Effect of Season, Genetic Line, and Sire on Growth Concentrations of Somatotropin in Serum of Holstein Cows in Early Lactation

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ABSTRACT
To determine the effect of selection for milk yield on somatotropin concentrations, blood samples were collected from 128 Holstein cows whose sires had either high or average predicted differences for milk. The phenotypic difference in milk yield between the high and average yielding groups was 1726 kg on a 305-d mature equivalent basis. At 37 ± 7 d postpartum, four blood samples were taken from each cow at 1-h intervals beginning at 0800 h. The statistical model contained genetic line, sires within line, and season as whole-plot effects and time of sampling as a subplot effect. The concentration of growth hormone was significantly higher in the high yielding group (1.89) than in the average yielding group (1.49). Cows sampled in summer had the highest concentrations of growth hormone, whereas cows sampled in spring had the lowest concentrations. Sires did not significantly influence the somatotropin concentration of their progeny.

INTRODUCTION
Daughters of sires selected for milk yield exhibit higher concentrations of endogenous somatotropin (ST) than do randomly bred cows (1). In several other studies, higher ST concentrations were likewise reported for lactating cows of superior genetic merit (3, 9, 14, 16). Somatotropin was elevated in dairy cattle compared with beef cattle (8). The variation in ST suggests the possibility of using this physiological trait as a means to intensify selection for milk yield. Perhaps variations in the ST concentrations of high and average yielding cows could be explained by differences between sires selected for high or average transmitting abilities for milk, fat, or protein. The number of animals in previous studies was too small, however, to enable the investigators to consider sire effects. Klindt (15) compared ST concentrations in sires with milk yield of their progeny. He found that sires' ST concentrations could be a predictor for yield differences in their progeny. The purposes of this study were to investigate cows of two distinct genetic lines for differences in circulating concentrations of ST and to examine the influence of sires on the concentration of ST in their progeny. In addition, season and parity effects were considered.

MATERIALS AND METHODS
Animals
Data were collected at the Iowa State University dairy breeding research farm located at Ankerta, IA, a facility that has been described in detail (21). Briefly, two genetic lines were
established in 1968 to start measuring accumulated responses to selection for milk yield. Pairs of foundation heifers were purchased based on high and low pedigree estimates of transmitting ability for milk. These heifers were randomly bred to sires with high or average predicted difference for milk (PDM), forming a 2 by 2 factorial design. For mating, either sires with the highest PDM available or breed-average sires from commercial AI were selected. In subsequent years, the progeny of high PDM sires were bred to high PDM sires and the progeny of average PDM sires to average PDM sires, thereby accumulating differences in milk yield. Three new sires were introduced every year in each selection line and subsequently used for 2 yr, resulting in a total of 12 sires being used at once. As a result of ongoing selection, the high yielding group outproduced their breed-average group herd mates by 1726 kg. Standardized twice daily milking, 305-d mature equivalent lactation records averaged 10,219 kg for the high yielding and 8493 kg for the average yielding group.

Cattle were housed in free-stall barns with access to paved lots and milked twice daily in a parlor. All animals were fed the same mixed ration for ad libitum intake, plus approximately 3.6 kg of alfalfa hay each day. The ration consisted of corn silage, a liquid protein supplement, and a purchased grain concentrate. Analyses of samples of the alfalfa hay and corn silage plus an analysis of the liquid supplement were used to determine the dietary make-up of the grain concentrate. The mixed ration was fed twice daily at a rate supporting maintenance, growth, and production of 36.5 kg/d of milk. The feed remained the same over the entire year. Management was as similar as possible for the high and average yielding groups. All health information was routinely recorded for all cows. Milk weight on the preceding milking and that on the morning blood samples were collected, and total milking time was recorded. Parity ranged from 1 to 6.

Blood samples (6 ml) were taken from the tail of each cow between 30 and 44 d postpartum. This period was selected because ST is relatively constant during this stage of lactation and then begins to decline (14, 20). No differences in ST concentrations were found between high and low yielding cows at the end of lactation (3, 9, 14). Four serial blood samples per cow were collected at hourly intervals beginning at 0800 h, after milking. During the sampling, the cows were in stanchions and had free access to hay. The blood was allowed to clot at room temperature for at least 2 h. After centrifugation, the serum was harvested and stored at −20°C until radioimmunoassay at a later date at the laboratories of the Upjohn Company. Circulating concentrations of ST were measured without administration of exogenous ST.

Procedures

Serum ST was quantified by a double antibody radioimmunoassay, using the procedures described by Mosely et al. (17). Amounts of ST ranged from .046 to 12.52 ng/ml. Antibody against purified ST was prepared in guinea pigs, and the antiserum against guinea pig gamma globulin was prepared in sheep. The specific antiserum bound 36% of radiolabeled ST. Interassay and intrassay coefficients of variation for a sample with mean 3.4 ng/ml and a standard error of .25 ng/ml were 7.3 and 6.0%, respectively. The crossreactivity of the ST antiserum with bovine prolactin, bovine insulin, bovine thyroid-stimulating hormone, and bovine luteinizing hormone was less than 1%. The reference standard used was AFP8500 (A.F. Parlow, Harbor-UCLA Medical Center, Torrance, CA). The lower limit of sensitivity was .03 ng/ml ST.

Statistical Analysis

Samples taken from November 16, 1988 through January 3, 1990 were used. One record per cow was used. Cows for which no mature equivalent lactation record was available, because the lactation was terminated early or was too short to extend, and cows with abnormal records (illness) were deleted. All sires had at least three daughters. The data consisted of 60 high yielding cows by 11 sires and 68 average yielding cows by 12 sires. Four ST samples per cow were taken, but two samples were lost for two cows; therefore, the total number of observations was 508. The data were analyzed by the general linear models procedures of SAS (19) using a split-plot design with re-
peated measurements (6). The model was

\[
Y_{ijklm} = \mu + s_i + l_j + s_{jk} + c_{ijkl} + t_m + e_{ijklm}
\]

where

\[Y_{ijklm}\] is the vector of ST observations;
\[\mu\] is the overall mean;
\[s_i\] is the fixed effect of season \(i\) (1 = November to February; 2 = March to June; 3 = July to October);
\[l_j\] is the fixed effect of line \(j\) (1 = high yielding cows; 2 = average yielding cows);
\[s_{jk}\] is the random effect of sire \(k\) (\(k = 1, \ldots, 12\)) within line \(j\);
\[c_{ijkl}\] is the random effect of cow \(l\) (\(l = 1, \ldots, 6\)) within season \(\times\) sire \(\times\) line interaction \(ijkl\);
\[t_m\] is the fixed effect of the time of sampling \(m\) (\(m = 1, 2, 3, 4\));
and
\[e_{ijklm}\] is the random residual.

No consistent correlation between means and standard error could be detected; therefore, the natural log transformation of the hormone data, usually performed to eliminate this correlation, was not used.

Sires, cows, and error were assumed to be independently distributed random variables with variances \(\sigma_s^2\), \(\sigma_c^2\), and \(\sigma_e^2\), respectively.

Preliminary analysis indicated that the season \(\times\) line interaction and all interactions with time of sampling were small and not significantly different from zero, and, therefore, these terms were omitted from the model. Overall effects were tested using \(F\) tests from the analysis of variance. Season, line, and sires were whole-plot effects, whereas time of sampling was a subplot effect. Therefore, the appropriate error term to test for season and sire differences was the mean square for cows. The error term to test line effects was the mean square for sires. Because a characteristic of repeated measurements in time is that the errors on the same experimental unit are correlated, to test time effects, conservative degrees of freedom were used to calculate probability levels (6). The experiment was designed so that a cow was measured in only one season; therefore, the cow effect was nested within the season effect. Season was defined after the means of monthly ST measurements were graphed (Figure 1). Three seasons were used: winter (November to February), spring (March to June), and summer (July to October).

In addition to the full data set (data set 1), a modified data set (data set 2) was created. In data set 2, peak concentrations of ST were deleted when they were larger than \(\mu + 3\sigma\); the mean was 1.72 ng/ml and the standard deviation 1.20 ng/ml. The total number of observations in this data set was thus decreased by 6 to 502.

Estimates of repeatability between measurements on the same animal were calculated as an intraclass correlation

\[
r = \frac{\hat{\sigma}_c^2 + \hat{\sigma}_s^2}{\hat{\sigma}_c^2 + \hat{\sigma}_s^2 + \hat{\sigma}_e^2}
\]

where \(\hat{\sigma}_c^2\) and \(\hat{\sigma}_s^2\) are estimates of cow and error variance components, respectively, and \(\hat{\sigma}_s^2\) is the estimate of the sire (within line) variance. Heritability was estimated by

\[
h^2 = \frac{4\hat{\sigma}_c^2}{\hat{\sigma}_c^2 + \hat{\sigma}_s^2 + \hat{\sigma}_e^2}.
\]

Estimates of variance components were determined by the Henderson method 3 (10). With so little data, the estimates of heritability can
**RESULTS AND DISCUSSION**

Means and standard errors of the means for both individual ST measures and daily milk yield are summarized by genetic line and season in Table 1. The concentrations of ST were lower than those found in previous investigations of lactating cattle of similar age and in similar stages of lactation (1, 9, 14). Enright et al. (5) found values similar to those in our study. The values obtained in a particular radioimmunoassay depend mainly on the antibody and reference standards used. The reference standard and antibody used by Enright et al. (5) and in our study differed from those used in other studies (1, 7), which may explain the observed differences in ST concentration.

High yielding cows had significantly higher ST concentrations than average yielding cows (Tables 1, 2, 3). Season influenced ST concentrations markedly, with the highest concentration of ST occurring during summer. As expected, daily milk yield tended to be lower in summer because of increased temperature (23). Means and standard errors for ST by genetic line and time of sampling are in Table 2. Concentrations of ST differed slightly with time of sampling.

### TABLE 1. Means and standard errors of somatotropin (ST) concentrations and actual daily milk yield by season for high and average yielding cows.

<table>
<thead>
<tr>
<th>Genetic line</th>
<th>Season¹</th>
<th>n</th>
<th>Mean ST</th>
<th>Actual daily milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(ng/ml)</td>
<td>(kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\bar{x}$</td>
<td>SE</td>
</tr>
<tr>
<td>High</td>
<td>1</td>
<td>27</td>
<td>1.75 .10</td>
<td>33.63 .71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13</td>
<td>1.28 .09</td>
<td>37.72 1.11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>20</td>
<td>2.49 .19</td>
<td>30.75  .59</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>60</td>
<td>1.89 .08</td>
<td>33.56 .47</td>
</tr>
<tr>
<td>Average</td>
<td>1</td>
<td>27</td>
<td>1.47 .12</td>
<td>32.61 .59</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17</td>
<td>.87 .08</td>
<td>29.88 1.15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24</td>
<td>1.93 .09</td>
<td>29.22  .69</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>68</td>
<td>1.49 .07</td>
<td>30.73  .55</td>
</tr>
</tbody>
</table>

¹Season 1 = winter; season 2 = spring; season 3 = summer.

be expected to have large sampling errors, but because so little is known about genetic differences in ST, the heritability estimate is given. Approximate standard errors were from Swiger et al. (22).

### Analysis of All Data

Season was a large and very significant source of variation in ST (Table 3). Genetic line was also a significant source of variation in ST. Sires, however, were not. Cows contributed significantly to the variability in ST concentration. Differences between times when samples were taken were not significant.

Our finding that ST differed with genetic line confirmed previous work, although the differences found were not as extreme as those in smaller samples (1, 3, 9, 16). Differences between sires were not significant, when all data were analyzed, and the estimated heritability from the sire component was rather low (12) (Table 4).

Concentrations of ST in the present study were highest in the summer (Figure 1), which

### TABLE 2. Means and standard errors of serum somatotropin concentration by time of the day sampled for high and average yielding cows.

<table>
<thead>
<tr>
<th>Time of sampling¹</th>
<th>High yielding</th>
<th>Average yielding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>SE</td>
</tr>
<tr>
<td>1</td>
<td>1.80 .13</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.11 .23</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.78 .17</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.88 .12</td>
<td></td>
</tr>
</tbody>
</table>

¹Approximate time: 1 = 0800 h; 2 = 0900 h; 3 = 1000 h; 4 = 1100 h.
TABLE 3. Analysis of variance of somatotropin (all data). 1

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season (S)</td>
<td>2</td>
<td>34.81</td>
<td>12.28***</td>
</tr>
<tr>
<td>Line (L)</td>
<td>1</td>
<td>16.15</td>
<td>4.32†</td>
</tr>
<tr>
<td>Sire (within line)</td>
<td>21</td>
<td>3.74</td>
<td>1.32</td>
</tr>
<tr>
<td>Cow (S x Sire x L)2</td>
<td>103</td>
<td>2.83</td>
<td>3.98***</td>
</tr>
<tr>
<td>Time</td>
<td>3</td>
<td>1.96</td>
<td>2.75</td>
</tr>
<tr>
<td>Error</td>
<td>377</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

1Data set 1.
2\(\sigma_{S}^2 = .04; \sigma_{L}^2 = .54; \sigma_{S}^2 = .71.\)
3\(P < .10.\)
4\(* * * P < .001.\)

...was in contrast with the low values reported by others (11, 12) in summer. Short-term exposure to heat results in an increase in ST concentrations and long-term exposure a decrease (13). In contrast, Tucker and Wetteman (25) found no effect of ambient temperature on ST although there was a tendency for ST concentration to increase with increasing temperature. In several other studies, no relation could be detected between temperature and ST concentration (7, 18). The cause of an elevated ST concentration in summer, as reported in our study, is not known.

There was an indication that time of sampling affected ST concentration, which was in agreement with a report by Bonczek et al. (3). Diurnal variation in ST concentration among cows was observed, but the pattern was inconsistent and primarily a characteristic of individual animals (26). In the present study, the variation between times of sampling was small (Table 2) and not significant (Table 3).

In the present study, in which data were collected for four consecutive hourly samples, the repeatability estimate was .45 (Table 4). In another experiment, day to day repeatability was low (.21), probably as a result of secretory variation (11). Vasilatos and Wungsness (26) found a high day to day repeatability of .92 based on hourly samples collected for 48 h, but Davis et al. (4), who measured blood samples at 15-min intervals, found a repeatability of .79 for lambs.

When parity was added to model [1], no significant differences in ST concentration could be detected between parities, and therefore the term was omitted from the final model. The lack of significant effect of parity agrees with the findings of previous reports (3, 11).

Analysis of Data Without Peak Values

In Table 5, probabilities are shown from the analysis of data without the peak values (data set 2). Seasonal differences were still highly significant. Genetic line and sire effects accounted for a significant amount of variation in ST. No differences between times of sampling were observed (\(P > .10.\) The mean squares associated with cow effects decreased considerably after peak values were deleted (2.83 to 1.57), thus indicating that the peak values were out of range from the base values, and, because the sire mean square was tested with the cow mean square, sire effects were significant (Table 5). Several of the cows having these peak values...
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TABLE 5. Probabilities from F tests for effects of season, line, and sire within line on somatotropin concentrations for different data sets.

<table>
<thead>
<tr>
<th>Source</th>
<th>All data</th>
<th>Peak values deleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season (S)</td>
<td>.0001***</td>
<td>.0001***</td>
</tr>
<tr>
<td>Line (L)</td>
<td>.05†</td>
<td>.06†</td>
</tr>
<tr>
<td>Sire (within line)</td>
<td>.18</td>
<td>.02*</td>
</tr>
<tr>
<td>Cow (S x Sire x L)</td>
<td>.0001***</td>
<td>.0001***</td>
</tr>
<tr>
<td>Time</td>
<td>.10</td>
<td>.11</td>
</tr>
</tbody>
</table>

†p < .10.
* p < .05.
*** p < .001.

values were extremely high yielding; thus, the high yielding cows may generally have had the highest spikes. A cause of increased milk yield could be either peak values or higher baseline concentrations of ST, but too few observations per cow were available to calculate frequency, amplitude, and baseline concentrations of ST from these data. In data set 1, a small number of peak values considerably increased the variation in ST. Therefore, deleting the peak values, which represented only 1.1% of the observations, reduced the large contributions of the cows with these high peaks. This deletion increased the R² from 65 to 69% (Table 4). The peak values were not associated with specific sires. If that were the case, the mean squares between sires would also have been reduced.

If it can be shown that peak values are associated with increased milk yield, they should be kept in the analysis of data. Because of higher spikes, more elevated plasma concentrations were found at 30 d postpartum than at 90 d postpartum, whereas baseline concentrations of ST and frequency were similar (26). Bines et al. (2) observed very high, brief amplitudes at irregular intervals in high yielding cows at peak lactation. Schams et al. (20), however, found that at peak lactation there was an increase in baseline concentration and amplitude and that frequency did not change with later stages of lactation. Infusion of ST-releasing factor increased pulse frequency, baseline, duration of ST, and milk yield (5). Klindt (15) found a negative correlation between the frequency of ST peaks in sires and milk production of their daughters. High yield was associated with higher baseline concentrations rather than with peak frequency and peak amplitude. The relation between peak values and milk yield is not clear. Therefore, analyses both with and without peak values are presented (Table 5).

Compared with data set 1, within-animal repeatability was increased from .45 to .49 after the peak values were deleted (Table 4). Heritability increased to .37, primarily because of a decreased error variance. Few heritability estimates are given in the literature. Tilakaratne et al. (24) found that the genetic merit of the sire did not affect the concentration of any of the metabolites studied in calves. In contrast, in the present study, sires accounted for a significant part of the variation in ST when the peak values were deleted.

CONCLUSIONS

Concentrations of ST were higher (P < .10) for the high yielding group. After deleting the peak values, the estimated heritability increased from .12 to .37. The significant contribution of the sire component suggests the possibility of using ST concentrations as an aid to sire selection for yield traits. Both ST characteristics of sires of cows differing in genetic merit and the correlation between ST concentration of sires and their progeny need further investigation to determine whether measures of hormones can be used as a basis for selection of yield traits.

ACKNOWLEDGMENTS

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REFERENCES


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