

1. Introduction

In mammals, colostrum is secreted from the mammary glands directly in the very early days postpartum. Especially high levels of immunoglobulins in the first milking have been reported by many researchers (Perez et al., 1990; Levieux and Ollier, 1999; Mainer et al., 2000). A sharp drop in the IgG concentration in the colostrum takes place in few days after parturition (Mainer et al., 2000; Levieux et al., 2002). It is well known that in ruminants, the placenta prevents the passage of antibodies from the mother to the fetus during pregnancy. Therefore, providing the newly born lambs with colostrum is essential in the early 12 h after birth till they gain the needed active immunity. In fact, ruminant embryo is capable to produce protecting antibodies to bacteria and viruses which penetrated through the placenta, at the third term of pregnancy. In other words, the immune system of the newborn is quite well developed, but being protected by the placenta, it is not immunologically challenged. Passive IgG absorption in lambs, takes place in the first 24 h after birth, hence after 12 h the absorption is negligible and has no practical value. After this short period, transmission of immunoglobulin through intestinal epithelium in the digestive system of the newly born animal is not possible (Dominguez et al., 2001). IgG levels can be taken as indicator of the milk status compared to the colostrum (Levieux and Ollier, 1999; Mata et al., 2001; Conesa et al., 2005; Raynal-Ljutovac et al., 2005).

Measurement of IgG levels can be performed by various techniques, namely radial immunodiffusion (Fleenor and Stott, 1980), nephelometry (Collin et al., 2002) or enzyme-linked immunosorbent assay (ELISA) (Kummer et al., 1992). Many researches have been performed on the colostrum of cows and goats where high IgG levels were measured in the first milking (30–200 mg/mL in cows and 48 mg/mL in goats) (Brian et al., 2016; Levieux et al., 2002). However, no thorough investigations have been performed on ewe's milk.

It should be taken into consideration that the above mentioned conventional methods for determination of IgG are expensive and time consuming. In addition, such techniques are not suitable for real time and *in situ* analysis. This shows that, it is of great importance to develop another method to evaluate IgG in colostrum avoiding the above mentioned drawbacks of the conventional methods.

Laser Induced Fluorescence (LIF) is a well-known highly sensitive spectrochemical analytical technique widely used in molecular analysis. This analytical technique is characterized by being completely nondestructive, noninvasive, needs no or minimal sample preparation, fast and cost effective. This makes LIF most suitable for the analysis of biological samples, including milk and colostrum.

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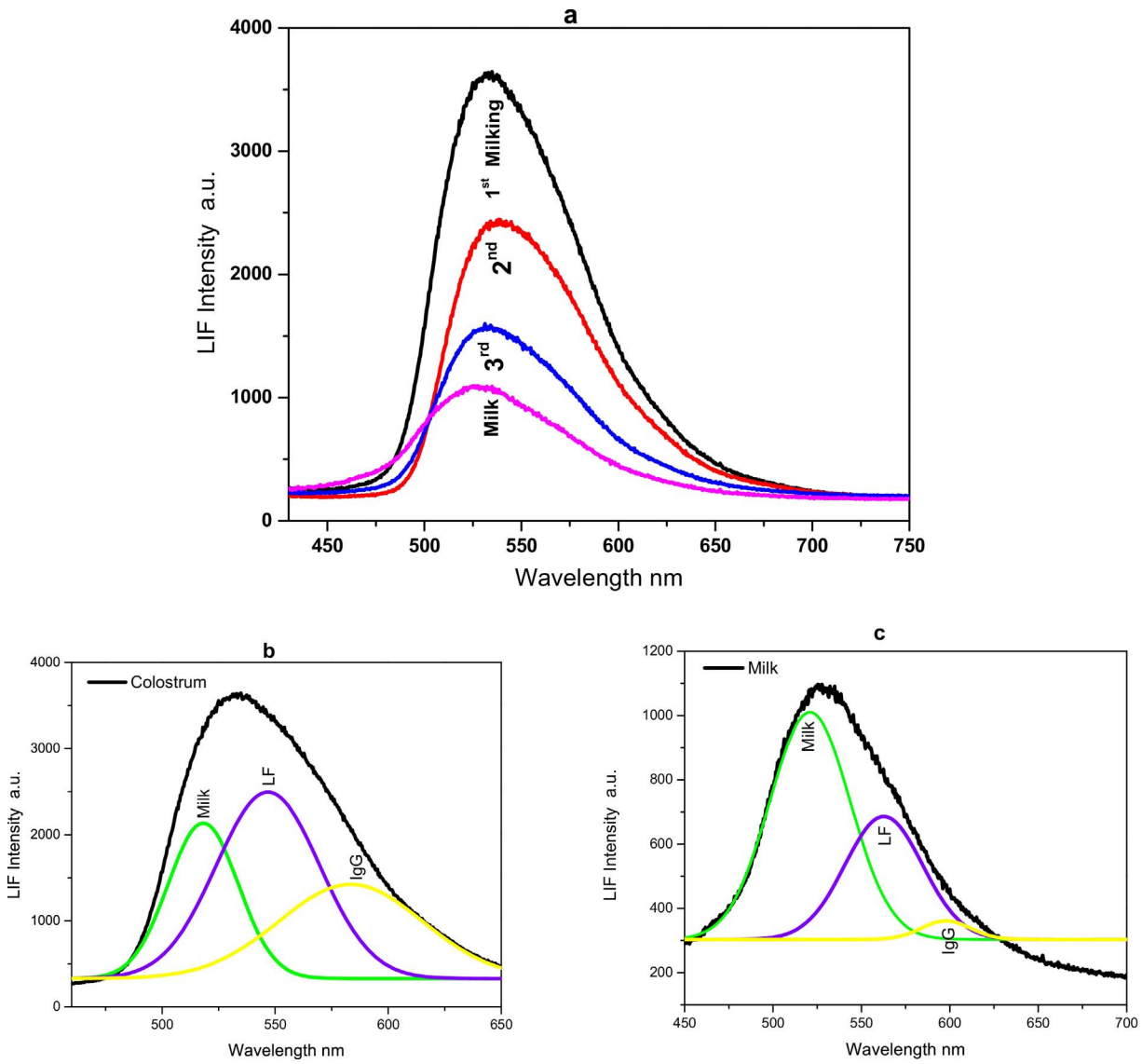

Fig. 1. Typical LIF spectra of the first three milking compared with that of mature milk sample (a). Deconvolution of the fluorescence peak shows the contribution of the IgG and Lactoferrin fluorescence with respect to the normal milk contribution in colostrum (b) and in milk (c).

In fact, LIF applications in biology are not new, and it has been reported previously in the literature by many researchers (Mu et al., 2016; Yang et al., 2017). Simplicity of running the compact equipment of LIF setup makes it suitable to be used for real time and *in situ* measurements in biological applications. This can be performed for example, in animal production centers and dairy farms. The technique has been used in diagnosis of animals' diseases (Abdel-Salam et al., 2015), sperm count (Abdel-Salam and Harith, 2012), evaluation of colostrum in milk (Abdel-Salam et al., 2014), follow up of spoilage of white meat (Abdel-Salam et al., 2017) and numerous other applications.

The aim of the present work was to explore the potential of LIF as an accurate and fast technique to evaluate proteins in sheep colostrum (especially immunoglobulin and lactoferrin) at different milking times. This is very important for setting feeding programs for newly born lambs and for the successful transfer of passive immunity from the ewe to the lamb.

2. Material and methods

2.1. Collection of sheep colostrum

Samples were obtained from Barki ewes in the sheep farm located in agricultural research and experimental station, Faculty of Agriculture, Cairo University. Colostrum milk samples were collected from each of 30 ewes, from the first three milking postpartum. The three milking times were 12 h apart, *i.e.* samples have been collected 0, 12 and 24 h after lambing. The colostrum samples were immediately frozen and stored at −20 °C. A fourth sample representing the ewes' milk has been obtained from milking one week after lambing from each ewe.

2.2. Determination of total IgG and total protein

IgGs in sheep's colostrum and milk were analyzed using enzyme-linked immunosorbent assay (ELISA- Biotek ELX808, USA) with Sheep IgG ELISA Kit, E-35G (ICL Inc. OR, USA) that allows determining the concentration of specific IgG in sheep's milk. Quantitative determination of total IgG in colostrum and milk was performed using the ELISA kit according to the manufactures' instructions. IgG concentration of each sample was determined from the calibration curve of the absorbance values obtained for the standards.

Total protein content in all samples under investigation was determined by routine laboratory procedures using an automated infrared milk analyser (MilkoScan FT1, FOSS, Demark) at the central laboratory of Faculty of Agriculture, Cairo University.