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## Diet and genotype affect milk lactoferrin concentrations in late lactation

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### ABSTRACT

Although lactoferrin (Lf) is a valued milk protein, little information is available on factors that affect its concentration in milk. Milk samples were collected during late lactation to determine if diet or cow genotype affected the concentration and yield of Lf in bovine milk. Holstein-Friesian (HF) cows (n=52) of either overseas (OS) or New Zealand (NZ) ancestry were fed either an all-pasture diet or a total mixed ration (TMR). Milk samples were collected from each cow during an AM milking in early March and late April. NZHF had a higher ( $P < 0.05$ ) Lf concentration than OSHF even though they produced less ( $P < 0.05$ ) milk. Genotype did not affect Lf yield, somatic cell count or the proportion of bacteriologically negative quarters. The Lf concentration in the milk of cows fed TMR was higher ( $P < 0.05$ ) than cows grazing pasture in April but not in March. Cows fed TMR also produced more ( $P < 0.05$ ) milk and a greater ( $P < 0.001$ ) yield of Lf than cows grazing pasture. Diet did not affect SCC, however only cows fed TMR had any bacteriologically positive quarters. A positive within-cow correlation ( $P < 0.001$ ) was found between Lf and SCC. The effect of diet on the concentration of Lf in milk requires further investigation. The genotype results suggest that NZHF have higher concentrations of Lf in milk than OSHF. Naturally high concentrations allow improved extraction efficiencies of Lf during processing.

**Keywords:** lactoferrin; pasture; diet; genotype; Holstein-Friesian.

### INTRODUCTION

Bovine milk contains a wide range of proteins, many of which are known for their bioactive properties. Lactoferrin (Lf) is one such protein. Lf is an iron-binding glycoprotein that is found in the whey fraction of milk (Nuijens *et al.*, 1996; Conneely, 2001). This protein has well known antimicrobial and immunomodulatory activities including bacteriostasis, inhibition of cytokine release and stimulation of natural killer cells (Nuijens *et al.*, 1996; Conneely, 2001). Because of the potential benefits to human health of these properties, a market has been established for Lf as an ingredient in products such as infant formulae, nutritional supplements and cosmetics.

The concentration of Lf in milk changes throughout lactation. Concentrations of Lf in colostrum can vary from 0.8 to 2 mg/ml on the first day postpartum and decline rapidly thereafter (Sanchez *et al.*, 1988; Schanbacher *et al.*, 1993). Milk collected during established lactation contains much lower concentrations of Lf (0.03 to 0.3 mg/ml; Rainard *et al.*, 1982; Farr *et al.*, 2002). Mammary secretions collected from the involuting gland one month following the cessation of regular milking contained an average Lf concentration of 25-35 mg/ml (Welty *et al.*, 1976).

There are few data available regarding the effect of cow breed or genotype on Lf concentration in milk. Lf concentrations in colostrum are significantly higher in Holstein-Friesian (HF) and Jersey animals compared with Japanese beef breeds although no difference was apparent between the Lf concentrations in HF and Jersey animals (Tsuji *et al.*, 1990). Similarly, Farr *et al.* (2002) found that Lf concentrations in milk collected at the PM milking from Jersey and Friesian cows during mid-lactation were not different. However, Friesian cows produced milk with higher Lf concentrations and Lf yields than Jersey cows at the AM milking.

The effects of diet on Lf concentrations are not known. Although Kolver *et al.* (2000; 2002) reported similar milk protein concentration in cows offered pasture or total mixed ration (TMR), nutrition, specifically energy intake, has been shown to influence the concentrations of whey proteins, including  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin (Gray & Mackenzie, 1987; Mackle *et al.*, 1999).

The reported research investigated the effect that diet (all-pasture or TMR) and cow genotype (HF cows of overseas (OS) or New Zealand (NZ) ancestry) have on Lf concentrations and yields in milk.

### MATERIALS AND METHODS

#### Experimental design

The 52 Holstein-Friesian (HF) cows used in this experiment were of either overseas (OS) or New Zealand (NZ) ancestry and were fed either an all-pasture diet or a total mixed ration (TMR). The management and dietary details of these animals has been previously described in detail (Kolver *et al.*, 2000; 2002; Roche *et al.*, 2001). Briefly, grazing cows were offered a generous allowance of pasture (>45 kg DM/cow/d) as fresh breaks, twice daily at 0700 and 1500 h. Group pasture intakes were calculated based upon visual assessment of pre- and post-grazing yields. Assessors were calibrated weekly through cutting a range of pasture yields, representative of pre- and post-grazing yields (O'Donovan, 2000; Roche *et al.*, 2001). Average pre-grazing and post-grazing pasture masses were  $3188 \pm 130$  and  $2443 \pm 154$  kg DM/ha in March and  $3513 \pm 149$  and  $2743 \pm 179$  kg DM/ha, in April respectively. The average pasture intake over the week of the 1<sup>st</sup> March and 23<sup>rd</sup> April were  $15 \pm 2.2$  and  $16 \pm 0.3$  kg DM/cow/day for OSHF and  $15 \pm 2.2$  and  $15 \pm 2.7$  kg DM/cow/day for NZHF, respectively. TMR cows were confined to a free-draining feed pad (11.5 m<sup>2</sup> per cow). TMR was fed between 0800 and 0900 h and between 1500 and 1630 h in 5-m long mobile fibreglass troughs. The TMR diets,

on a DM basis, consisted of 25% maize silage, 19.5% grass silage, 7.5% hay, 10% whole cottonseed and 38% compounded concentrate, with the NZHF and OSHF cows receiving the same TMR mix. The cows were fed TMR to achieve a 10% refusal rate. Group TMR intakes were calculated daily from measurement of the feed offered and refused. TMR intakes were 26±0.8 and 27±0.7 kg DM/cow/day for the OSHF, and 23±1.0 and 23±1.2 kg DM/cow/day for the NZHF during the weeks of the 1<sup>st</sup> March and 23<sup>rd</sup> April respectively.

### Animal measurements

Twice during the 2001/2002 season (early March and late April), foremilk samples were collected aseptically, by hand, from each quarter of 52 (March) and 44 (April) cows at the AM milking. Milk yield of each cow was also recorded.

All experimental animals were used with the approval of the Ruakura Animal Ethics Committee.

### Milk sample analyses

All foremilk samples were analysed for somatic cell count (SCC), Lf and bacteriological status. SCC was measured using an automated cell counter (Fossomatic 5000; Hillerød, Denmark). Lf concentrations in foremilk samples were measured using a bovine Lf ELISA quantification kit (Bethyl Laboratories, Inc, Montgomery, TX, USA) with the following modifications. Following incubation with a goat anti-bovine Lf antibody, the plates were incubated with Tris-buffered saline (TBS; 50 mM Tris, 0.14 M NaCl, pH 8.0) containing 2% bovine serum albumin (BSA). The goat anti-bovine Lf-horseradish peroxidase conjugate was diluted 1/100,000 in TBS containing 1% BSA and 0.05% Tween20. The plates were developed by the addition of 100 µl/well of 0.2 mg/ml 3,3',5,5'-tetramethylbenzidine (Sigma Chemical Co., St Louis, MO, USA), 1 mg/ml urea hydrogen peroxidase (Sigma Chemical Co.) and 11% dimethylformamide (BDH Chemicals, Poole, England) in 0.1 M citrate buffer. Following a 10-minute incubation at room temperature the reaction was stopped by the addition of 100 µl/well of 1 M H<sub>2</sub>SO<sub>4</sub>. The absorbance of the samples was then

read at 450 nm using an ELx800 Universal Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA).

Bacteriological assessment of quarter fore-milk samples was carried out as described by Lacy-Hulbert *et al.* (2002).

### Statistical analyses

Data were analysed using residual maximum likelihood (REML). Treatment differences were tested using the submodel procedure (Welham & Thompson, 1997) in GenStat (GenStat, 2002). SCC and Lf concentration were log<sub>10</sub> transformed to stabilise the variance before statistical analysis. Lf yield (g/AM/cow) was estimated by calculating the average Lf concentration per cow and multiplying by the total AM milk yield for that cow. Diet and genotype effects on the proportion of quarters per cow that were bacteriologically negative were analysed using generalised linear models (GLM) with binomial error distribution. The relationship between log<sub>10</sub> Lf concentration and log<sub>10</sub> SCC within-cow, was investigated using random coefficient regression models in REML.

## RESULTS

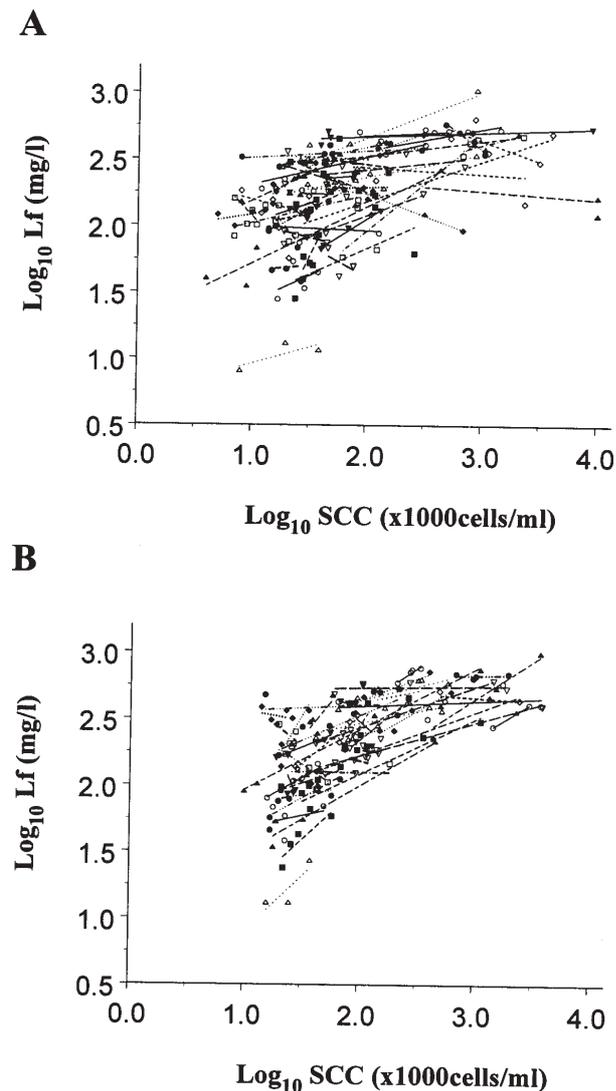
There was a higher ( $P < 0.05$ ) concentration (mg/l) of Lf in the milk of NZHF cows than in the milk of OSHF animals in both March and April (Table 1). In contrast, genotype did not affect the estimated Lf yield (Table 1) even though AM milk yield was higher ( $P < 0.05$ ) in OSHF than in NZHF cows in both March and April (Table 1). Genotype did not affect SCC or the proportion of bacteriologically negative quarters (Table 1).

Diet did not affect the Lf concentrations in March, however, in April, there was significantly more Lf in the milk of cows fed TMR (Table 1). Milk yield was also higher ( $P < 0.05$ ) in TMR-fed cows, resulting in greater ( $P < 0.001$ ) yields of Lf compared with pasture-fed cows (Table 1). There was an effect ( $P < 0.001$ ) of diet on the proportion of bacteriologically negative quarters (Table 1), those animals grazing pasture showing no signs of bacterial infection in either March or April. All 27 infections diagnosed were in cows fed TMR.

**TABLE 1:** Effect of genotype (Overseas Holstein-Friesian (OSHF) or New Zealand Holstein-Friesian (NZHF)) and diet (Pasture or Total mixed ration (TMR)) on log<sub>10</sub> lactoferrin (Lf) concentrations, estimated Lf yield, milk yield, SCC in milk and the proportion of quarters that were bacteriologically negative. Back-transformed Lf concentrations (mg/l) are presented in parenthesis.

	Genotype			Diet			Genotype	P Diet	G*D
	NZHF	OSHF	sed	Pasture	TMR	sed			
<b>March</b>									
Log <sub>10</sub> Lf conc. (mg/l)	2.38 (239.9)	2.08 (119.4)	0.081	2.16 (145.6)	2.29 (196.3)	0.081	<0.001	0.118	0.525
Lf yield (g/AM/cow)	3.1	2.3	0.441	1.7	3.6	0.441	0.083	<0.001	0.723
Milk yield (litres/AM)	11.2	13.8	1.228	9.4	15.5	1.228	0.040	<0.001	0.088
Log <sub>10</sub> SCC (x 1000 cells/ml)	1.9	1.7	0.147	1.7	2.0	0.147	0.154	0.058	0.794
Proportion negative quarters	0.896	0.959	0.034	1.000	0.852	0.035	0.068	<0.001	0.992
<b>April</b>									
Log <sub>10</sub> Lf conc. (mg/l)	2.47 (292.8)	2.22 (165.5)	0.096	2.25 (175.9)	2.44 (275.5)		0.013	0.047	0.215
Lf yield (g/AM/cow)	3.1	2.5	0.434	1.9	3.8	0.434	0.199	<0.001	0.282
Milk yield (litres/AM)	9.1	11.4	0.969	8.5	11.9	0.969	0.023	0.001	0.419
Log <sub>10</sub> SCC (x 1000 cells/ml)	2.0	1.9	0.151	1.9	2.1	0.151	0.416	0.169	1.000
Proportion negative quarters	0.908	0.947	0.038	1.000	0.849	0.040	0.296	<0.001	0.100

**FIGURE 1:** Within-cow correlation between  $\log_{10}$  lactoferrin in milk (Lf; mg/l) and  $\log_{10}$  SCC (x 1000 cells/ml milk). A: March. B: April.



A positive within-cow correlation ( $P < 0.001$ ) was found between  $\log_{10}$  Lf and  $\log_{10}$  SCC in both March and April (Figure 1). The observed increase in Lf concentrations with increasing SCC varied between the two months.

## DISCUSSION

NZHF cows produced milk with higher Lf concentrations than OSHF. Cow breed effects on Lf concentration have been previously reported for HF versus Jersey cows by Farr *et al.* (2002). However, in contrast to the results of Farr *et al.* (2002) genotype did not significantly influence Lf yield in this study. Both genotype and diet affected milk yield, however, no genotype x diet interaction was apparent. This is in contrast to the results reported in the previous season for these animals (Kolver *et al.*, 2002).

Diet effects on Lf concentration were inconclusive. Diet did not affect milk Lf concentration in March, however, cows fed TMR had a higher concentration of Lf in the milk in April. Nutritional influences on various milk proteins have been previously reported (Gray & Mackenzie, 1987; Mackle *et al.*, 1999; Auld *et al.*, 2000) with effects particularly apparent on the blood-derived proteins, and mammary-synthesised whey proteins. Diet effects on Lf do not appear to have been reported previously in the literature.

Lacy-Hulbert *et al.* (2002) reported a higher incidence of mastitis, in particular coliform mastitis in TMR-fed animals. In studies in which mastitis is experimentally induced, Lf concentrations in the milk of infected quarters increases and peaks within several days. There is a wide variation between animals in the time to peak Lf concentration as well as the maximum concentration reached (Harmon *et al.*, 1976; Rainard *et al.*, 1982). Given the reported relationship between Lf and mastitis (Harmon *et al.*, 1976; Rainard *et al.*, 1982) and SCC in this study, the reported diet effects in April may also reflect differences in the bacteriological status of the animals and may not be solely due to nutrition. All infected quarters were from cows offered TMR. SCC did not appear to be influenced by genotype nor diet in this study. Previous examination of seasonal variation in SCC in these animals showed inconsistent treatment effects (Lacy-Hulbert *et al.*, 2002).

The relationship between Lf and SCC has been previously reported (Rainard *et al.*, 1982), supporting the findings of this study. The variation between March and April in the within-cow correlations is interesting. In studies in which mastitis is experimentally induced, SCC peaks within hours of infection, whereas Lf takes several days until peak concentrations are apparent (Harmon *et al.*, 1976; Rainard, 1983). In the current study, the developmental stage of infection for each (quarter) animal is unknown. This could impact on the Lf concentration and SCC of the milk at the time of measurement and, therefore, the relationship between the two. Due to the small number of infected quarters (27), the effects of individual pathogens were not investigated in further detail. Kawai *et al.* (1999) suggested that the effect of pathogens on Lf concentration was dependent on the type of pathogen present. This variable could further contribute to differences in the strength of the Lf/SCC relationship apparent in this current study.

This paper has provided the first information on the effect of diet on Lf concentration and yield in bovine milk and, although the results between the two sampling times were not consistent, provides evidence of further factors that may affect Lf concentrations in milk. The genotype effects on Lf concentration provide vital information for the dairy industry. As Lf is becoming a common addition to many health supplements and infant formulae due to its potential health benefits this research provides the first evidence that NZHF may be more suited to an industry in which Lf is extracted. The timeliness of this information is appropriate, given the increasing use of overseas genetics in the NZ dairy industry.

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