Relationships between major milk whey proteins in blood plasma and milk yield in dairy cattle

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ABSTRACT

The objective of this study was to investigate phenotypic variation in α-lactalbumin (α-lac) and ß-lactoglobulin (ß-lg) concentrations in the blood plasma of cows immediately before calving, and to evaluate their use as predictors of the subsequent lactation yields of milk or milk solids. Fifty-nine Jerseys (all ages) at Ruakura No. 2 and 148 Jerseys (all 2-year-olds) in a private Waikato herd were blood sampled in July immediately before calving. Data for plasma $\log_e$ α-lac and $\log_e$ ß-lg concentrations were adjusted for date of calving and (in the Ruakura herd) for stocking rate and age of cow, and regressions and correlations were estimated with subsequent lactation yields of milk, milkfat, protein and lactose (also adjusted for known fixed effects including lactation length). Correlations of adjusted $\log_e$ α-lac with adjusted yields in the Ruakura herd were 0.33 ($P<0.05$) for milk, 0.29 ($P<0.05$) for milkfat, 0.25 for protein and 0.32 ($P<0.05$) for lactose. The residual standard deviation for milkfat was reduced from 16.5 to 16.0 kg. Correlations were smaller and not significant in the private herd. After adjusting yields additionally in the Ruakura herd for live weight, correlations of adjusted $\log_e$ α-lac with adjusted yields ranged from 0.35 to 0.40 (mostly $P<0.01$), and residual standard deviations of yield were further reduced (e.g. from 15.3 to 14.3 kg for milkfat). Correlations between adjusted $\log_e$ ß-lg and adjusted yield were small and not significant in either herd. Adjusted $\log_e$ α-lac and adjusted $\log_e$ ß-lg had a correlation of 0.56 in herd 1 and 0.14 in herd 2. The use of $\log_e$ α-lac to predict adjusted yield in the subsequent lactation deserves further assessment.

Keywords: cattle; milksolids yield; α-lactalbumin; ß-lactoglobulin.

INTRODUCTION

α-Lactalbumin (α-lac) and ß-lactoglobulin (ß-lg) are the two major whey proteins in cow's milk. α-lac has important biochemical and physiological roles in milk production, whilst the function of ß-lg has not been clearly defined. Milk proteins leak from the udder into the blood plasma, so they may be measured in plasma just before calving.

Wisconsin workers have demonstrated that α-lac and ß-lg concentrations in the blood serum of heifers in late pregnancy were both correlated with milk and fat yields in the subsequent lactation (Mao *et al.*, 1991). This information was tested in Wisconsin as a possible screening method for lactation potential ("mammary status") in heifers before calving. We have followed up this work here by testing for the same phenotypic relationships under the quite different farming conditions in New Zealand.

A long-term aim of this type of study is to find an early predictor of milk yield for use in the young sire proving scheme. Obtaining preliminary sire proofs when daughters are 23 months of age could reduce the interval between progeny testing and use of a proven sire by one year. Thus, the age when young sires are proven and used again could potentially be reduced from 5.25 to 4.25 years. It could alternatively provide the opportunity to identify bull dams one year earlier, and reduce the generation interval by this pathway. The merit of using this indicator, probably with others in an index, would then depend on the heritability of the index and the genetic correlation between the index and milk solids yield.

MATERIALS AND METHODS

Herd Descriptions

Fifty-nine Jerseys (2 to 8 years of age) at Ruakura No. 2 Dairy (herd 1) and 148 Jerseys (2-year-olds only) in a private Waikato herd (herd 2) were blood sampled on July 10 and July 12 1991, respectively, immediately before the start of calving. Animals were the progeny of 14 and 9 sires respectively.

The start of calving in the two herds was July 6 at Ruakura and July 15 in herd 2, with calving spreads of 51 and 63 days, respectively (excluding cows induced to calve). Five Ruakura cows, whose records were retained in the data, calved on or before the blood sampling day, but inspection of the final regression analyses showed that including their data had little effect on the slope. For the α-lac and ß-lg analyses, data were excluded for cows which aborted, and in herd 2 results were also omitted for 7 other animals with <70 days in milk by the mid-lactation sampling in November.

At Ruakura, the herd was also involved in a stocking rate trial, and consisted of two subherds at high (4.5 cows/ha) and low (3.6 cows/ha) stocking rates.

Blood Sample Analyses

The blood samples, which had been collected from the tail of each animal into heparinised vacutainer tubes, were centrifuged and the plasma stored at -20°C, to await subsequent assay.

α-lac was analysed by radioimmunoassay using an antibody (kindly provided by Dr M.R. Grigor, University of

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Otago), raised in rabbits against human α-lac. Radiolabelled tracer was made as described by Salacinski *et al.* (1981), by iodinating affinity-purified bovine α-lac (Lindahl and Vogel, 1984) using Iodo-Gen™ (Pierce, P.O. Box 117, Rockford, Ill. 61105, U.S.A.). Samples were assayed in duplicate, and assay performance was evaluated using the procedure described by Chard (1987). Inter- and intra-assay coefficients of variation were both 10%.

ß-lg was analysed by competitive ELISA using triplicate analyses as described by Mao *et al.* (1991). The antibody, raised in rabbits, was obtained from Nordic Immunology (Tilburg, The Netherlands). Biotinylated tracer was made from bovine ß-lg obtained from the Sigma Chemical Co. (P.O. Box 14508, St Louis, Mo. 63178, U.S.A.) using ImmunoPure^{R} NHS-LC-Biotin (Pierce). Inter- and intra-assay coefficients of variation were 20 and 10% respectively.

Data Analyses

Data were analysed using the statistical package Genstat (5, Release 2.1). In view of the wide range of concentrations (e.g. about 130-fold, in herd 2) for both α-lac and ß-lg, the data were $\log_e$ transformed. For herd 1, fixed effects were fitted for stocking rate and age of cow (2, 3, 4, 5, >5 years), with a covariate for the lactation parameters (i.e. a date of calving adjustment for the blood proteins, and a lactation length adjustment for the yield data consisting of milk, milkfat, protein and lactose). After the above adjustments, a residual was calculated for each animal as the difference between the actual and predicted values, and regressions and correlations between residual blood data and residual yield data were computed. For analyses where $\log_e$ α-lac and $\log_e$ ß-lg were fitted together, prediction accuracy was assessed by comparing residual standard deviations. Although data from cows older than 2 years may not be relevant to using α-lac or ß-lg in a genetic study, data on older cows may be useful at a phenotypic level.

The effect of live weight before calving was found to approach significance for α-lac. However, this effect could have been used to predict milk yield in the absence of blood samples. Thus, we took the approach of additionally adjusting yield for live weight, calculating another set of residuals for each yield, and then re-estimating regressions and correlations of $\log_e$ α-lac with residual yield.

Similar procedures were applied to the data from herd 2, except that only one age group of cows was involved and there were no stocking rate differences. Liveweight data were available from half of the animals at the time of mating in the previous October.

RESULTS

Age of cow, stocking rate and calving date

Table 1 shows the effects of age of cow on $\log_e$ α-lac and $\log_e$ ß-lg in herd 1. Concentrations of both proteins in blood plasma were generally greater for 2- and 3-year-old calvers than for older cows. Two-year-old values in herd 1 were similar to the overall mean in herd 2, which consisted entirely of 2-year-olds. Stocking rate effects on $\log_e$ α-lac and $\log_e$ ß-lg in herd 1 were small and non-significant.

Blood concentrations of both whey proteins rose as calving date approached, and this was reflected in negative regressions of concentration on calving date or "days to calving" (given that blood sampling was on a fixed date). In other words, the regressions of concentration on "days from conception" were positive. For example, $\log_e$ α-lac increased by 43% in herd 1 if sampling was 30 days closer to calving. Covariates for date of calving were about 2 to 3 times as large in herd 1 as in herd 2.

Residual standard deviations

Residual standard deviations for $\log_e$ α-lac and $\log_e$ ß-lg were about twice as large in herd 1 as in herd 2. Coefficients of variation for $\log_e$ α-lac and $\log_e$ ß-lg were 23 and 18% in herd 1, and 9 and 7% in herd 2, respectively.

Regressions/correlations between blood parameters and yield

Table 2 shows the regressions of yield traits (adjusted for age of cow, stocking rate and lactation length) in herd 1

TABLE 1: Factors affecting variation in the logarithms of α-lactalbumin (α-lac) or ß-lactoglobulin (ß-lg) concentrations (ng/ml) in pre-calving samples of blood plasma

Item	Herd		$\log_e$ α-lac	$\log_e$ ß-lg
Number of records analysed	1		47	55
	2		100	100
Overall mean	1		4.22 (68)[1]	5.01 (150)
	2		4.56 (96)	6.02 (412)
Stocking rate effect	1	significance	n.s.	n.s.
Age of cow effect	1	significance	***	**
		2 years	4.77 (118)	5.60 (270)
		3 years	5.32 (204)	5.67 (290)
		4 years	3.93 (51)	4.54 (94)
		5 years	4.04 (57)	5.06 (158)
		>5 years	3.38 (29)	4.48 (88)
Date of calving covariate (d^{-1})	1		-0.060±0.012***	-0.031±0.010**
	2		-0.020±0.002***	-0.014±0.002***
Residual standard deviation	1		0.95	0.90
	2		0.42	0.40

[1] Antilog value in brackets

TABLE 2: Regression parameters for predicting adjusted lactation yield from $\log_e$ α-lac or $\log_e$ ß-lg in herd 1

Item	d.f.	Milk, l	Milkfat, kg	Protein, kg	Lactose, kg
Overall mean		3060	189	127	151
Regression of yield 1[1]					
on $\log_e$ α-lac		114* (0.33)[2]	5.4* (0.29)	3.3 (0.25)	6.0* (0.32)
on $\log_e$ ß-lg		85 (0.23)	0.9 (0.05)	1.9 (0.13)	5.2 (0.26)
residual SD:					
initial	46	316	16.5	12.1	17.0
with $\log_e$ α-lac	45	302	16.0	11.9	16.4
with $\log_e$ ß-lg	45	311	16.7	12.1	16.6
with both	44	305	16.0	12.0	16.5
with wt	45	310	16.3	11.7	16.7
wt + $\log_e$ α-lac	44	291	15.5	11.3	15.7
Regression of yield 2[1]					
on $\log_e$ α-lac		132** (0.40)	6.5** (0.38)	4.2* (0.35)	7.0** (0.40)
residual SD:					
initial	46	297	15.3	10.9	15.9
with $\log_e$ α-lac	45	275	14.3	10.3	14.8

[1] Yield 1 adjusted for lactation length, stocking rate and age; yield 2 also adjusted for live weight; blood sample data ($\log_e$ ng/ml) adjusted for age of cow and date of calving.

[2] Correlation in brackets

on blood data adjusted for age of cow and date of calving. Three of the four regressions for adjusted $\log_e$ α-lac were significant, whereas adjusted $\log_e$ ß-lg showed no effect. The correlation between adjusted $\log_e$ α-lac and $\log_e$ ß-lg was 0.56, but the residual standard deviations showed that fitting values for both blood proteins together was no better than fitting $\log_e$ α-lac alone.

Live weight approached significance as a covariate for $\log_e$ α-lac (-0.0071±0.0048 ng/ml per kg), but not for $\log_e$ ß-lg. The size of the udder is likely to determine in part the quantity of α-lac secreted into the circulation, and live weight is related to the size of the plasma pool. Using live weight and $\log_e$ α-lac to predict adjusted yields, residual standard deviations showed that the two together were more accurate than either alone. However, live weight was a significant covariate for milk yield ($P<0.10$) and for yields of fat, protein and lactose (all $P<0.05$), after making allowance for age of cow and stocking rate. In a second set of analyses (yield 2 in Table 2) we adjusted all yields for liveweight and for the other fixed effects above. The correlations of adjusted $\log_e$ α-lac with adjusted yields in this second set were significant at $P<0.01$, except for protein yield ($P<0.05$). The best prediction equations used $\log_e$ α-lac and weight-adjusted yield.

Table 3 shows the corresponding analyses in herd 2. Log_e α-lac or $\log_e$ ß-lg were not significant in predicting adjusted yield in this herd, but the sign was generally as for herd 1 and the sizes of regression covariates were the same order of magnitude as those for herd 1 in Table 2. The correlation between adjusted $\log_e$ α-lac and $\log_e$ ß-lg was 0.14. The covariate for liveweight in herd 2 was not significant for any of the three yield traits. This may have been the result of only having a weight record nine months earlier, at conception, and the fact that some weights were not taken.

TABLE 3 Regression parameters for predicting adjusted lactation yield from $\log_e$ α-lac or $\log_e$ ß-lg in herd 2.

Item	d.f.	Milk, l	Milkfat, kg	Protein, kg
Overall mean		2290	149	96
Regression of yield[1]				
on $\log_e$ α-lac		41 (0.07)[2]	2.5 (0.07)	0.15 (0.01)
on $\log_e$ ß-lg		104 (0.17)	3.3 (0.09)	2.9 (0.13)
residual SD				
initial	98	252	14.3	9.2
with $\log_e$ α-lac	97	253	14.4	9.2
with $\log_e$ ß-lg	97	250	14.3	9.2
with both	96	251	14.4	9.2

[1] Yield adjusted for lactation length; blood sample data ($\log_e$ ng/ml) adjusted for date of calving.

[2] Correlation in brackets.

DISCUSSION

There was considerable variation in concentrations of the two blood proteins as shown by the residual SDs relative to their means (Table 1), particularly for α-lac. If either protein was to be used in predictions of yield, then adjustments would be required for the significant fixed effects and covariates. In herd 1, where animals of various ages were sampled, concentrations of α-lac and ß-lg were generally higher in 2- and 3-year-olds than in older cows. The large rise in blood protein concentration with approaching calving date was similar to that reported from Wisconsin by Mao *et al.* (1991).

Log_e α-lac was significantly correlated with adjusted yield, and this led to reduced residual SDs for yield, relative to the initial values for residual SDs (Table 2). In the case where liveweight was also included, the correlations ranged from 0.35 to 0.40, and residual SDs were reduced even further, e.g. from an original 16.5 kg to 14.3 kg for lactation

fat yield. From the regression coefficient, animals with a 2 SD difference in $\log_e$ α-lac have a difference of 12.4 kg (or 7%) in fat yield, compared with a 2 SD difference in live weight being associated with a difference of 7.6 kg (or 4%) in fat yield. Mao *et al.* (1991) found a correlation of 0.43 between mature-equivalent fat yield and $\log_{10}$ α-lac ($P<0.05$) measured four weeks before first calving, but the value was not significant if the sample was taken before the second calving. ß-lg was not significantly correlated with yield in our data. Significant correlations of similar size to those with $\log_{10}$ α-lac were found for yield with $\log_{10}$ ß-lg in the Wisconsin data.

Our values were not significant in herd 2. It should be noted that these animals were light (190 kg) as yearlings in October and in low body condition, which was later reflected in low 2-year-old lactation yield data (Table 3). It is not known whether this could explain our findings in this herd.

Overall, we conclude that α-lac deserves further investigation as a possible predictor of yield, as it can be measured in blood plasma before calving. Used alone, or preferably with live weight, it warrants investigation for use in identifying dams for contract mating one year earlier than normal. Estimates of genetic regression of yield on $\log_e$ α-lac would be needed first from a sample of herds with cows of known sire pedigree, e.g. as in the young sire proving scheme. In view of the Wisconsin results, ß-lg should perhaps also be tested further.

ACKNOWLEDGEMENTS

To the staff at Ruakura No. 2 Dairy and to the owner of the private herd who kindly agreed to collaborate in July 1991, to the Livestock Improvement Corporation Ltd for access to milk yield data, to Dr K.L. Macmillan for access to the liveweight data in the private herd, to Miss J.M. Brown for assistance with early analyses, and to Dr H.V. Henderson for advice and discussion during the analyses of these data.

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