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## Diurnal variation in the protein composition of bovine milk

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### ABSTRACT

Two trials were conducted to define the variation in concentrations of  $\beta$ -lactoglobulin ( $\beta$ -LG) in bovine milk following different milking intervals.

In the first, milk samples were collected from three herds of 20 cows. At the time of sampling in early summer, one herd was in each of early, mid and late lactation (30, 120 and 210 days post calving, respectively). Average  $\beta$ -LG concentrations were 41, 37 and 50% higher ( $p<0.05$ ) after a 16h than 8h milking interval for early, mid and late lactation, respectively.

In the second trial, bulk milk was sampled from four herds of 12 cows. All cows were at the same stage of lactation and the milking interval was 16:8h. Samples were collected on three separate occasions when cows were on unrestricted pasture intake and once when intake was restricted by 50%. On unrestricted pasture intake, concentrations of  $\beta$ -LG were 11% higher in milk collected after 16h than 8h milking interval, whilst on restricted intake, concentrations were 44% higher. There were no differences in concentrations of  $\alpha$ -lactalbumin ( $\alpha$ -LA).

Both trials show a significant difference in  $\beta$ -LG levels in milk collected after 16h versus 8h milking interval, although only 71% of all samples collected in the first trial exhibited this difference and it was less in the second trial using bulk milk.

**Keywords:** Diurnal variation; bovine milk; protein composition;  $\alpha$ -lactalbumin;  $\beta$ -lactoglobulin;  $\beta$ -casein.

### INTRODUCTION

$\beta$ -Lactoglobulin ( $\beta$ -LG) is the principle protein in the whey fraction of bovine milk, comprising around 10% of total milk protein. Much interest recently has been focused on the relationship between  $\beta$ -LG phenotypes and protein composition in milk from New Zealand cows (Hill, 1993; Hill and Paterson, 1994). However,  $\beta$ -LG concentrations vary considerably within a lactation (Caffin *et al.*, 1985) and there is evidence that the level of nutrition can influence the concentration of whey fraction components of milk (Gray and Mackenzie, 1987).

Since  $\beta$ -LG can readily influence the quality of milk products by imparting distinct processing properties to milk (McLean *et al.*, 1987), the potential to manipulate  $\beta$ -LG concentration is an important consideration for dairy processors.

In this study we investigated the effect of stage of lactation, milking interval (16:8h), and feed restriction on the concentrations of  $\beta$ -LG and other milk components.

### MATERIALS AND METHODS

#### Experimental design

Two trials were conducted. All animals used in both trials were Friesian cows of mixed  $\beta$ -LG phenotype.

In the first trial, individual milk samples were obtained at morning and afternoon milkings from 3 herds each of 20 cows, all milked at 0700 and 1500h (16:8h milking interval). The trial was conducted in early summer and calving was synchronised such that each of the 3 herds

was in early, mid or late stage of lactation; 30, 120 and 210 days post calving respectively. An aliquot of milk was analysed for fat, protein and lactose by Milkoscan and another was defatted by centrifugation and analysed for  $\beta$ -LG by ELISA (Prosser and McLaren, 1997).

The second trial was designed to determine the effect of pasture intake on milk composition and consisted of 4 herds of 12 animals, all at the same stage of lactation. All animals were on a 16:8h milking interval. The cows were offered unrestricted access to pasture during *ad libitum* intake and then restricted to 50% of required metabolisable energy intake. Bulk milk was obtained from each herd in the morning and afternoon on three separate occasions during *ad libitum* pasture intake and once at the end of 7 days of restricted pasture intake. Concentrations of  $\alpha$ -LA in skim milk samples were determined by RIA (Prosser *et al.*, 1992) and both  $\beta$ -casein and  $\beta$ -LG by ELISA (Prosser and McLaren, 1997).

### RESULTS

#### Trial 1

Table 2 shows that the differences in milk composition between morning and afternoon milk collections from trial 1, expressed as am/pm ratios, were restricted to  $\beta$ -LG levels and % fat. The highest mean concentrations of  $\beta$ -LG were in am milk and in pm milk for fat. Lactose and total protein were unchanged (Table 1). These ratios were independent of stage of lactation and the differences in  $\beta$ -LG concentrations between am and pm milk were significant ( $P<0.05$ ) at all stages of lactation; 41, 37 and 50% for early, mid and late lactation respectively.

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**TABLE 1:** Mean morning and afternoon levels of  $\beta$ -LG total protein, fat and lactose from trial 1 across three stages of lactation in summer. Values in parenthesis are  $\pm$  s.e.m.

	STAGE OF LACTATION					
	EARLY		MID		LATE	
	AM	PM	AM	PM	AM	PM
$\beta$ -LG (mg/ml)	1.74 ( $\pm$ 0.18)	1.23 ( $\pm$ 0.13)	1.88 ( $\pm$ 0.24)	1.37 ( $\pm$ 0.12)	1.84 ( $\pm$ 0.21)	1.21 ( $\pm$ 0.15)
Protein (g/100ml)	3.23 ( $\pm$ 0.05)	3.31 ( $\pm$ 0.05)	3.45 ( $\pm$ 0.06)	3.61 ( $\pm$ 0.07)	3.5 ( $\pm$ 0.05)	3.89 ( $\pm$ 0.06)
Fat %	3.82 ( $\pm$ 0.19)	5.11 ( $\pm$ 0.20)	4.24 ( $\pm$ 0.13)	5.16 ( $\pm$ 0.17)	4.20 ( $\pm$ 0.11)	6.10 ( $\pm$ 0.19)
Lactose (g/L)	4.89 ( $\pm$ 0.05)	4.99 ( $\pm$ 0.05)	4.95 ( $\pm$ 0.07)	4.97 ( $\pm$ 0.07)	4.96 ( $\pm$ 0.06)	4.96 ( $\pm$ 0.06)

**TABLE 2:** Morning and afternoon milk composition differences from trial 1 expressed as mean am/pm ratio across three stages of lactation. Values in parenthesis are  $\pm$  s.e.m.

	STAGE OF LACTATION		
	EARLY	MID	LATE
$\beta$ -Lg	1.53 ( $\pm$ 0.14)	1.52 ( $\pm$ 0.23)	1.63 ( $\pm$ 0.24)
Protein	0.98 ( $\pm$ 0.01)	0.97 ( $\pm$ 0.01)	0.90 ( $\pm$ 0.01)
%Fat	0.77 ( $\pm$ 0.05)	0.83 ( $\pm$ 0.03)	0.70 ( $\pm$ 0.02)
Lactose	0.98 ( $\pm$ 0.01)	1.0 ( $\pm$ 0.01)	1.0 ( $\pm$ 0.01)

Although only 71% of all cows sampled had a higher concentration of  $\beta$ -LG in am milk, the overall difference across all stages of lactation in summer was 43%.

## Trial 2.

In the second trial, mean  $\beta$ -LG concentrations were 15% higher in bulk milk samples collected after 16h than 8h milking interval with unrestricted pasture intake, but 44% higher during restricted feeding (Table 3). As in trial 1, 71% of all bulk samples showed an am/pm ratio greater than 1.0 for  $\beta$ -LG. There was also a significant difference between treatment groups in am/pm ratios for  $\beta$ -casein ( $p < 0.05$ ), in this instance restricted feeding reduced the am/pm ratio (Table 3). There was no diurnal difference in the concentrations of  $\alpha$ -LA within and between treatment groups.

## DISCUSSION

In this study we found consistent and significant differences in mean  $\beta$ -LG concentrations between milk collected after 16h (am) versus 8h (pm) milking interval which, over both trials, were independent of stage of lactation, milking season (spring versus summer) and  $\beta$ -

LG phenotype. In a previous study on Argentinean cattle by Sbodio *et al.*, (1985), no differences in total protein nor in casein concentration were found between milk collected in the morning or afternoon. This report, as far as we are aware, is the only other to have investigated an apparent diurnal variation in individual milk proteins.

In the first trial, the mean percentage difference in  $\beta$ -LG concentration between morning and afternoon milk across all stages of lactation was 43%. This is higher than the 31% difference in  $\beta$ -LG concentration between  $\beta$ -LG phenotypes AA and BB in bulk milk samples (pooled from 4 successive milking) from New Zealand Friesian cows reported by Hill (1993). In the present study only, 71% of milk from individual cows (trial 1) or bulk herd milk samples (trial 2) showed a diurnal variation in  $\beta$ -LG concentrations. It is not known what, if any, influence  $\beta$ -LG phenotype has on the diurnal variation in  $\beta$ -LG.

The difference in  $\beta$ -LG and  $\beta$ -casein levels between morning and afternoon milk in the second trial were significantly enhanced by restricting feed intake. There was a 20% reduction in mean  $\beta$ -LG concentration in afternoon milk from the feed restricted group compared with the milk from the control *ad libitum* group, inducing a 30% increase in the am/pm ratio. In contrast, there were no differences between the morning and afternoon in  $\beta$ -casein levels in milk from the *ad libitum* fed group, while there was a 26% decrease in mean b-casein concentration in am milk from the feed restricted group. This resulted in a 19% decrease in the am/pm ratio for  $\beta$ -casein. These differences, due to pasture intake, were statistically significant ( $p < 0.05$ ). In a previous study, Gray and Mackenzie (1987) reported significant reductions in the concentration of whey proteins,  $\beta$ -LG, and  $\alpha$ -LA, in composite milk samples from cows on reduced levels of nutrition. This contrasted with increased milk concentrations of both serum albumin and immunoglobulin, although

**TABLE 3:** Mean am and pm concentrations and am/pm ratios for  $\beta$ -LG,  $\beta$ -Casein and  $\alpha$ -LA for *ab libitum* and feed restricted cows from trial 2. Values in parenthesis are  $\pm$  s.e.m.

	TREATMENT GROUP			FEED RESTRICTED		
	AD LIBITUM		AM/PM	FEED RESTRICTED		AM/PM
	AM(mg/ml)	PM(mg/ml)	AM/PM	AM(mg/ml)	PM(mg/ml)	AM/PM
$\beta$ -LG	5.5 ( $\pm$ 0.20)	5.0 ( $\pm$ 0.40)	1.11 ( $\pm$ 0.07)	5.4 ( $\pm$ 0.30)	4.0 ( $\pm$ 0.60)	1.44 ( $\pm$ 0.15)*
$\beta$ -casein	10.8 ( $\pm$ 0.40)	10.3 ( $\pm$ 0.50)	1.05 ( $\pm$ 0.03)	8.1 ( $\pm$ 0.30)	10.3 ( $\pm$ 0.80)	0.84 ( $\pm$ 0.10)*
$\alpha$ -LA	1.26 ( $\pm$ 0.02)	1.36 ( $\pm$ 0.03)	0.93 ( $\pm$ 0.03)	1.03 ( $\pm$ 0.05)	1.09 ( $\pm$ 0.04)	0.95 ( $\pm$ 0.07)

Level of significance between treatment groups \*  $p < 0.05$

only the change in the serum albumin level was significant. Casein levels were not investigated in their study. More recently, Prosser and McLaren (1997), reported on milk protein compositional changes following atropine induced reduction in availability of amino acids to the mammary gland of cows with different  $\beta$ -LG phenotypes. The differences in reported levels of  $\alpha$ -LA,  $\beta$ -LG,  $\beta$ -casein and serum albumin in milk were not significantly different between phenotypes within treatment groups, however  $\beta$ -LG and serum albumin concentrations varied significantly between treatments.

There are several possible explanations for the diurnal variations in individual milk proteins, including the different intervals between milkings as well as changes in grazing behaviour over the 24 h period. It is not known which of these is the direct cause of the differences observed. Nevertheless, the results from this present study demonstrate the potential to alter milk composition through management practices such as altered milking interval and/or pasture allowance. However, further work is required to determine the underlying mechanisms that influence these milk compositional changes since not all animals show a consistent response.

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