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Caprine immunoglobulin G, β-lactoglobulin, α-lactalbumin and serum albumin in colostrum and milk during the early *post partum* period

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Summary. Colostrum and milk samples from 20 goats were analysed for concentrations of immunoglobulin G (IgG), β-lactoglobulin (β-lg), α-lactalbumin (α-la) and serum albumin (CSA) throughout the first 14 milkings *post partum* (7 d of lactation) using single radial immunodiffusion assay. Concentrations (mg/ml, means ± SD) at first milking were IgG 47·9 ± 25·5, β-lg 30·7 ± 10·4, α-la 2·77 ± 0·82 and CSA 2·97 ± 2·46 mg/ml. Large variations were recorded for IgG concentrations (19·9–94·5 mg/ml) and β-lg (9·3–49·8 mg/ml). Concentrations of IgG, β-lg and CSA dropped abruptly in the subsequent milkings and α-la concentration decreased slowly. Mean IgG concentration was < 2 mg/ml after 7 milkings and < 1 mg/ml after 11 milkings. However, IgG concentration does not differ significantly, at the 1% level, from milkings 7–14. The contribution of β-lg to the increase in whey proteins in early milks was greater than that of IgG from milkings 5 to 14. The results were tabulated to make it possible to calculate the excess of whey proteins that would be obtained if early milks were illegally added to milk supply.

Keywords: Goat, milk processing, contamination, detection.

The composition of ruminant colostrum is quite different from that of milk in established lactation. Proteins secreted by the mammary gland such as β-lactoglobulin (β-lg), α-lactalbumin (α-la) and lactoferrin, or proteinase inhibitors and proteins derived from blood such as albumin, α_2-macroglobulin and transferrin are present in higher concentrations in colostrum (reviewed by Levieux, 1999). However, colostrum is mostly characterized by its very high level of immunoglobulin G (IgG), essentially from the IgG_1 subclass which is actively concentrated from the serum to the mammary gland during the last weeks prior to parturition (Pahud & Mach, 1970; Micusan & Borduas, 1976; Butler, 1981).

Presence of colostrum in milk is undesirable for the dairy industry. In cow milk it causes problems such as reduced heat stability, low cheese yield, weak curd formation and poor curd characteristics (Feagan, 1979). Owing to the temperature sensitivity of IgG, the use of milk containing colostrum necessitates more frequent and comprehensive cleaning of heat transfer surfaces and other equipment in dairy

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plants (Zawistowski & MacKinnon, 1993). Similar problems have been observed by cheese producers using goat milk although quantitative data have not been published.

Because of these undesirable properties, commercial milk should be free from colostrum. However, regulations for the period of withholding post-parturition milk vary across countries. This situation arises largely because there is no clear delineation between colostrum and milk: there are gradual changes in protein composition from colostrum to definitive milk during the first week *post partum* and from milk to colostrum during the last weeks *ante partum*. A large number of published investigations on goat colostrum have centred on the evolution of its main chemical components in the first days *post partum* (reviewed by Quiles *et al*. 1991). However, to our knowledge there is no published information describing in detail the variations in concentration of IgG and of other major whey proteins in colostrum and milk during early lactation.

The purpose of the present work was to study the concomitant variation in concentration of IgG, β-lg, α-la and serum albumin during the first week of lactation in the goat.

Materials and methods

Colostrum, milk and whey samples

Individual colostrum and milk samples were obtained from the first 14 milkings *post partum* (7 d of lactation) of 20 Alpine goats (INRA herd, Bourges, France). Colostrum and milk samples were also obtained at days 1, 3, 5, 9 and 13 from ten goats of the Saanen ($n = 4$) and Alpine ($n = 6$) breed. Samples were obtained twice a day by manual milking, after discarding the first jets ejected, and kept frozen at −20 °C until analysed.

Rennet whey was obtained by coagulating cow bulk milk or colostrum at 37 °C with standard 1:10000 rennet added at 0·5 ml/l. After centrifugation at 16000 ***g*** for 20 min, the supernatant was collected, clarified with Frigen (300 ml/l; Behring, F-92504 Rueil Malmaison, France) and centrifuged at 2300 ***g*** for 20 min. The whey retentate was filtered through a 0·8-μm filter and stored at −20 °C until used for the purification of the different proteins.

Purified proteins

Immunoglobulins G_1 were purified from goat colostrum by a combination of gel permeation chromatography on Sephadex G200 (Pharmacia Biotech, S-751 84 Uppsala, Sweden) and ion exchange chromatography on DEAE Cellulose (Serva, D-69042 Heidelberg, Germany) as described previously for bovine IgG_1 (Levieux, 1974). IgG_1 was separated from IgG_2 by affinity chromatography on protein A-agarose (Pharmacia Biotech) as described by Delacroix & Vaerman (1979).

β-lg and α-la were purified from bulk milk whey by gel permeation chromatography on Sephadex G-100) at a flow rate of 50 ml/h on a column (950 × 50 mm; Pharmacia Biotech) previously equilibrated with 0·02 M-Tris-HCl, pH 7·0. The β-lg/α-la fractions were loaded onto a Mono-Q anion-exchange column (HR 10/10; Pharmacia Biotech) equilibrated with the same buffer. Elution was performed at a flow rate of 1 ml/min over a 0·0–0·5-M gradient using HPLC equipment (gradient former GF 425, pump 420, detector 430; Kontron Instruments, F-78180 Montigny les Bretonneux, France). The β-lg and α-la fractions were then extensively dialysed in water and freeze-dried. As assessed from PAGE analysis, the β-lg and α-la were at least 98% pure.

Caprine serum albumin (CSA) was purchased from Sigma Aldrich Chimie (F-38297 St-Quentin Fallavier, France).

Production of antisera

Antibodies were produced in rabbits for caprine IgG_1. The protein, emulsified in Freund's complete (first injection) or incomplete (booster injections) adjuvant (1:1, v/v) was administered at doses of 1 mg/rabbit by multiple intradermal injections. Animals were bled 7–9 d after each monthly booster injection. The specificity of the antisera was assessed by double-immunodiffusion and agar gel immunoelectrophoresis. Rabbit or sheep antisera against bovine β-lg, α-la and serum albumin were obtained as previously described (Levieux & Ollier, 1999). Their extensive cross-reaction with the homologous caprine proteins was assessed by double immunodiffusion.

Immunochemical assay of proteins

Concentrations of β-lg, α-la, IgG and CSA in individual colostrum and milk samples were determined by SRID assay (Mancini *et al*. 1965) using 1·85 mm-thick agar plates containing 12 g Noble agar/l in 0·05 M-veronal buffer, pH 7·2 (complement fixation test diluent tablets, Oxoid, Unipath, F-69570 Dardilly, France) containing 1 g sodium azide/l and appropriate quantities of each specific antiserum. Circular wells (2 mm diam.) were punched out in the gel and filled with 3-μl portions of adequately diluted samples in the veronal buffer or 3 μl of purified proteins of known concentrations as standards. Purified proteins were dissolved in the veronal buffer containing 1 g human serum albumin/l and 1 g sodium azide/l. Plates were incubated in a moist box at 37 °C for 20–22 h and the diameter of the ring-shaped precipitates measured automatically using a magnifying video camera system (Levieux, 1991). Standard curves were constructed by plotting the diameter of the precipitating ring against the square root of the protein concentration. With the diffusion time used, a linear regression was always obtained. Samples and controls were plated in duplicate. CVs of the assays were 3–5 %, values similar to those found previously for quantification of bovine milk proteins (Levieux & Ollier, 1999).

Electrophoresis

Electrophoresis was performed on cellulose acetate strips with Ponceau-red staining as described previously (Levieux & Vénien, 1991). For quantification by densitometry, whey samples were diluted 1 in 10 in 0·05 M-veronal buffer, pH 7·2 containing 5 mg/ml bovine serum as internal standard. Calibration was done using electrophoresis of known quantities of pure β-lg and α-la.

Statistical analysis

Probability and significance were calculated by Student's t test.

RESULTS

Values are given as means $\pm$ SD. IgG concentrations at the first milking were 19·9–94·5 (mean 47) mg/ml. Thereafter, they fell sharply to 1·05 mg/ml at the 11th milking (Fig. 1). IgG concentration in milkings 1–6 were significantly ($P < 0·01$) higher than that for milking 14. At the 13th milking IgG levels were 0·20–2·21 (mean 0·68) mg/ml. In the second herd, IgG concentrations at the first milking were 39–89 (mean 59·9) mg/ml. At the 13th milking, values were 0·32–1·18 (mean 0·73) mg/ml.

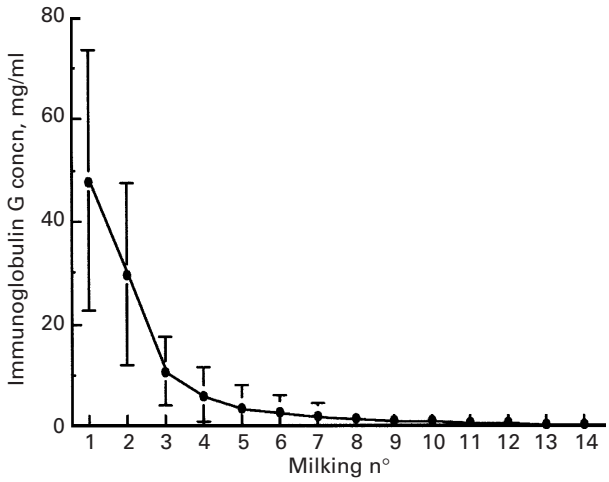

Fig. 1. Changes in IgG concentrations of caprine milk during early lactation. Values are means with SD indicated by vertical bars. Values for milkings 1–6 were significantly different from those for milking 14: $P < 0{\cdot}01$.

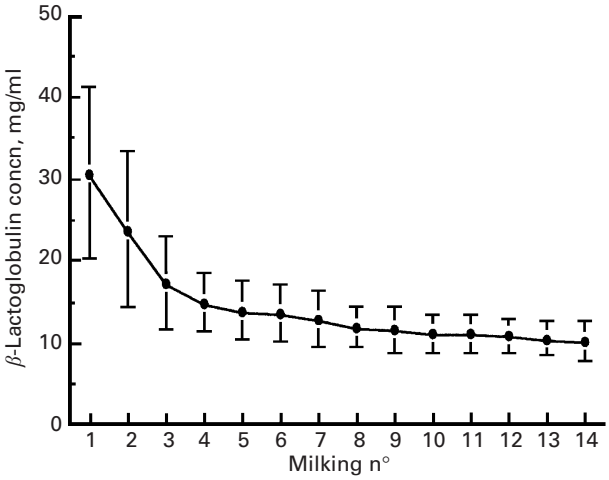

Fig. 2. Changes in β-lg concentration in caprine milk during early lactation. Values are means with SD indicated by vertical bars. Values for milkings 1–7 were significantly different from those for milking 14: $P < 0{\cdot}01$.

β-Lg concentrations at the first milking were 9·3–49·8 (mean 30·7) mg/ml, falling first sharply and then decreasing more slowly up to the 14th milking (Fig. 2). Concentrations in milkings 1–7 were significantly ($P < 0{\cdot}01$) higher than that for milking 14.

Levels of α-la decreased slowly and regularly (Fig. 3). Concentrations in milkings 1–2 were significantly ($P < 0{\cdot}01$) higher than that for milking 14.

Serum albumin concentrations at the first milking were 0·94–11·44 (mean 2·97) mg/ml, falling sharply between milkings 1 and 3 and then decreasing more slowly (Fig. 4). Concentrations in milkings 1–3 were significantly ($P < 0{\cdot}01$) higher than that for milking 14.

The high concentrations of β-lg found in the first milking were confirmed by electrophoresis of whey proteins on cellulose acetate strips. A typical profile is presented in Fig. 5*a*. Major peaks were quantified by densitometry using bovine

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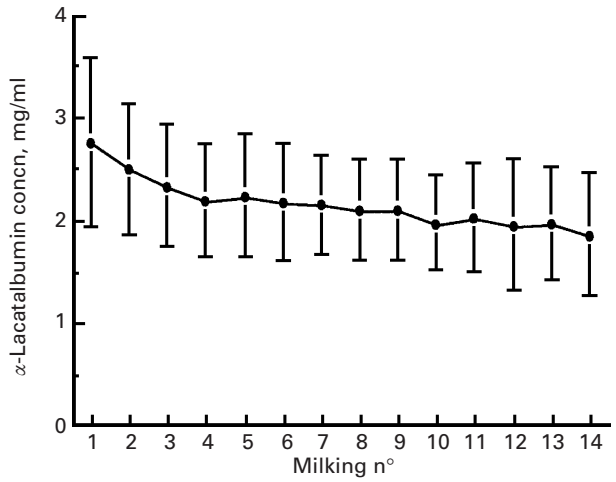

Fig. 3. Changes in α-la concentration in caprine milk during early lactation. Values are means with SD indicated by vertical bars. Values for milkings 1–2 were significantly different from those for milking 14: $P < 0{\cdot}01$.

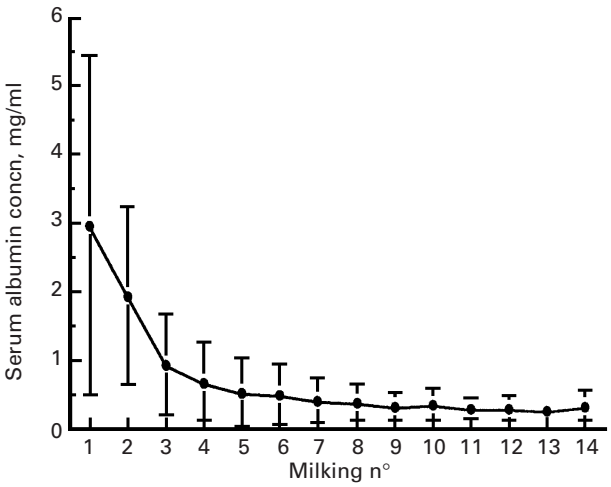

Fig. 4. Changes in serum albumin concentration in caprine milk during early lactation. Values are means with SD indicated by vertical bars. Values for milkings 1–3 were significantly different from those for milking 14: $P < 0{\cdot}01$.

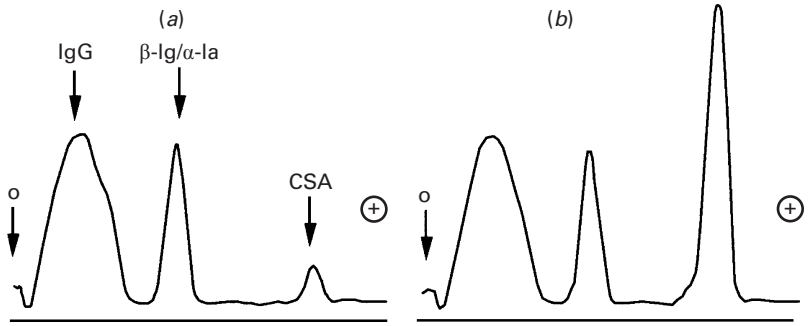

Fig. 5. Electrophoretic analysis of a caprine whey sample obtained from the first milking post partum and analysed diluted 1 in 10 before (*a*) or after (*b*) supplementation with bovine serum albumin (5 mg/ml). Cellulose acetates were stained with Ponceau red.

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Table 1. *Excess concentration of the four major whey proteins* (*immunoglobulin G*, *β-lactoglobulin*, *α-lactalbumin and caprine serum albumin*) *in the milk of goats during early lactation*

	Excess protein* mg/ml				
Milking no.	IgG	β-lg	α-la	CSA	Total
1	47·3	20·7	0·9	2·6	71·5
2	29·0	13·9	0·7	1·6	45·2
3	10·1	7·2	0·5	0·6	18·4
4	5·5	4·9	0·4	0·4	11·2
5	3·3	4·0	0·4	0·2	7·9
6	2·2	3·7	0·3	0·2	6·4
7	1·4	2·9	0·3	0·1	4·7
8	1·0	1·9	0·2	0·0	3·1
9	0·7	1·5	0·3	0·0	2·5
10	0·6	1·1	0·1	0·0	1·8
11	0·4	1·1	0·2	−0·1	1·6
12	0·2	0·8	0·1	0·0	1·1
13	0·1	0·6	0·1	−0·1	0·7

* Excess values are calculated with reference to values for milking n°14.

serum albumin as an internal marker (Fig. 5*b*). Values obtained for IgG (92·9 mg/ml), and β-lg/α-la (32·1 mg/ml) confirmed those obtained by SRID (94·5 and 33·2 mg/ml, respectively).

The excess of whey proteins in early milks relative to samples obtained at the 14th milking is presented in Table 1. As expected, IgG was the predominant whey protein in excess in milkings 1–4. Thereafter, β-lg was the most important protein in excess.

DISCUSSION

SRID was used to quantify milk proteins because this technique makes it possible to analyse whole colostrum or milk directly, as recommended by Fleenor & Stott (1981). Moreover, the technique can be automated (Levieux, 1991) and it allows precise quantification of low IgG concentrations in milk which cannot be obtained by electrophoresis or HPLC analysis. For the SRID quantification of goat IgG, special care must be taken in the choice of antisera and standards because the immunological cross reaction between the two IgG subclasses, IgG_1 and IgG_2, is not complete (Gray *et al.* 1969). In colostrum and milk, IgG_1 is the predominant IgG subclass with a concentration approximately 20-times higher than that of IgG_2 (Pahud & Mach, 1970; Micusan & Borduas, 1977). In contrast, serum IgG fractions are mostly IgG_2 and overestimation of IgG_1 levels would occur when using such fractions for antibody production or for use as a standard. This could partly explain the very high colostral IgG concentrations ($\geqslant$ 100 mg/ml) reported by Ha *et al.* (1986) for Korean native goats or by N'Diaye-Wereme *et al.* (1999) for Dwarf Mossi goats. Conversely, a very low colostral IgG concentration ($10{\cdot}9 \pm 2{\cdot}2$) has been reported by Ferrer *et al.* (1997). As these authors indicated that colostrum sampling was from the morning milking, it may be supposed that kids had had access to the udder during the night before sampling and that, therefore, the colostrum sample was not really the first milking. Quiles *et al.* (1991) also found relatively low IgG concentration ($\leqslant 35{\cdot}1$ mg/ml) in goat colostrum using polyacrylamide-SDS gel electrophoresis. However, these authors indicated that IgG were in an unresolved fraction entitled 'other whey proteins'. Our IgG concentrations (48–60 mg/ml) are consistent with

those reported by Pahud & Mach (1970), Micusan & Borduas (1977) and Chen *et al.* (1998).

After parturition, IgG concentrations fell by half at each milking for the first 2 d of lactation (Fig. 2*a*). A level below 2 mg/ml was attained after six milkings (3 d of lactation). After 7 d of lactation, the legal time for withholding post parturition milk in France, the mean IgG concentration was < 1 mg/ml. These results are consistent with those reported by Pahud & Mach (1970) and Ha *et al.* (1986). Very high concentrations of IgG in mature milk (5 mg/ml) were reported by Ferrer *et al.* (1997); however, these authors used a rabbit antisera against serum IgG. Interestingly, our results indicate that the IgG concentration in milking 14 is not significantly different from those in milkings 7–13.

For measurement of β-lg, α-la and CSA, we used antisera obtained against the homologous bovine proteins. However, purified caprine proteins were used as standards to prevent overestimation of the results. Mean concentrations of β-lg and α-la were higher in colostrum than in mature milk, in agreement with the findings of Quiles *et al.* (1991). However, these authors found lower levels of β-lg (22·3 *v.* 30·7 mg/ml) and higher levels of α-la (9·4 *v.* 2·8 mg/ml). To confirm our results, we submitted samples particularly rich in β-lg to quantitative electrophoresis on cellulose acetate strips with Ponceau red staining. Results were in good agreement with those obtained by SRID analysis. The ability of β-lg to bind about 2·5 moles sodium dodecyl sulfate per mole protein and, consequently, to aggregate into particles of larger size (Jenness, 1980) could explain the low β-lg level found by Quiles *et al.* (1991) using SDS-PAGE electrophoresis. Interestingly, Liberatori *et al.* (1975), using gel-filtration on Sephadex G-200, reported an increase of β-lg and α-la during the first 8 d of lactation.

The considerable variation in CSA concentration observed in the first milking (0·94–11·44 mg/ml) reflects the inflammatory status of the udder at parturition since only 10–20 % of milk serum albumin is synthesized by the mammary gland (Phillippy & McCarthy, 1979). Thereafter protein concentration dropped abruptly during the first five milkings, as found in early lactation in dairy cows (Perez *et al.* 1989; Levieux & Ollier, 1999).

Because the IgG content of colostrum is much higher than that of mature milk, it has been suggested that measurement of IgG concentration could provide a means of detecting illegal addition of colostrum to milk (Lebreton *et al.* 1981; Levieux, 1991; Zawistowski & MacKinnon, 1993). However, the consequences of such illegal practice are not limited to an excess of IgG since the other major whey proteins are also in higher concentrations in colostrum than in mature milk. We have therefore tabulated values for the total concentrations of the four major whey proteins throughout early lactation and calculated their total excess relative to samples of milk obtained at the 14th milking (Table 1). From these values, the effect of contaminating an authentic milk with one or more of the first 13 milkings can be estimated. For example, addition of 100 ml/l of milk from the first milking would give an excess whey protein concentration of 7·15 mg/ml, from which 4·7 mg/ml belongs to IgG and 2·1 to β-lg. On and after the 5th milking, the contribution of β-lg to the whey proteins excess is strikingly greater than that of IgG.

Information on the technological consequences for the milk industry of adding early milk is lacking for goat milk and is scarce and inconsistent for cow milk. Ibrahim *et al.* (1990) suggested that addition of bovine colostrum from the first day *post partum* to normal milk at 50 ml/l significantly affected its technological properties. This would give an IgG concentration of 3·44 mg/ml and an excess

concentration of 3·52 mg whey proteins/ml (Levieux & Ollier, 1999). In contrast, Suchanek *et al*. (1978) suggested that early milk can be used from 5–6 d *post partum* without causing technological problems if added at no more than 100 ml/l (excess whey protein concentration of 0·35 mg/ml). This indicates the need for more precise studies of the technological implication of increased quantities of IgG and other whey proteins in milk in order to be able to harmonize legislation concerning the time for withholding post parturition milk.

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