Talanta 129 (2014) 15–19

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Evaluation of immunoglobulins in bovine colostrum using laser induced fluorescence

Z. Abdel-Salam [a], Sh. Abdel Ghany [b], M.A. Harith [a,*]

[a] National Institute of Laser Enhanced Science, Cairo University, Giza 12316, Egypt
[b] Animal Production Department, Faculty of Agriculture, Cairo University, Egypt

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form
11 April 2014
Accepted 15 April 2014
Available online 30 April 2014

Keywords:
LIF
Immunoglobulins evaluation
Bovine colostrum

ABSTRACT

The objective of the present study was to exploit laser induced fluorescence (LIF) as a spectrochemical analytical technique for evaluation of immunoglobulin (IgG) in bovine colostrum. Colostrum samples were collected from different American Holstein cows at different times after calving. Four samples were gathered from each cow; the first three samples were obtained from the first three milkings (colostrum) and the fourth sample (milk) was obtained a week after calving. It has been demonstrated that LIF can be used as a simple, fast, sensitive and less costly spectrochemical analytical technique for qualitative estimation of IgG in colostrum. LIF results have been confirmed via the quantitative evaluation of IgG in the same samples adopting the single radial immunodiffusion conventional technique and a very good agreement has been obtained. Through LIF it was possible to evaluate bovine colostrum after different milking times and to differentiate qualitatively between colostrum from different animals which may reflect their general health status. A fluorescence linear calibration curve for IgG concentrations from 0 up to 120 g L^{-1} has been obtained. In addition, it is feasible to adopt this technique for in situ measurements, i.e. in dairy cattle farms as a simple and fast method for evaluation of IgG in bovine colostrum instead of using lengthy and complicated conventional techniques in laboratories.

1. Introduction

Colostrum is one of the most immunologically important nutrients for neonates of mammals. It is produced and stored in the mammary glands shortly before calving. In addition to the milk constituents, colostrum is characterized by its high content of IgG necessary for the immunity of newborns. Unequivocally, this natural nutrient product is the principal source of IgG during the very early period of the calves' life [1,2]. There are many factors affecting immunoglobulin's concentration in colostrum such as calving season, lactation number, age, breed, etc. [3]. In addition, it is widely known that higher levels of immunoglobulin exist in colostrum of mature cows than in that of primiparous ones [4]. The adequate and inadequate intake of high quality colostrum is respectively related to the successful or failure of transfer of passive immunity (FTPI) to the neonates in mammals generally [5]. Newborn calves fed on poor-quality colostrum usually have an increased incidence of morbidity and consequently high mortality [6]. A calf's serum IgG concentration of less than 10 g L^{-1} at 24 h after birth characterizes failure of passive transfer of immunity that leads to the aforementioned consequences of losing the ability of disease resistance and high mortality [7].

Conventionally, determination of IgG level in colostrum was being conducted by using several immunochemical methods such as radial immuneodiffusion (RID), single radial immunodiffusion (SRID), radio immuno assay (RIA), electrophoresis, or color evaluation, enzyme-linked immunosorbent assay (ELISA) [8,9], nephelometric immunoassay [2,10,11], or turbidimetric assay[12]. These conventional techniques are usually used on a large scale in most cattle farms for determination of IgG level for first lactation which gives a prediction for the health status of newborn calves consuming colostrum. In general, such techniques are mostly complicated, time consuming and expensive.

Conventional diagnostic fluorescence experiments using spectrofluorimeters were being conducted early on drugs and milk [13]. Recently, laser-induced fluorescence (LIF) is a spectrochemical analytical technique used for detection of selective species and studying the structure of molecules. LIF is an appealing technique for biological samples analysis as it is rapid, and is time saving, requires no or minimal sample preparation and it is also completely nondestructive and noninvasive. Typically, compact and simple equipment are used in LIF setups. This makes this technique a very good candidate for performing in situ measurements which facilitate its use in field applications such as in dairy cattle

* Corresponding author. Tel./fax: +202 35675335.
E-mail address: mharithm@niles.edu.eg (M.A. Harith).

http://dx.doi.org/10.1016/j.talanta.2014.04.033
0039-9140/

farms for example. Thus, as has been already mentioned, LIF is one of the most appropriate molecular analysis techniques in biological applications. The technique has also been used in characterization of plant material [14]. Recent approaches have been used to estimate sperm count of semen using LIF via follow up of ATP fluorescence in sperm [15].

The current work aims to assess the feasibility of using LIF as a fast, simple and cost effective technique for evaluation of IgG level in bovine colostrum from a number of different cows at the early milking times just after partum.

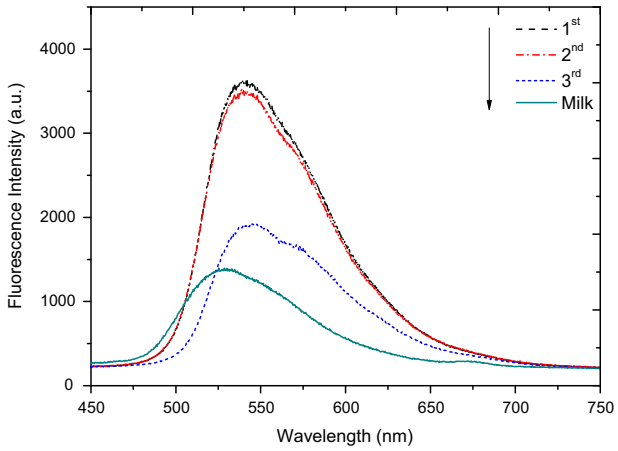

Fig. 1. The fluorescence spectra of the first three milking compared with that of milk.

2. Material and methods

2.1. Collection of bovine colostrum

Colostrum samples were collected from the first three milkings postpartum from 30 healthy American Holstein cows at nearly the same body conditions in an accredited commercial cattle farm in the vicinity of Cairo. The cows were milked manually twice a day after suckling. The first three milkings were 12 h apart, i.e. 0, 12 and 24 h after calving. The fourth sample (the milk) has been obtained from milking the cows one week after calving. All the cows were at their first calving and subjected to the same environmental circumstances and feed conditions. The colostrum and milk samples were properly stored and kept frozen at −20 °C.

2.2. Laboratory determination of total protein and total IgG

Colostrum protein content in all under investigation samples was determined by routine laboratory procedures using an automated infrared method with a Bentley 150 infrared milk analyzer (Chaska, MN, USA), at the central laboratory of the Faculty of Agriculture, Cairo University.

The total IgG (g L^{-1}) was conventionally quantified by using the single radial immunodiffusion technique (SRID, DIFFU-PLATE)

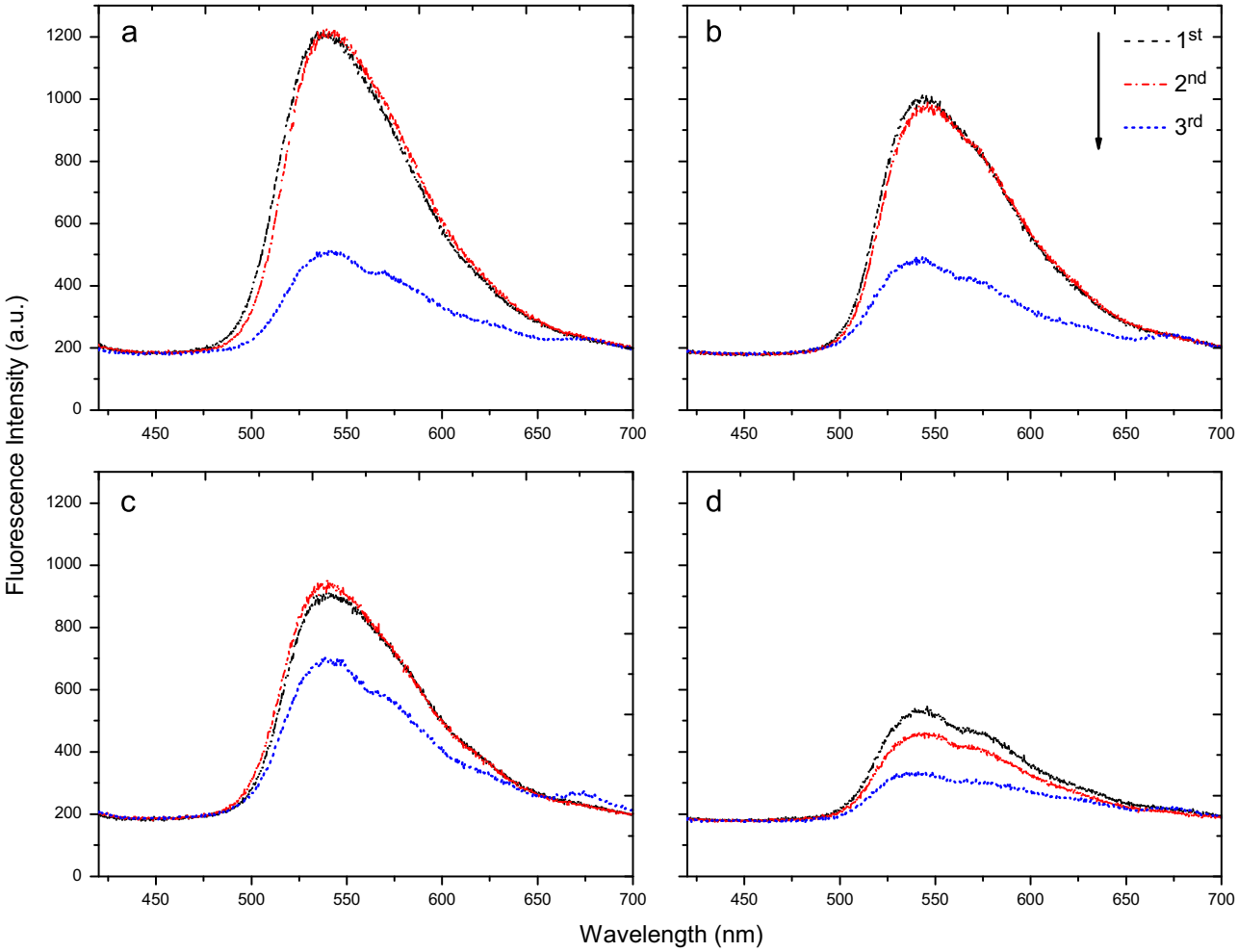

Fig. 2. Typical LIF spectra of the first three postpartum milking of four different animals.

as described by Fleenor and Stott [16]. The procedure makes use of immunoprecipitation in agarose gel between an antigen and its homologous antibody. The antigen diffuses radially out of the well into the surrounding gel-antibody mixture and a visible ring of the precipitation is formed where the antigen and antibody reacted. A quantitative relationship exists between the ring diameter and the antigen concentration. As the precipitate expands, the relation between ring diameter and antigen concentration logarithm is approximately linear.

2.3. Fluorescence microscopy procedure

The colostrum and milk samples were examined via a fluorescence microscope DM6000 B (Leica Microsystems, Wetzlar, Germany) provided with a Lica HCX APO 100X objective and epifluorescence filter cube Leica: I3. The microscope was equipped with a high sensitivity camera Leica DFC360FX for imaging the samples under examination.

2.4. LIF arrangement

In LIF laser light excites atoms or molecules, which send out radiation when they undergo de-excitation. In the time interval between the excitation and de-excitation nonradiative processes can occur that may lead to population of new states. Since many different states are populated, fluorescence can take place on several wavelengths and it is possible to observe an emission spectrum. Because both the wavelengths that excite the molecule and the one that is observed could be chosen, this makes an excellent choice to study atoms and smaller molecules. An atom or a molecule can be excited in one way, by absorbing a photon, but it can be de-excited in several ways. It can be spontaneously emit a photon, it can be stimulated to emit a photon, it can be quenched, or it can be photo-ionized and pre-dissociated. The excited species will be de-excited after some time, usually in the order of few nanoseconds to microseconds, and emit light at a characteristic wavelength longer than the excitation wavelength. Such de-excitation emitted light can be measured for identification of the species under investigation and for other different tasks.

The experimental setup of the LIF equipment used throughout the present work was described in details elsewhere [15]. The laser used was a CW-DPSS laser [Changchun New Industries Optoelectronics Tech Co, Ltd. (China)] of 40 mW power at $\lambda = 405$ nm. The focused laser beam was directed via an optical fiber to one side of the quartz cuvette containing 1 ml colostrum (or milk) sample. The fluorescent light was collected perpendicularly via another optical fiber coupled to the spectrometer (USB2000 FLG, Ocean Optics, FL, USA). Acquisition and further processing and analysis of the obtained spectra were accomplished using the commercial Spectra-Suit software (Ocean Optics, FL, USA). The final processing of the data for presentation was performed with Origin 8.5 software (Origin Lab. Corp., MA, USA).

3. Results and discussion

It is well known that colostrum contains important nutrients in addition to a high level of immunoglobulins (Igs). As mentioned above, newborn calves have immature immune system thus they depend upon first-milking maternal colostrum to obtain antibodies which offer significant protection against diseases.

Fig. 1 shows the fluorescence spectra of the first three milkings compared with that of the milk. Obviously, the highest fluorescence intensity was for the first couple milkings then it drops sharply approaching the milk fluorescence level. These spectra demonstrate the correlation between the IgG level in the measured samples and the fluorescence intensity. The spectra depict the clear difference between the colostrum fluorescence and that of the milk. The fluorescence band extends from 475 nm up to 650 nm peaking at 530 nm for the milk and around 550 nm for the bovine colostrum samples. In fact the fluorescence emission intensity of the second milking sample approaches that of the first milking and they are nearly overlapping. This indicates nearly equal levels of IgG in both samples, which drop sharply in the following milking samples. The obtained results are in good agreement with the fact that the concentration of the total IgG is very high in the first milking, then it decreases sharply postpartum and reaches the final concentration around the first or second week of lactation [17]. Gapper et al. [18] reported that the concentration of total IgG is in the range 32–212 mg mL^{-1} in colostrum, but in milk it is only 0.72 mg mL^{-1}. It is important to pay attention to the fact that in bovine colostrum and milk, immunoglobulin G (IgG; subclasses IgG1 and IgG2) is the major immune component, although low levels of IgA and IgM are also present [19,20]

By using LIF, it was not only possible to evaluate bovine colostrum of different milking after calving but it was also possible to differentiate between different cows in view of the colostrum quality they are giving in the same milking times and under the same conditions (e.g., age, race, calving season, lactation number, dry period length, inter calving interval…etc.) [3]. Fig. 2,(a–d) shows a comparison of fluorescence band intensities of colostrum milking from four cows. According to the depicted results, samples

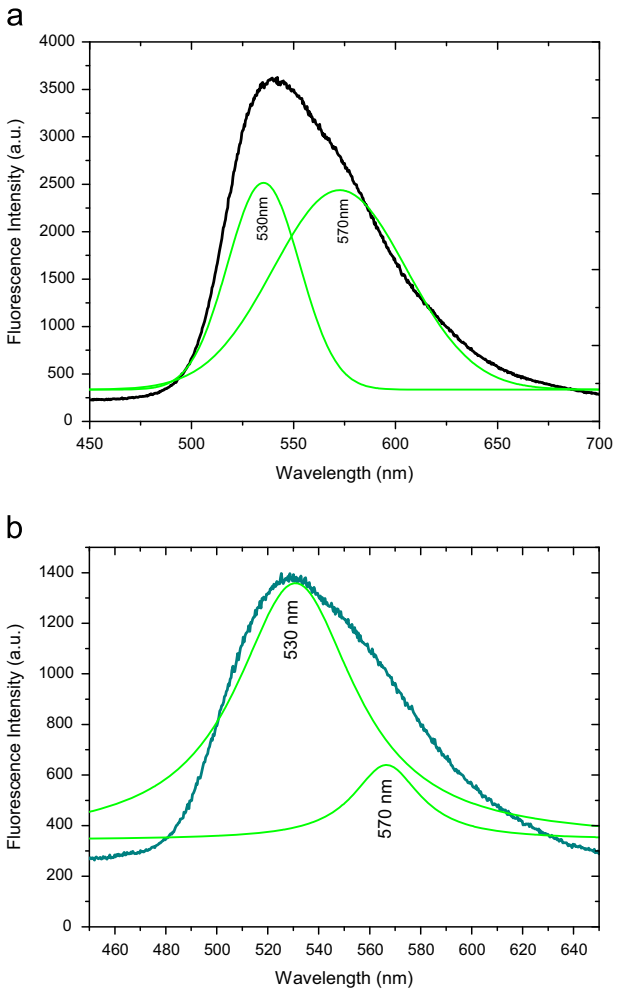

Fig. 3. Deconvolution of the 1st milking fluorescence peak showing the contribution of the IgG fluorescence at 570 nm with respect to the milk contribution at 530 nm in colostrum (a) and in milk (b).

of cow (a) give the highest fluorescence intensity in the first two milkings indicating highest IgG content and consequently best colostrum quality compared with the other samples from the other three cows. It is clear that the lowest fluorescence intensities and consequently the worst colostrum, qualitatively, are that of cow (d). Such results may be very useful in choosing the most suitable animal for lactation during the first few days after calving to raise the calves' immunity by providing them with the optimum quantity of IgG.

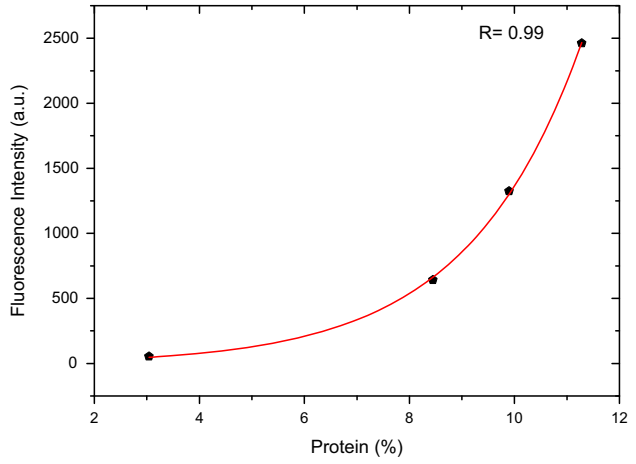

Fig. 4. IgG fluorescence intensity at 570 nm in colostrum versus the total protein (%). The solid line is the exponential fitting curve of the experimental points.

In Fig. 3a and b, deconvolution of the fluorescence peaks of colostrum and milk are demonstrated. It is clear that milk protein is peaking at 530 nm while IgG is peaking at 570 nm. Although the fluorescence intensity of the milk sample is much less than that of the colostrum sample there is still a contribution of IgG under the milk peak though it is relatively very low (Fig. 3b). The exponential relation between the measured colostrum total protein content and the corresponding fluorescence intensity is depicted in Fig. 4. This curve can be used in estimation of the protein content in other milk, and colostrum samples by just measuring their fluorescence intensity.

Examining samples of first and second milkings after calving and the milk sample (obtained seven days after calving) via the fluorescence microscope, provided interesting results as shown in the insets in Fig. 5. The micrograph of each sample is displayed with the corresponding LIF spectral signal. The green and green–yellow fluorescence are very clear in the first and second milkings while the yellow green completely disappeared in the milk sample micrograph demonstrating the very weak contribution of IgG. For clarification, the spectral location of the emission of all colostrum components is displayed below the graph [21].

In order to confirm the above obtained results, the same samples subjected to the LIF measurements were used to evaluate their quantitative contents of IgG via the conventional SRID technique. The linearity of the relation between the IgG concentration and the corresponding laser induced fluorescence verifies the above obtained results (see Fig. 6). The zero IgG fluorescence (the intercept with the *y*-axis) indicates the milk relevant fluorescence. In fact, the plot in Fig. 6 can be used as a standard

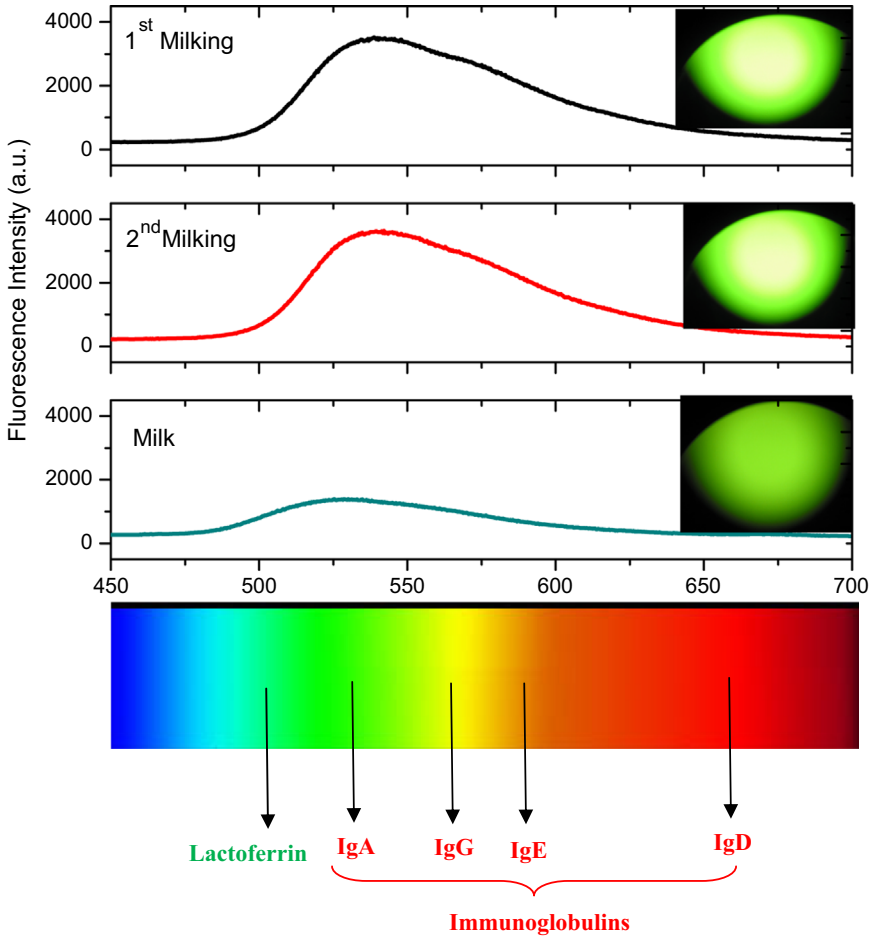

Fig. 5. Fluorescence microscopy micrographs of each sample displayed with the corresponding LIF spectral signal. The spectral emission color locations of different colostrum components are also shown.

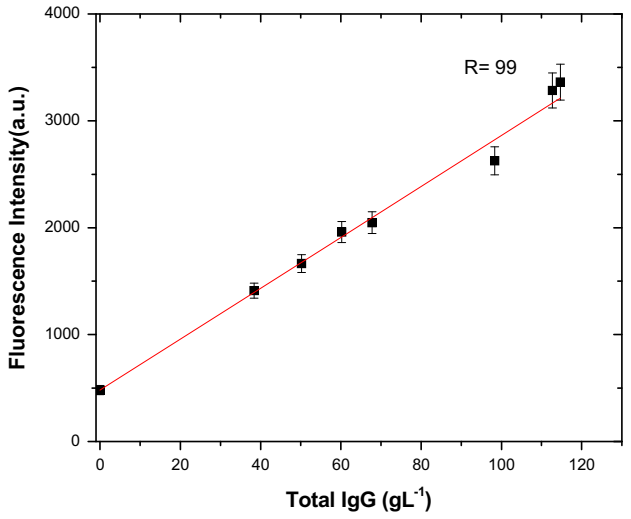

Fig. 6. Calibration curve for fluorescence intensity values versus the total IgG ($g\,L^{-1}$) determined conventionally. The error bars are the experimental standard deviation.

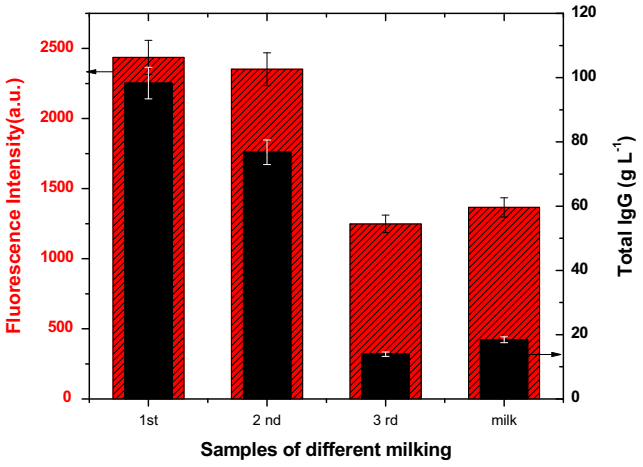

Fig. 7. Trends of integrated fluorescence intensity values and total IgG ($g\,L^{-1}$) at different milking. The error bars are the experimental standard deviation.

calibration curve for the quantitative determination of IgG concentration in any bovine colostrum samples by measuring its laser induced fluorescence intensity. The bar graph shown in Fig. 7, demonstrates that both fluorescence intensity and IgG concentration have the same trend in the analyzed samples.

4. Conclusions

In conclusion, LIF as a spectrochemical analytical technique has been used to provide qualitative and quantitative information about IgG in bovine colostrum as well as in milk. To the best of our knowledge this is the first use of LIF in this field. The obtained results demonstrated that adopting LIF it was possible to evaluate bovine colostrum after different milking times and to differentiate qualitatively between colostrum from different animals which may reflect their health status in general. Potentials of LIF were evinced in relating the proteins content of the samples and the corresponding fluorescence intensity of the laser induced spectral bands. Using the SRID conventional technique, the IgG concentrations in the analyzed samples have been determined and found to coincide with the LIF results. Ultimately, this work offers distinct advantages when compared with other previously published works in this field as it presents a successful spectrochemical analytical technique, namely LIF as noninvasive, nondestructive and fast analysis method of IgG of bovine colostrum. In addition, it is feasible to adopt this technique for in situ measurements, i.e. in dairy cattle farms as a simple and fast method for colostrum evaluation instead of using more costly, lengthy, and complicated conventional techniques in the laboratory.

References

[1] J.L. Linzell, M. Peaker, JPN. J. Physiol. 24 (1974) 129–151.
[2] I. Moreno-Indias, D. Sánchez-Macías, N. Castro, A. Morales-delaNuez, L. E. Hernández-Castellanoa, J. Capote, A. Argüello, Small Ruminant Res. 103 (2012) 220–224.
[3] J.E. Devery-Pocius, B.L. Larson, J. Dairy Sci. 66 (1983) 221–226.
[4] J.W. Tyler, B.J. Steevens, D.E. Hostetler, J.M. Holle, J.L. Denbigh, Am. J. Vet. Res. 60 (1999) 1136–1139.
[5] S.B. Constant, M.M. Leblanc, E.F. Klaptein, D.E. Beebe, H.M. Leneau, C.J. Nunier, J. Am. Vet. Med. Assoc. 205 (1994) 1759–1762.
[6] E. Kolb, S. Kaskous, Bestandteile des Kolostrums und der Milch von Ziegen und deren Bedeutung für die Gesundheit der Ziegenlämmer, Tierärztl. Umschau 58 (2003) 140–146.
[7] D.M. Weaver, J.W. Tyler, D.C. Van Metre, D.E. Hostetler, G.M. Barrington, J. Vet. Inter. Med. 14 (2000) 569–577.
[8] A. Argüello, N. Castro, S. Alvarez, J. Capote, Small Ruminant Res. 64 (2006) 53–59.
[9] L. Elfstrand, H. Lindmark-Månsson, M. Paulsson, L. Nyberg, B. Åkesson, Int. Dairy J. 12 (2002) 879–887.
[10] C. Conesa, M. Lavilla, L. Sánchez, M.D. Pérez, L. Mata, P. RazquÍn, M. Calvo, Eur. Food Res. Technol. 220 (2005) 222–225.
[11] A. Rudovsky, L. Locher, A. Zeyner, A. Sobiraj, T. Wittek, Small Ruminant Res. 74 (2008) 265–269.
[12] L.R. Etzel, R.E. Strohbehn, J.K. McVicker, Am. J. Vet. Res. 58 (1997) 1201.
[13] M.C. Mahedero, T. Galeano Díaz, S. Galán Pascual, Talanta 57 (2002) 1–6.
[14] J.P. Andersen, C. Sternberg, L.K. Poulsen, S.P. Bjorn, M. Givskov, S. Molin, Appl. Environ. Microbiol. 64 (1998) 2240–2246.
[15] Z. Abdel- Salam, M.A. Harith, Talanta 99 (2012) 140–145.
[16] W.A. Fleenor, G.H. Stott, J. Dairy Sci. 64 (1980) 640–747.
[17] G. Mainer, M.D. Perez, L. Sanchez, P. Puyol, M.A. Millan, J.M. Dominguez, E. Ena, M. Calvo, Milchwissenschaft 55 (2000) 613–617.
[18] L.W. Gapper, E.J. Copestake, D.E. Otter, H.E. Indyk, Anal. Bioanal. Chem. 89 (2007) 93–109.
[19] D. Blakeslee, J. Rapacz, J.E. Butler, J. Dairy Sci. 54 (1971) 1319–1320.
[20] J.E. Butler, A.J. Winter, G.G. Wagner, J. Dairy Sci. 54 (1971) 1309–1914.
[21] ⟨http://www.colostrum.de/en/products/c-live-color-scale/⟩ (February 2014).