Hot Topic: An Association Between a Leptin Single Nucleotide Polymorphism and Milk and Protein Yield

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ABSTRACT

Allelic variation (C to T transition that results in an Arg25Cys) in the leptin gene has been associated with increased fat deposition in beef cattle. We report that this same genetic variant is also present in dairy breeds. Body fat reserves play an important role in sustaining high milk production in early lactation, when energy intake is limited. To test for an association between the leptin single nucleotide polymorphism and milk productivity, we genotyped 416 Holstein cows and compared lactation performance data using a mixed model. Animals homozygous for the T allele produced more milk (1.5 kg/d vs. CC animals) and had higher somatic cell count linear scores, without significantly affecting milk fat or protein percent over the entire lactation. The increase in milk yield is most prominent in the first 100 d of lactation (2.44 kg/d), declining to 1.74 kg/d between 101 and 200 d in lactation. The milk yield advantage, observed in cows homozygous for the T allele, could represent a major economic advantage to dairy producers.

(Key words: leptin gene, allelic variation, milk productivity, immune response)

Abbreviation key: SNP = single nucleotide polymorphism.

Leptin is a hormone secreted predominantly from white adipose tissue and performs important roles in the control of BW, feed intake, immune function, and reproduction (Santos-Alvarez et al., 1999; Kadokawa et al., 2000; Block et al., 2001). The primary factors affecting plasma leptin levels include body fat mass and energy balance (Block et al., 2001). Before calving,

increased body condition provides energy stores to support milk production. Nevertheless, during early lactation dairy cattle are in a negative energy balance. Leptin has also been shown to regulate the immune response (Santos-Alvarez et al., 1999), and a delay in the recovery of leptin secretion postpartum increases the delay to first ovulation in Holstein dairy cows (Kadokawa et al., 2000). We have previously shown that a C to T transition in exon 2 of leptin that encodes an Arg25Cys substitution (position four of the secreted peptide) is associated with body fat deposition in beef cattle (Buchanan et al., 2002). The T allele is associated with increased fat deposition and higher leptin mRNA levels in adipose tissue.

Using PCR-RFLP to distinguish the alleles (Buchanan et al., 2002), we genotyped individuals from six dairy breeds. The single nucleotide polymorphism (SNP) was present in all breeds examined (Table 1). Using DHI records for 416 Holstein cows and a total of 9584 observations (from 11 Saskatchewan herds; Table 2) associations between milk production, milk fat percentage, milk protein percentage, SCC linear score, and leptin genotype were analyzed. Many of the cows were not registered, and, therefore, sire-group information was not available. Data were analyzed using a mixed model (SAS v. 8.0 for Windows (PROC MIXED); SAS Institute, Cary, NC). The model included a random effect for cow nested within herd to account for the repeated test dates and lactations within cow and the clustering of observations at the herd level. A compound symmetry covariance structure was specified in which all observations within cows within each herd were assumed to be equally correlated. This choice of random effect and covariance structure produced the model with the best R^2 . Initial analyses were examined by looking at the association between each individual production outcome (milk production, fat percent, protein percent, and linear score) and genotype (TT, TC, and CC). Genotype was analyzed as a fixed effect, with CC as the referent category. Potentially important covariates were then introduced for each outcome using a manual step-wise process to produce the final models

Received March 25, 2003.

Accepted July 23, 2003.

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Table 1. Frequency of the C and T alleles at the leptin single nucleotide polymorphism in dairy breeds.

Breed	Number of animals	T allele	C allele
Holstein	416	0.46	0.54
Ayrshire	17	0.62	0.38
Brown Swiss	21	0.45	0.55
Canadienne	9	0.11	0.89
Guernsey	16	0.06	0.94
Jersey	20	0.53	0.47

for milk production, fat percentage, protein percentage, and, finally, SCC linear score. Additional covariates introduced into the model for milk production as fixed effects included milk fat percent, milk protein percent, DIM, lactation number, month the lactation started (for potential seasonal effects), and SSC linear score. Models for fat percent, protein percent, and SSC linear score included fixed effects for milk production, lactation number, month at the start of lactation and protein, fat, or SSC linear score as appropriate. The main-effects model in each case was assessed for first-order interactions where genotype and one or more covariates remained in the model with $P < 0.05$. Model diagnostics included visual examination of the raw and standardized residuals (SAS, 2000). The residuals were plotted against predicted values of each observation. Rankit plots and Wilk-Shapiro tests were used to assess the normality of the residuals (SAS, 2000).

Our analysis demonstrated a striking impact of leptin genotype on milk yield, particularly in early lactation. Table 3 provides an estimate of the increase in milk yield of TT and TC genotypes relative to the CC genotype. Over the entire lactation, the TT genotype was associated with an increase in test-day milk yield of 1.5 kg/d vs. the CC genotype. This effect was most prominent in early lactation (2.44 kg/d), declining to 1.74 kg/d between 101 and 200 DIM.

We observed a decrease in fat percent associated with the T allele that was not significant over the entire lactation (TT = -0.1% and TC = -0.07%; $P = 0.19$) but approached significance between 101 to 200 DIM (TT = -0.15% and TC = -0.12%; $P = 0.06$). As a result, yield of 3.5% FCM was not significantly affected by genotype

(data not shown). An increase of test-day milk protein yield was observed between 1 to 100 DIM (TT = 72 and TC = 50 g/d; $P < 0.02$) and approached significance between 101 to 200 DIM ($P < 0.08$) in TT (47 g/d) and TC (37 g/d) cows vs. CC cows. Over the entire lactation, milk protein yield approached significance (TT = 43 g/d, $P = 0.06$) for TT vs. CC cows. Our analysis also showed a significant impact of genotype at the leptin SNP on milk SSC linear score. Cows homozygous for the T allele demonstrated a significant increase in SSC linear score over the entire lactation ($P < 0.01$) and within each of the early- ($P = 0.02$), mid- ($P = 0.04$), and late- ($P = 0.03$) lactation periods. Daughter groups may exist in this dataset that share alleles at other genes for higher milk production coincidentally with the T allele at leptin. However, because this was a random sampling of Saskatchewan herds this possibility is unlikely.

An unpaired cysteine exists in chicken leptin, three amino acids from the N-terminus of the secreted protein. Dridi et al. (2000) suggest that after substituting this unpaired cysteine with serine that it does not alter bioactivity. However, a heterologous bioassay system was used to compare avian leptins (wild type and mutated) against mammalian receptors. In the in vivo assay reported, the serine form of leptin suppressed accumulated food intake in chickens more than the wild type but not significantly so. It is possible that, similar to our previous observation in beef cattle in which the T allele was associated with increased gene expression and fat deposition (Buchanan et al., 2002), animals homozygous for the T allele may have had increased body fat reserves. Further we speculate that TT animals have increased feed intake, but the effect of genotype on appetite in early lactation would be limited as cows are in a negative energy balance and leptin levels would be low (Kadokawa et al., 2000; Block et al., 2001). The mechanism of action may also be independent of changes in adipose tissue reserves. For example, Silva et al. (2002) demonstrated a direct inhibitory effect of leptin on proliferation of a mammary epithelial cell line (Mac T cells) in vitro. Liefers et al. (2002) using a different, intronic, SNP in the leptin gene also found an increase in milk and protein yield in Holsteins. However, in their population the favorable allele was very

Table 2. Description of 11 Saskatchewan dairy herds used in study.

Item	Mean	Minimum	Maximum
Number of milking cows	71	36	129
Herd average milk yield (kg/d)	30.5	19.0	36.8
Herd average fat percent (%)	3.68	2.94	4.51
Herd average protein percent (%)	3.22	3.01	3.44
Herd average SCC linear score	3	2	4
Herd average SCC (cells/ml)	300,000	81,000	518,000

Table 3. Effect of a T allele at the leptin single nucleotide polymorphism on test-day milk yield (kg/d).

Genotype ¹	Estimate (kg/d)	df	Probability <i>P</i>	Lower confidence limit (kg/d)	Upper confidence limit (kg/d)
Entire lactation					
TT	1.50	9149	0.04	0.05	2.95
TC	0.91	9149	0.12	-0.24	2.07
CC	...	...	...	...	...
0–100 DIM					
TT	2.44	2499	<0.01	0.78	4.11
TC	1.74	2499	0.01	0.41	3.07
CC	...	...	...	...	...
101–200 DIM					
TT	1.74	2507	0.04	0.11	3.37
TC	1.38	2507	0.04	0.08	2.68
CC	...	...	...	...	...
>200 DIM					
TT	0.24	3299	0.73	-1.14	1.63
TC	0.22	3299	0.69	-0.88	1.32
CC	...	...	...	...	...

¹Covariates included milk fat percent, milk protein percent, DIM, lactation number, month that the lactation started (for potential seasonal effects), and somatic cell linear score.

rare (only 1 of 613 homozygous) compared with the high frequency of the SNP reported in this study.

Higher SCC can be symptomatic of mastitis infection. However, we show an increase in milk yield and SSC linear score associated with the T allele. This may reflect a possible increase in mastitis incidence associated with higher milk yield or the role of leptin in modulating an immune response. Leptin has been shown to act as a proinflammatory cytokine, stimulating the proliferation and activation of peripheral blood leukocytes (Santos-Alvarez et al., 1999). Further investigation will be necessary to resolve the relationship between leptin genotype and SCC.

These results indicate that the leptin TT genotype is associated with increased milk and protein yield, without changing yield of milk fat. These observations are of economic interest. A more extensive study is under way in which issues of sire information and immune function are to be addressed. Although in this study we measured the effect of leptin genotype on production in Holsteins only, we have noted that all dairy breeds tested showed the presence of the favorable T allele. This preliminary study suggests that the leptin genotype may play an important role in regulating milk and protein yield.

ACKNOWLEDGMENTS

This project received financial support from Dairy Farmers of Canada and the Natural Sciences and Engineering Research Council-Collaborative Research De-

velopment. Access to the 416 Holstein blood samples was provided through the Western Canadian Dairy Herd Improvement Services with permission of the 11 producers. Data records on these animals were provided with permission from Holstein Canada from the Canadian Dairy Network. Thanks to Max Rothschild for comments on the manuscript.

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