GENETIC VARIATION IN THE CONCENTRATION OF HORMONES IN THE BLOOD OF HOLSTEIN CATTLE

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SUMMARY

Samples of blood from ten pairs of split embryo, monozygous twin Holstein heifers were collected twice a week from January 1987 through December of 1989. The members of a given pair were from different surrogate mothers. Half of each sample was stored at -70°C and the other half was stored but subsequently and periodically assayed for the concentration (Ng/ml) of growth hormone, insulin, T-3, T-4, cortisol, progesterone, LH and FSH; this paper is concerned with the first four hormones listed. The differences among pairs were significant at the .0001 level of probability for growth hormone, insulin, T-3 and T-4 respectively. The components of variance associated with differences among pairs were 0.63, 1.59, 173 and 0.29 for growth hormone, insulin, T-3 and T-4 respectively. The corresponding components of variance for differences between animals of the same pair were 0.06, 0.15, 134 and 0.11 respectively. The magnitude of the component of variance among pairs is from 1.2 to 10.5 times that for variation between members of the same pair. The concentration (Ng/ml) of growth hormone decreased essentially linearly from 6.0 ± 0.25 at 8 months of age to 2.0 ± 0.25 at 24 months and subsequent ages. The mean for the 2003 samples assayed was 3.11 ± 0.25. Insulin increased essentially linearly with age from 12.1 to 18.5 (Ng/ml) with a mean of 15.11 ± 0.11 for the 1170 samples. T-3 decreased at a decreasing rate from 210 (Ng/ml) at eight months to 170 (Ng/ml) at 24 months; the mean of the 1170 samples assayed was 181 ± 0.92. T-4 decreased essentially linearly with age from a mean of 6.7 (Ng/ml) at 8 months to 5.6 (Ng/ml) at 24 months, the mean of the 1030 samples assayed was 6.19 ± 0.03 (Ng/ml). Sampling of high and low pairs of dams and daughters in several large commercial herds is in progress to estimate additive genetic variance and genetic covariation with production.

INTRODUCTION

The response of lactating dairy cows to injections of BST suggests that hormones contribute substantially to variation in milk production; however, little has been done to obtain estimates of variation in levels of hormones occurring naturally, and even less data are available on the fraction of the variance attributable to genetic differences among cows.

Dairy cows kept in the same herd and receiving the same care and feeding vary in milk production per year from a few thousand (4 or 5) to almost forty thousand pounds. This range of approximately ten-fold is generally attributed to both genetic and environmental differences among cows without specifically defining physiologically such differences. Such general terms as "higher" rates of metabolism", "greater ability to ingest and digest large amounts of feed" and "greater capacity" are used to explain such differences.

To effectively use the techniques of genetic engineering it is essential that genetic and phenotypic variation among lactating cows for various
physiological traits be determined in an objective and quantitative way and with adequate precision. Further, the extent to which genetic variation in physiological traits determines variation in milk, butterfat and protein production must be established.

MATERIALS AND METHODS

In 1985 an effort was initiated to produce a group of split embryo, identical twins Holstein heifers as an appropriate group from which to determine the magnitude of hereditary variance in the concentrations of hormones in the blood of dairy cattle. As a result of this effort, twelve pairs of identical twin, female dairy calves were produced along with approximately the same number of identical twin male calves. The male calves were sold as calves, and we have lost two pairs of the identical twin females. In nine of the twelve cases the members of the same pair of twin heifers were by different surrogate mothers.

Beginning in January of 1986 we began taking blood samples twice a week from each member of each pair. Half of each sample was placed in a freezer and is being kept at -70°C Centigrade for future studies to corroborate what was done and to undertake other studies. The other half of each sample was placed in a freezer and kept at -20°C Centigrade. Samples from this freezer have been used to assay the blood for the concentration (Ng/ml) of various hormones in the blood of the identical twin heifers. Hormones assayed to date are bovine growth hormone, insulin, thyroxine (T₄), triiodothyronine (T₃), progesterone, cortisol, LH and FSH. A brief description of the assay procedure used for assaying four hormones is given below.

Bovine Growth Hormone (GH):

A double antibody method of radioimmunoassay as modified from Glick et al., 1963 Nature 199, 784-787 was used to measure GH in bovine plasma. Synthetic bovine somatotropin (bST) CP104301, a gift from Monsanto Agricultural Co., St. Louis, Missouri, was used for iodination and standards. The bST was dissolved in .01M saline buffer containing .025M NaHCO₃ with pH 9.4. Aliquots were stored at -70°C. Iodination of the bST was carried out using a modified Hunter & Greenwood technique. The labeled hormone was stored at -70°C until the day of the assay at which time it was further purified by passing through a Sephadex G-100 column.

The best immunoreactive label peak was used in the assay. Cleaning of the bST was necessary to improve assay sensitivity and to decrease non-specific binding.

INSULIN (Using Micromedics (ICN) R.I.A. Kit)

The kit utilizes a treated tube procedure which allows a rapid and complete separation of antibody-bound from free radioactively labeled antigen.

Polystyrene assay tubes (12x75) are treated with an antibody to insulin produced in guinea pigs. In the assay, radioiodinated porcine insulin and endogenous insulin from cows are incubated in the treated tube. After the incubation period is complete, the insulin-antibody complex is separated from
free insulin by decanting and washing of the assay tube. The tube is then counted in an auto gamma counter.

TRIIODOTHYRONINE (T₃) R.I.A. (Using ICN Biomedicals Kit)

The antibody is covalently bound to the inner surface of a polypropylene tube. This antibody-bound antigen is also bound to the tube wall. At the conclusion of the assay free antigen is decanted leaving only antibody-bound antigen. The coated tube is then counted in a gamma counter.

THYROXINE (T₄) R.I.A. (Using ICN Biomedicals Kit)

The antibody is covalently bound to the inner surface of a polypropylene tube. This antibody-bound antigen is also bound to the tube wall. At the conclusion of the assay free antigen is decanted leaving only antibody-bound antigen. The coated tube is then counted in a gamma counter.

RESULTS

Shown in Table 1 are the analyses of variance of the data resulting from assays for growth hormone, insulin, T-3 and T-4. As is obvious from Table 1 there are large variations in concentration (Ng/ml) of hormones in the blood of dairy heifers associated with age and assays. This indicates that any comparison of animals should be made at like ages and on a basis of the same assay or label. Even with the commercial kits one finds that an assay conducted this week on 600 samples, will have a consistent an additive difference from an assay of the same 600 samples conducted next week using the same kind of kit. Variation associated with stage of lactation will be explored in a subsequent study.

Table 1. Analysis of Variance for Concentration (Ng/ml) of Growth Hormone, Insulin, T-3 and T-4 in the Blood of Split Embryo Identical Twin Holstein Heifers.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>GH</th>
<th>Mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH</td>
</tr>
<tr>
<td>Assays</td>
<td>(3-5)</td>
<td>45.3</td>
<td>2813</td>
</tr>
<tr>
<td>Pairs</td>
<td>9</td>
<td>40.5</td>
<td>344</td>
</tr>
<tr>
<td>Heifers/Pairs</td>
<td>10</td>
<td>7.9</td>
<td>26*</td>
</tr>
<tr>
<td>Age (Linear)</td>
<td>1</td>
<td>31.1</td>
<td>584</td>
</tr>
<tr>
<td>Age (Quadratic)</td>
<td>1</td>
<td>46.2</td>
<td>62*</td>
</tr>
<tr>
<td>Error</td>
<td>(1006-1977)</td>
<td>2.4</td>
<td>15</td>
</tr>
</tbody>
</table>

* All mean squares in the table except these two were significant at the .001 level of probability when tested against the respective error terms; these two are not significant.

The data in Table 1, indicate that the mean squares among pairs of identical twins are from 4.9 to 13.2 times as large as the mean squares associated with differences between heifers of the same pair. This suggests that there are large hereditary differences among dairy cattle for the concentration of hormones in the blood.
Shown in Table 2 are the components of variance associated with difference among pairs and differences between heifers of the same pair. These components indicate that there are substantial hereditary differences among dairy heifers for concentration (Ng/ml) of hormones in the blood.

The large error variance in Tables 1 and 2 indicate that there is much to learn relative to the appropriate sampling techniques to measure level of hormones in the blood of dairy heifers. About all that one can suggest at the present is that an adequate number of samples be taken. The number of samples assayed for growth hormone, insulin T-3 and T-4 were 2003, 1170, 1170 and 1030 respectively.

Table 2. Components of Variance for the Concentration (Ng/ml) of Four Hormones in the Blood of Identical Twin Holstein Heifers at an Average Age of 18 months.

<table>
<thead>
<tr>
<th>Component</th>
<th>G.H.</th>
<th>Insulin</th>
<th>T-3</th>
<th>T-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pairs (P)</td>
<td>0.63</td>
<td>1.59</td>
<td>173</td>
<td>0.29</td>
</tr>
<tr>
<td>Heifers/Pairs (H)</td>
<td>0.06</td>
<td>0.15</td>
<td>134</td>
<td>0.11</td>
</tr>
<tr>
<td>Error (E)</td>
<td>2.40</td>
<td>15.39</td>
<td>423</td>
<td>0.73</td>
</tr>
<tr>
<td>(P + H + E)</td>
<td>0.20</td>
<td>0.09</td>
<td>0.24</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Mean Concentration (Ng/ml)

\[
\begin{array}{cccc}
\text{G.H.} & \text{Insulin} & \text{T-3} & \text{T-4} \\
3.11 & 15.11 & 181 & 6.19 \\
\end{array}
\]

DISCUSSION

As a result of the evidence of sizeable amounts of hereditary variance for levels of hormone in the blood of dairy cattle a field sampling study is underway. Pairs of high dams and their daughters and low dams and their daughters are being routinely sampled in 6 large dairy herds. These data should provide data on the additive genetic variance of hormone level and the genetic covariance with production.

REFERENCES

