Variation in milk whey composition throughout lactation in cows of different ß-lactoglobulin phenotypes

S-A. TURNER, M.J. AULDIST[1], P.C. MOLAN[2] AND C.G. PROSSER.

Dairy Science Group, AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand

ABSTRACT

The association between ß-lactoglobulin (ß-LG) variants and milk composition throughout lactation was determined by sampling from 44 Friesian cows (22 homozygous for ß-LG AA and 22 homozygous for ß-LG BB) in early (day 6 postpartum), established (1-3 months postpartum) and late lactation (on the day of drying off).

Results indicated that differences between ß-LG AA and BB phenotype milk (expressed as a percentage of the level in phenotype AA) in concentrations of bovine serum albumin (BSA; -15.9%, -32.0%, -42.5%) and ß-LG (5.9%, 16.0%, 32.2%), in early, established and late lactation respectively, increased steadily as lactation progressed. The differences in α-lactalbumin concentrations were less variable (1.2%, -9.0%, -9.1%).

These results provide new evidence that compositional differences are not seen between ß-LG AA and BB phenotype milk until established lactation.

Keywords: milk composition; ß-lactoglobulin phentoypes; whey proteins.

INTRODUCTION

Variants of ß-lactoglobulin (ß-LG) are associated with differences in milk composition during established lactation (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1987; Hill, 1993; Hill & Paterson, 1994). For instance, the milk of ß-LG AA phenotype cows contains more whey and less casein protein than the milk of ß-LG BB phenotype cows (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1986; Hill, 1993).

Recent research has shown that milk proteins have different physicochemical properties and can thus alter the chemical and physical properties of milk or milk products (Hill *et al*., 1996). Therefore this could lead to utilisation of milk with particular proteins being processed into specific products.

The very early stages of lactation are associated with some of the largest compositional changes seen in lactation (Hartmann, 1973) when the production of milk proteins is being 'switched on'. Thus, the period just following calving offers a unique opportunity to study the mechanisms controlling this. Although many studies have investigated the milk compositional changes that occur throughout this period, there is no information regarding the influence of ß-LG variants.

This study examined the associations between ß-LG phenotypes and milk composition across lactation. Milk composition was examined during the very early and late stages of lactation in an attempt to determine if the differences were apparent from the beginning of lactation, and to examine whether the compositional differences persisted during late lactation.

MATERIALS AND METHODS

Cow selection and management

Forty-four Friesian cows, of which 22 were homozygous for ß-LG AA and 22 for ß-LG BB, were utilised in this study. The groups were balanced as far as possible for previous production history, breeding index, parity, condition score prior to calving and κ-casein phenotype. The cows were run as a mixed herd at the Dairying Research Corporation's No 5 Dairy. All animals had *ad libitum* access to pasture and water.

Sample collection and preparation

Whole milk samples (each approximately 10 ml) were collected from each cow both AM (0700) and PM (1500) on day 6 postpartum, using in-line milk meters. Composite samples (PM + AM) were collected from all cows during established lactation (1-3 months postpartum) and also on the day the cows were dried off (187-270 days postpartum). Milk weights were recorded at each milking.

To reduce proteolysis during storage, 150 µl of 1 Mε-amino-n-caproic acid (Sigma Chemical Co., St Louis, MO, USA) and 50 µl of 7 mg/ml phenylmethylsulfonyl fluoride (Boehringer Mannheim GmbH, Mannheim, Germany) were added to 10 ml of whole milk. The samples were then centrifuged at 1150 x g for 10 minutes at 4°C. The skim milk was collected and stored at -20°C until required.

Sample analysis

α-LA concentrations in the milk samples were measured by radioimmunoassay as previously described (Prosser *et al*., 1992).

BSA concentrations were analysed by ELISA based upon the method of Stelwagen *et al*. (1994) with the following changes. The rabbit anti-bovine serum albumin (DAKO Corporation, Carpintera, CA, USA) primary antibody was used at a dilution of 1:32 000 and the assay plates were

[1] Dairying Research Corporation Ltd, Private Bag 3123, Hamilton, New Zealand
[2] Biological Sciences Department, University of Waikato, Hamilton, New Zealand

developed by the addition of 100 µl of 0.05 M phosphate/citrate buffer, pH 5.0, containing 10 mg/ml 3,3',5,5' tetramethylbenzidine in dimethyl sulphoxide with 0.3 µl/ml 30% [v/v] H_2O_2 to each well.

ß-LG concentrations in the milk samples were measured by ELISA based upon the method of Prosser and McLaren (1997) with the following change. On the day of assay skim milk samples were thawed and diluted 1:1 (v/v) with Triton-X100/EDTA buffer (8.5% [w/v] ethylenediaminetetraacetic acid [EDTA] and 16% [w/v] Triton-X100) prior to being diluted 1:50 (v/v) with ELISA diluent (50 mM phosphate, pH 7.5, 0.15 M NaCl containing 0.05% [w/v] Tween-20 with 0.1% [w/v] polyvinylpyrrolidone [PVP]).

Somatic cell counts were measured using an automated cell counter (Fossomatic 450; Foss Electric, Hillerød, Denmark).

Statistical analysis

A Student's 't' test was used to examine whether the compositional differences between ß-LG AA and ß-LG BB phenotype cows were significant. Differences were considered significant at *P* - 0.05.

RESULTS

Table 1 shows the concentrations of α-LA, ß-LG and BSA in milk and milk yields of the two phenotypes during the different stages of lactation and Table 2 shows the percentage difference between ß-LG AA and ß-LG BB phenotype animals. The differences increased steadily for both ß-LG and BSA concentrations as lactation progressed. At all stages of lactation, ß-LG AA phenotype animals produced milk that contained the highest amounts of ß-LG, while ß-LG BB phenotype animals produced milk containing higher amounts of BSA. The differences seen in α-LA concentrations between the phenotypes remained constant between established and late lactation. ß-LG AA phenotype animals had slightly higher concentrations of α-LA during early lactation, however, during established and late lactation, the ß-LG BB phenotype animals had the highest concentrations. The differences seen in milk yields between the phenotypes were not statistically different at any stage of lactation.

The compositional differences between the phenotypes (Table 2) were not significant at the very beginning of lactation, but were significant during both established and late lactation ($P < 0.05$) for BSA, while only significant during late lactation ($P < 0.05$) for ß-LG. The differences seen in α-LA concentrations and milk yields were not significant at any stage of lactation.

TABLE 2: Comparison of differences in milk whey composition between ß-lactoglobulin AA and ß-lactoglobulin BB phenotype cows throughout lactation. Values presented are differences between the phenotypes expressed as a percentage of the level in ß-lactoglobulin AA phenotype milk. Statistical significance is presented for the differences (*$P < 0.05$). Early: Day 6 postpartum; Established: 1-3 months postpartum; Late: 187-270 days postpartum.

	Early	Established (AA-BB/AA x 100)	Late
α-lactalbumin	1.2	-9.0	-9.1
ß-lactoglobulin	5.9	16.0	32.2*
Bovine serum albumin	-15.9	-32.0*	-42.5*
Milk Yield	3.3	1.8	11.5

* $P < 0.05$

DISCUSSION

Compositional analysis during established lactation showed similar trends to those previously reported for both ß-LG and α-LA, although the absolute differences seen were much smaller (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1987; Hill, 1993). This is possibly due to differences between cows as the study of Hill and Paterson (1994) showed the variation in milk composition between cows can be as large as or greater than the differences between phenotypes.

Similar to the study by Ng-Kwai-Hang *et al*. (1987), but in contrast to that of Hill *et al*. (1995) the ß-LG BB cows in the present study contained more BSA than ß-LG AA phenotype cows. It has been suggested that increased somatic cell counts are associated with increased BSA concentrations (Lacy-Hulbert *et al*., 1995). Somatic cell counts are also used as an indicator of mastitis with cows showing elevated levels during subclinical or clinical bouts of mastitis (Holmes & Woolford, 1992). Somatic cell counts were examined (data not presented) for all animals, and there was no indication that these levels were elevated. The mean SCC's for both phenotypes were not significantly different.

During early lactation there were small differences between the phenotypes for BSA, ß-LG and α-LA, but none of these were significant. These increased later in lactation, suggesting that the mechanism(s) controlling

TABLE 1: Comparison of milk whey composition between ß-lactoglobulin AA and ß-lactoglobulin BB phenotype cows throughout lactation. Values are presented ± standard error of the mean. Significant differences between AA and BB phenotypes are shown (*$P < 0.05$). Early: Day 6 postpartum; Established: 1-3 months postpartum; Late: 187-270 days postpartum.

	Early		Established		Late	
	AA	BB	AA	BB	AA	BB
α-LA[1]; mg/ml	1.48 ± 0.12	1.46 ± 0.10	1.51 ± 0.06	1.65 ± 0.06	1.12 ± 0.06	1.23 ± 0.07
ß-LG[2]; mg/ml	7.92 ± 0.74	7.46 ± 0.60	5.39 ± 0.40	4.53 ± 0.37	5.14 ± 0.38	3.49 ± 0.19*
BSA[3]; mg/ml	0.19 ± 0.03	0.22 ± 0.02	0.23 ± 0.02	0.31 ± 0.02*	0.27 ± 0.03	0.38 ± 0.05*
MY[4]; Kg/day	10.58 ± 0.44	10.24 ± 0.48	20.93 ± 1.04	20.55 ± 1.15	12.21 ± 0.55	10.81 ± 0.61

[1] α-Lactalbumin [2] ß-Lactoglobulin [3] Bovine serum albumin [4] Milk Yield * $P < 0.05$

these were not activated until sometime between day 6 of lactation and established lactation.

Lactogenesis is associated with rapid and substantial increases in milk production and milk composition and is regulated by defined hormonal changes (Hartmann, 1973). It is possible that it is not until the mammary gland reaches full production potential, that the compositional differences associated with ß-LG phenotypes are manifested. This suggests that the mechanisms controlling these differences may be affected by the state of the mammary gland either during the early stages of lactation when milk production is being switched on, resulting in the composition remaining similar between variants, or later in established lactation when the mammary gland cells are at maximal production, thus resulting in the reported compositional differences. It is unlikely that nutritional effects are playing a role in the differences as Prosser and McLaren (1997) reported that cows with different phenotypes responded similarly to a reduction in amino acid supply. Additionally, it is unlikely that milk volume alone plays a role in this as the differences between variants are also seen during late lactation when milk volume is relatively small.

The exaggeration of the differences between the phenotypes for BSA and ß-LG at the end of lactation may suggest that the mechanism controlling the concentrations of these proteins is more affected by the declining milk yields and involution than α-LA, where the difference between the phenotypes remained relatively constant across lactation. It is interesting that the greatest differences in milk composition between the phenotypes occurred at the end of lactation when there was the greatest difference between the phenotypes in terms of milk yields.

These results indicate that compositional differences associated with the ß-LG phenotypes vary across lactation. However, more research is required during the early stages of lactation to determine the time when the phenotypic differences become apparent.

ACKNOWLEDGEMENT

This work was supported by the Foundation for Research Science and Technology.

REFERENCES

Hartmann, P. E. 1973. Changes in the composition and yield of the mammary secretion of cows during the initiation of lactation. *Journal of Endocrinology* **59:** 231-247.

Hill, J. P. 1993. The relationship between ß-lactoglobulin phenotypes and milk composition in New Zealand dairy cattle. *Journal of Dairy Science* **76:** 281-286.

Hill, J. P.; Paterson, G. R. 1994. The variation in milk composition from individual ß-lactoglobulin AA and BB phenotype cows. *Proceedings of the New Zealand Society of Animal Production* **54:** 293-295.

Hill, J. P.; Paterson, G. R.; Lowe, R.; Wakelin, M. 1995. The effect of season and beta-lactoglobulin phenotype on milk composition. *Proceedings of the New Zealand Society of Animal Production* **55:** 94-96.

Hill, J. P.; Thresher, W. C.; Boland, M. J.; Creamer, L. K.; Anema, S. G.; Manderson, G.; Otter, D. E.; Paterson, G. R.; Lowe, R.; Burr, R. G.; Motion, R. L.; Winkelman, A.; Wickham, B. 1996. The polymorphism of the milk protein ß-Lactoglobulin. A Review. *In* Milk Composition, Production and Biotechnology (ed. Welch, R. A. S.; Burns, D. J. W.; Davis, S. R.; Popay, A. I.; Prosser, C. G.), pp. 173-202. Wallingford, England, CAB International.

Holmes, C. W.; Woolford, M. W. 1992. Why are somatic cells important? *Proceedings of the Ruakura Farmers' Conference* **44:** 131-135

Lacy-Hulbert, S. J.; Woolford, M. W.; Bryant, A. M. 1995. End of season milk. *Proceedings of the Ruakura Farmers' Conference* **47:** 71-77

McLean, D. M.; Graham, E. R. B.; Ponzoni, R. W.; McKenzie, H. A. 1984. Effects of milk protein genetic variants on milk yield and composition. *Journal of Dairy Research* **51:** 531-546.

Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E.; Monardes, H. G. 1986. Relationships between milk protein polymorphisms and major milk constituents in Holstein-Friesian cows. *Journal of Dairy Science* **69:** 22-26.

Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E.; Monardes, H. G. 1987. Variation in milk protein concentrations associated with genetic polymorphism and environmental factors. *Journal of Dairy Science* **70:** 563-570.

Prosser, C. G.; Eichler, S. J.; Farr, V. C.; Davis, S. R. 1992. Effect of colostrum intake on alpha-lactalbumin concentrations in serum of calves. *Research in Veterinary Science* **53:** 219-222.

Prosser, C. G.; McLaren, C. G. 1997. Effect of atropine on milk protein yield by dairy cows with different ß-lactoglobulin phenotypes. *Journal of Dairy Science* **80:** 1281-1287.

Stelwagen, K.; Davis, S. R.; Farr, V. C.; Eichler, S. J.; Politis, I. 1994. Effect of once daily milking and concurrent somatotropin on mammary tight junction permeability and yield of cows. *Journal of Dairy Science* **77:** 2994-3001.