SHORT COMMUNICATIONS

Milk whey protein concentration and mRNA associated with β-lactoglobulin phenotype

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Two common genetic variants of β-lactoglobulin (β-lg), A and B, exist as co-dominant alleles in dairy cattle (Aschaffenburg, 1968). Numerous studies have shown that cows homozygous for β-lg A have more β-lg and less α-lactalbumin (α-la) and casein in their milk than cows expressing only the B variant of β-lg (Ng-Kwai-Hang *et al*. 1987; Graml *et al*. 1989; Hill, 1993; Hill *et al*. 1995, 1997). These differences have a significant impact on the processing characteristics of the milk. For instance, the moisture-adjusted yield of Cheddar cheese is up to 10% higher using milk from cows of the β-lg BB phenotype compared with milk from cows expressing only the A variant (Hill *et al*. 1997). All these studies, however, describe compositional differences associated with β-lg phenotype in established lactation only. No information is available on the first few weeks of lactation, when there are marked changes in the concentrations of β-lg and α-la (Pérez *et al*. 1990).

In cows expressing both β-lg A and B, increased output of the A over the B variant in milk correlated with higher levels of mRNA for β-lg A compared with B (Wilkins *et al*. 1995). Further, Geldermann *et al*. (1996) and Folch *et al*. (1999) showed that the level of expression of a reporter gene attached to the β-lg A promoter is greater than that using the β-lg B promoter. These studies suggest that expression of the β-lg A allele is greater than that of the B allele, but they do not address the impact of expression of both A alleles in homozygous animals on the mRNA for other milk proteins. It is likely that the differential production of milk proteins is due to different levels of mRNA, but it cannot be assumed that levels of mRNA always determine the output of individual proteins. In late gestation in the rat, differences in levels of individual milk proteins mirror the variation in their respective mRNA (Nakhasi & Qasba, 1979; Rosen, 1987), but the mRNA profile during days 4 and 10 of lactation does not match that of the proteins (Qasba & Nakhasi, 1978; Geursen & Grigor, 1987).

The objective of the current study was to determine the relative pattern of change in whey protein composition from cows homozygous for β-lg A *v*. B in early

lactation and the role of mRNA in determining the differential production of α-la and β-lg in milk.

Materials and methods

Cows and management

Animals. Cows in each of the trials were run as a mixed herd and had access to a generous allowance of pasture and water *ad lib.* The β-lg phenotypes of the cows were determined in milk samples using non-denaturing gel electrophoresis (Lowe *et al.* 1995).

Trial 1. Milk from 44 Friesian cows, half homozygous for β-lg A and half for β-lg B, was collected on or near to days 6, 60, 180 and 220 post calving. The groups were balanced as far as possible for previous production history, breeding index, parity, condition score on calving and κ-casein phenotype. Blood from the coccygeal vein of each cow was collected into vacutainers containing EDTA on days 60 and 180. Plasma was obtained by centrifugation and analysed for lactose (Stelwagen *et al.* 1997).

Trial 2. Milk from 29 Friesian cows, 14 homozygous for β-lg A and 15 for β-lg B was collected at weekly intervals from week 1 to week 6 post calving.

Trial 3. Mammary tissue was obtained by biopsy from six cows homozygous for β-lg A and six homozygous for β-lg B following the protocol developed and utilized extensively in this laboratory (Farr *et al.* 1996). The cows were between 90 and 120 d post calving. Total RNA was extracted from ∼ 500 mg tissue using Trizol according to the manufacturer's instructions (Gibco BRL, Auckland, New Zealand) and quantified by absorbance at 260 nm. RNA (10 μg) from each sample was separated on an agarose gel (16 g/l) containing 0·66 M-formaldehyde–20 mM-3-(*N*-morpholino)propane-sulphonic acid–10 mM-EDTA–50 mM-sodium acetate, pH 7·0 prior to transfer to Hybond-N^+ membranes (Amersham, Auckland, New Zealand) by capillary action. RNA was visualized with u.v. and then probed with ^{32}P-labelled α-la and β-lg cDNA as detailed by Wheeler *et al.* (1995). The mRNA was measured by densitometric analysis on a Personal Densitometer with ImageQuant software (Molecular Dynamics, Sunnyvale, CA 94086-4520, USA). Densitometry was normalized to the RNA loading visualized under u.v. Three different exposures of the X-ray film were quantified and averaged to ensure exposures in the linear range.

Milk sample collection and analysis

Milk yields were recorded on each experimental day, and samples of milk were collected from each cow using in-line milk meters (Tru-test Ltd, Auckland, New Zealand). Milk lactose, protein and fat were determined using a Milkoscan 133B (Foss Electric, Hillerød, DK-3400, Denmark). A skim milk sample was also stored at −20 °C. To reduce potential proteolysis during storage, 150 μl 1 M-ε-amino-*n*-caproic acid (Sigma-Aldrich Pty, Castle Hill, NSW 2154, Australia) and 50 μl 40 mM-phenylmethylsulphonyl fluoride (Boehringer Mannheim NZ Ltd, Auckland, New Zealand) were added to whole milk. The samples were mixed, centrifuged at 1150 ***g*** and 4 °C for 10 min and a portion of skim milk removed and stored at −20 °C.

Concentrations of α-la in milk were determined by radioimmunoassay (Prosser *et al.* 1992) and β-lg and serum albumin by ELISA (Stelwagen *et al.* 1994; Prosser & McLaren, 1997).

Table 1. *Milk yields and concentrations of α-lactalbumin, β-lactoglobulin and serum albumin in milk from Friesian cows of β-lactoglobulin AA and β-lactoglobulin BB phenotypes*

(Values are for 22 cows of both phenotypes)

Days in milk	Milk yield, kg/d			β-Lactoglobulin, mg/ml			α-Lactalbumin, mg/ml			Serum albumin, μg/ml		
	AA	BB	SED	AA	BB	SED	AA	BB	SED	AA	BB	SED
6	10·6	10·2	0·7	7·8	7·5	0·9	1·5	1·5	0·2	187	209	34
60	23·6	22·4	1·2	5·2	3·5	0·2**	1·4	1·6	0·1*	157	183	11*
120	14·0	13·8	0·6	5·0	3·4	0·2**	1·0	1·2	0·08*	231	253	15
220	12·2	10·8	0·8	5·1	3·5	0·5**	1·1	1·2	0·1	268	381	57*

*$P < 0·05$, **$P < 0·01$.

Statistical analysis

Differences in milk composition due to phenotype were tested for significance using the *t* test or the General Linear Modelling procedure in MINITAB v. 12.1 (Minitab Inc., State College, PA 16801-3008, USA). Effects were considered significant at $P < 0·05$.

RESULTS

Trial 1

Table 1 shows the effect of stage of lactation on the whey protein composition of milk of cows of β-lg A or B phenotype. Significant ($P < 0·05$) differences in α-la, β-lg and serum albumin were found at day 60 post calving. The β-lg AA phenotype was associated with higher concentrations of β-lg and lower concentrations of α-la and serum albumin in milk than the β-lg BB phenotype. Differences in concentrations due to phenotype remained at days 180 and 220 for β-lg, but only at day 180 for α-la. Concentrations of serum albumin were also higher in milk from β-lg BB cows collected around 220 d, but not day 180. In contrast, at day 6 of lactation there were no significant differences due to phenotype in any of these constituents. Cows of different phenotypes produced similar volumes of milk at all stages of lactation.

Plasma lactose was 31 ± 6 and 42 ± 6 μM on day 60 and 47 ± 4 and 55 ± 4 μM on day 180 for β-lg AA and BB cows respectively. These were not significantly different.

Trial 2

The results of the first trial suggested that the phenotypic differences in whey proteins in milk are not apparent in early lactation. Collection of milk at weekly intervals from the first week of lactation confirmed this observation and further showed that the differences in α-la and β-lg were first manifested only during the third week of lactation (Fig. 1). Concentrations of α-la were initially ~ 2 mg/ml in milk from both β-lg AA or BB cows, but while concentrations in milk from β-lg AA cows fell 31% by week 4, those in milk from β-lg BB cows fell by only 11%. Conversely, concentrations of β-lg in milk, intitially ~ 5 mg/ml, changed little over the next 4 weeks in β-lg AA cows, but fell 23% for β-lg BB cows.

Trial 3

In agreement with the first two trials, the two groups of animals gave very similar yields and concentrations of total protein, but the ratio of concentrations of β-lg to

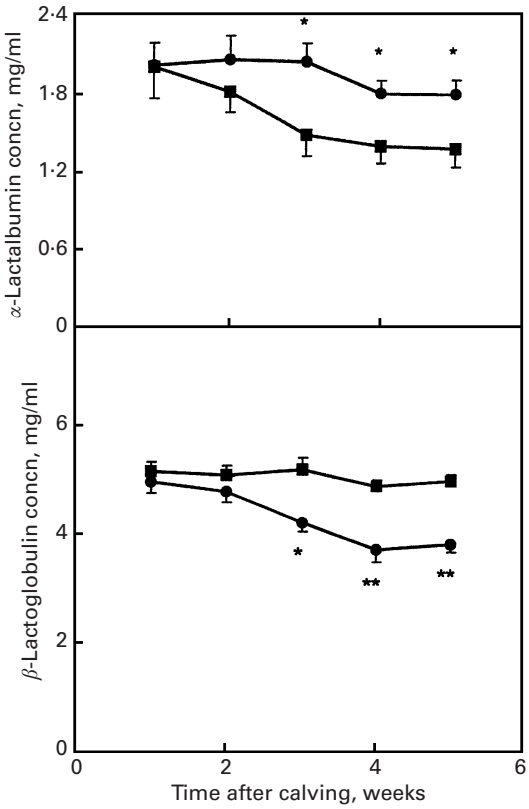

Fig. 1. Concentrations of (*a*) α-lactalbumin and (*b*) β-lactoglobulin in milk from Friesian cows of ■, β-lactoglobulin AA phenotype ($n = 14$) and ●, β-lactoglobulin BB phenotype ($n = 15$) at weekly intervals from week 1 post calving. Values are means ± SEM. Values for the two phenotypes were significantly different: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

Table 2. *Ratio of β-lactoglobulin to α-lactalbumin mRNA in mammary tissue and proteins in milk from cows homozygous for β-lactoglobulin A and β-lactoglobulin B*

(Values are means ± SE for $n = 6$)

Phenotype	Milk yield, kg/d	Milk protein content, g/kg	Ratio, β-lactoglobulin: α-lactalbumin	
			Protein	mRNA†
AA	14·4 ± 0·2	40 ± 2	5·7 ± 0·8	1·2 ± 0·2
BB	13·8 ± 0·2	40 ± 1	3·6 ± 0·4**	0·64 ± 0·08**

† Arbitrary values based on densitomentric analysis of mRNA blots.
Values were significantly different (by *t* test) from those for AA phenotype: $**P < 0{\cdot}01$.

α-la in milk from β-lg AA cows was 1·6 times that from β-lg BB cows (Table 2). The results of the northern blot analysis of β-lg and α-la mRNA are shown in Fig. 2. After normalizing to the amount of total RNA loaded, the relative values of β-lg mRNA were 9·7 and 6·8 for AA and BB phenotypes respectively, whereas the corresponding values for α-la were 7·8 and 10·6. Thus the ratio β-lg : α-la mRNA in udder tissue of cows homozygous for β-lg A was 1·9 times that for cows expressing only the B variant.

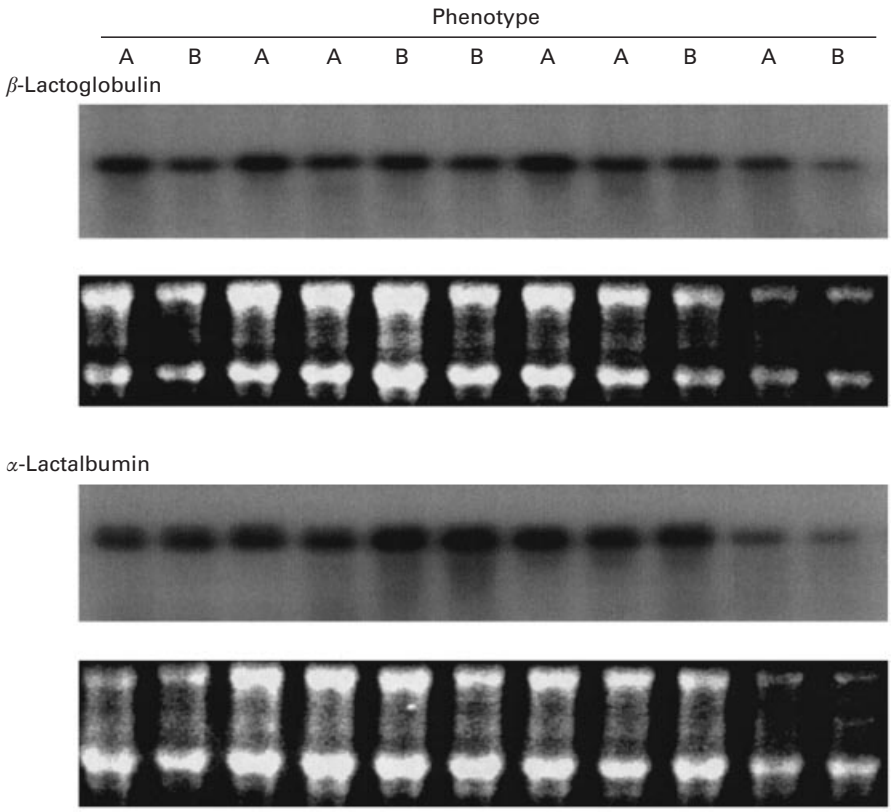

Fig. 2. Northern blot of RNA isolated from mammary tissue from lactating cows homozygous for β-lactoglobulin A or B. The top section was probed with β-lactoglobulin and the bottom section with α-lactalbumin. Total RNA loaded per lane was visualized with u.v. and is shown in the lower panels in each section.

DISCUSSION

Previous studies conducted in established lactation showed significantly higher concentrations of β-lg and lower concentrations of α-la in milk from cows homozygous for β-lg A as compared with β-lg B (Hill *et al*. 1997). The present studies, however, show that these differences are manifested only after the second week of lactation and were due to falls in the concentrations of β-lg in the β-lg B homozygous cows while those in β-lg A homozygous cows remained relatively constant. These results suggest it was not until mammary gland function reached a certain level that the compositional differences associated with different β-lg phenotypes were manifested.

The absence of a phenotypic effect immediately post calving was not due to the low level of milk production *per se*, as the phenotypic differences were apparent during the last few weeks prior to drying-off, when milk volumes were similar to those in the first weeks after calving. Moreover, a difference in response to nutritional status was unlikely, as β-lg phenotypic differences in milk composition are maintained when plasma amino acids are reduced by atropine (Prosser & McLaren, 1997) or when pasture is limited (M. J. Auldist, unpublished observations).

The present study also showed significantly higher concentrations of serum albumin in milk from cows of the β-lg BB phenotype at day 60 and also just prior to drying-off, but not at day 180 of lactation. The variation seen during different

stages of lactation may partly explain why some studies report a phenotypic difference for serum albumin (Atroshi *et al.* 1982; Ng-Kwai-Hang *et al.* 1987), while others do not (Hill *et al.* 1995). The physiological basis underlying the higher levels of serum albumin are not readily obvious. Albumin may be transferred into milk from blood via the paracellular pathway between mammary cells. Increased levels of plasma lactose have been shown to indicate movement of substances between cells via the paracellular pathway (Stelwagen *et al.* 1997), but no significant difference between phenotypes in plasma levels of lactose were found in this study. Thus, the present result cannot support differential activity of the paracellular pathway as the cause of the phenotypic differences in milk albumin.

Of greater significance was the observation that the relative levels of milk proteins in different β-lg phenotypes reflected the abundance of their respective mRNA, at least for α-la and β-lg. Allele-specific differences in the promoter region of β-lg have been documented (Wagner *et al.* 1994; Lum *et al.* 1997) and at least two cell transfection studies show greater expression of a reporter gene attached to the promoter region of β-lg A compared with B (Geldermann *et al.* 1996; Folch *et al.* 1999). These studies are consistent with altered transcription being the main cause of the higher expression of the A compared with the B allele of β-lg. One explanation of such a phenomenon is that the promoter region of the β-lg A allele has a higher affinity for transcription factions, thereby being expressed more efficiently (Lum *et al.* 1997). The present results show that expression of both β-lg A alleles in homozygous animals was associated with different levels of mRNA of other milk proteins. This raises the possibility of competition for expression between different milk protein genes as a means of regulating milk composition in normally lactating cows. A similar regulatory mechanism was proposed to account for the suppressive effect of a β-lg transgene on endogenous milk protein gene expression in mice (McClenaghan *et al.* 1995).

In conclusion, the results of this study, combined with previous reports, show major differences in the whey protein composition of milk due to β-lg phenotype throughout most of lactation, with the exception of the first 2 weeks post calving. In established lactation the phenotypic differences in the ratio β-lg:α-la in milk were reflected in similar differences in their respective mRNA in mammary tissue, suggesting that it was the level of mRNA that determined the whey protein profiles of these two phenotypes. The phenomenon of differential allelic expression is now well described for the β-lg gene and may also explain the differential production of the A *v.* B variant of κ-casein in heterozygous κ-casein cows (Van Eenennaam & Medrano, 1991). However, the present study represents the first report of the potential effect of homozygous expression of specific alleles on other milk proteins and provides an important insight into how milk protein composition may be regulated during established lactation. From a practical standpoint, these studies show that any benefit that may be derived from segregating milk from cows of different β-lg phenotypes, for example for cheese manufacture, can only be gained after lactation has become established.

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