Relationships among Mammary Nucleic Acids, Milk Yield, Serum Prolactin, and Growth Hormone in Heifers from 3 Months of Age to Lactation

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Abstract
Nucleic acid content was determined in the left halves of udders (removed surgically) of 28 Holstein heifers at 5 months of age and in contralateral halves of udders after 60 days of lactation. Mammary deoxyribonucleic acid increased 89 times and ribonucleic acid increased 415 times between 5 months of age and 60 days of lactation. Nucleic acid contents of udder-halves at 5 months were positively correlated ($r = .05$ to .27) with similar mammary tissue measurements after 60 days of lactation and milk production of contralateral udder-halves. Correlations of nucleic acids after 60 days of lactation with milk yield were consistently larger than similar correlations between nucleic acids at 5 months of age and milk yield.

Serum concentrations of prolactin and growth hormone were measured at many intervals between 3 months of age and 26 days of lactation. Phencyclidine hydrochloride (an anesthetic) caused release of prolactin. Various estimates of serum growth hormone among animals at different physiological states were positively correlated with each other, but similar correlations involving prolactin were consistently low and erratic. Correlation coefficients between various estimates of serum hormone concentrations and mammary nucleic acids or milk yield were low and often negative. The relatively low magnitude of correlation coefficients precludes use of these measures of mammary development or of serum prolactin or growth hormone in prepupal heifers as reliable means of predicting future lactational performance.

Introduction
Little selection of dairy cattle occurs until initial production records become available. Early selection of calves could be practiced if criteria existed for accurately predicting subsequent lactational performance. Swett et al. (15) devised an udder rating system for calves which was positively correlated with subsequent milk production, but correlation coefficients were low (8). Recently Rader et al. (12) reported significant correlations as large as .28 between certain qualitative measures of mammary development before puberty and milk yield at maturity. Hackett and Tucker (2) observed that mammary deoxyribonucleic acid (DNA), an estimate of cell numbers, and ribonucleic acid (RNA), an estimate of metabolic activity, in rats 35 days old were correlated with litter weight gains during the first lactation ($r = .34$ and .57).

Our first objective was to determine the relationship between mammary DNA and RNA in calves at 5 mo of age with subsequent DNA, RNA, and milk yield after 60 days of lactation. In addition, serum concentrations of prolactin and growth hormone (GH) measured at intervals between 3 mo of age and early lactation were correlated with mammary nucleic acid content and milk yield.

Materials and Methods
Twenty-nine Holstein heifers, purchased within 1 mo of birth, were managed under standard conditions at the Michigan State University Dairy Cattle Research Center. At 5 mo of age the heifers were trucked to the Michigan State University Large Animal Clinic for surgery. Phencyclidine hydrochloride ($2.2$ mg/kg body weight) was given intravenously to anesthetize the heifers, which were placed in dorsal recumbency for surgical removal of the left half of the udder (front and rear quarters). After removal of the left udder-half, the...
HORMONES, MAMMARY GROWTH, MILK

skin incision was closed with synthetic suture with the continuous lockstitch pattern. The left udder-half was frozen quickly at -20 C for subsequent nucleic acid determinations (16). The day following surgery heifers were returned to the Dairy Cattle Research Center.

Heifers were bred beginning at 14 mo of age. One heifer did not conceive. A mechanical milking machine with left front and rear teat cups occluded was used to milk the heifers. Animals were slaughtered after 60 days of lactation, and the right udder-halves were removed for nucleic acid determinations.

Initial blood samples (10 ml) were collected via jugular venipuncture at 3 mo of age. At 5 mo of age blood samples were taken about 1 hr after trucking animals 3.2 km to the large animal clinic and again at 40 to 60 min after each animal was anesthetized. Blood samples (40 ml) were collected during the first, fourth, and seventh estrous cycles on days 0 (estrus), 2, 4, 7, 11 of the estrous cycle and at 3, 2, 1, and 3 days before onset of the next estrus (14). Blood samples were collected at monthly intervals between days 30 and 270 of pregnancy (4). Blood samples were also collected twice weekly from 29 to 6 days postpartum, twice daily from 6 days before to 5 days after parturition, then twice weekly until 26 days postpartum or first estrus (3). Extra blood samples were collected around estrus and parturition, but only those samples corresponding to the times listed above were analyzed for prolactin and growth hormone. Blood samples were allowed to clot at room temperature for 2 to 4 hr, stored 10 to 20 hr at 5 C, and centrifuged at 6,000 x g for 10 to 15 min; serum was stored at -20 C. Prolactin was measured by method of Koprowski and Tucker (5) on all serum samples, and GH was measured as described by Purchas et al. (11) on all samples except those collected during estrous cycles.

Serum hormone concentrations from the 28 heifers in our study constituted a portion of the mean concentrations presented in other publications during the estrous cycle (14), pregnancy (4), and around parturition (3). For this reason mean hormone concentrations during these physiological states will not be presented, and only correlations between serum hormones and various estimates of nucleic acid content and milk yield will be shown.

Simple correlation coefficients were determined among mammary nucleic acid measurements and between nucleic acids and total milk yields for the first 30 and second 30 days of lactation. In addition, correlation coefficients were obtained among serum hormone measurements, between serum hormones and mammary nucleic acids, and between serum hormones and milk yield. In these latter calculations the multiple estimates of serum hormone concentrations were averaged for each heifer intra physiological state and this average was correlated with the respective hormone, nucleic acid, or milk yield. Physiological state groupings were blood samples collected between 3 and 5 mo of age; during the first, fourth, and seventh estrous cycles; at monthly intervals during pregnancy; from 29 to 30 days before parturition; and from parturition to 26 days of lactation.

Results and Discussion

Services per conception for the 28 hemimastectomized heifers was 1.3. Age at parturition ranged from 23 to 25 mo. Total milk production of the right udder-half for these heifers during the first 30 days of lactation ranged from 161 to 450 kg with an average of 312 kg (624 kg on a whole-udder basis). During the second 30 days of lactation milk yield ranged from 132 to 495 kg with an average of 308 kg (616 kg on a whole-udder basis). We concluded that the surgery and bleeding schedule for these heifers did not markedly alter reproductive or lactational performance from that expected in normal heifers.

Total mammary DNA increased 89 times whereas total RNA increased 415 times be-

### Table 1. Nucleic acid content of one-half of the udder at 5 mo of age and of the contralateral half after 60 days of lactation.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary wt, wet, kg</td>
<td>5.44 ± 0.29</td>
</tr>
<tr>
<td>DNA, g</td>
<td>12.40 ± 0.86</td>
</tr>
<tr>
<td>RNA, g</td>
<td>54.51 ± 3.85</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>4.52 ± 0.40</td>
</tr>
</tbody>
</table>

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*28 animals.
Serum prolactin and growth hormone concentrations in heifers at 3 and 5 mo of age and after phencyclidine hydrochloride anesthesia.

<table>
<thead>
<tr>
<th>Age</th>
<th>Prolactin (ng/ml)</th>
<th>Growth hormone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo</td>
<td>24.6±8.4</td>
<td>4.3±0.5</td>
</tr>
<tr>
<td>5 mo</td>
<td>29.2±5.4</td>
<td>10.0±1.0</td>
</tr>
<tr>
<td>5 mo, anesthesia</td>
<td>58.5±10.0</td>
<td>3.5±0.2</td>
</tr>
</tbody>
</table>

28 animals.

Means ± SE of means.

Significantly greater (P<.01) than other values in column.

Table 3. Correlations between measurements on udder-halves at 5 mo of age and contralateral halves after 60 days of lactation.

<table>
<thead>
<tr>
<th>At 5 mo of age</th>
<th>After 60 days lactation</th>
<th>Milk yield from half-udders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt</td>
<td>DNA</td>
</tr>
<tr>
<td>Wt</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>.93</td>
<td></td>
</tr>
<tr>
<td>RNA</td>
<td>.89</td>
<td>.94</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>0.01</td>
<td>.02</td>
</tr>
</tbody>
</table>

Table 2. Serum prolactin and growth hormone concentrations in heifers at 3 and 5 mo of age and after phencyclidine hydrochloride anesthesia.

Correlations in cattle between either mammary DNA or RNA measured after 60 days of lactation and milk production (Table 3). Although these coefficients were positive, they were generally lower than similar comparisons in rats between 35 days of age and lactation. Correlations between nucleic acid determinations in heifers in response to anesthesia agrees with data collected in rats after ether (10) or nembutal (1) anesthesia, meaning that anesthesia probably depresses the hypothalamic prolactin-inhibiting factor in heifers as it does in rats (9).

In contrast to prolactin, serum GH concentration increased (P < .01) between 3 and 5 mo of age (Table 2). However, there was no significant difference (P > .05) between concentration at 3 mo and that after anesthesia at 5 mo of age. It is unknown whether reduction in GH during anesthesia represents an effect of anesthesia or whether the initial 5-mo sample was elevated because of an effect of trucking the animals to the clinic before collecting blood samples.

Average serum prolactin and GH concentrations in heifers after 5 mo of age have been published elsewhere (3, 4, 14).

Mammary weight, DNA, and RNA content at 5 mo of age were positively correlated with similar measurements during lactation and with milk production (Table 3). Although these coefficients were positive, they were generally lower than similar comparisons in rats between 35 days of age and lactation. Correlations between nucleic acid determinations in cattle between either mammary DNA or RNA measured after 60 days of lactation and milk production were much greater than correlations between nucleic acid determinations in heifers in response to anesthesia.
Table 4. Correlations between serum growth hormone measured at 3 to 5 mo, during pregnancy, and shortly before and after parturition.

<table>
<thead>
<tr>
<th>Growth hormone</th>
<th>3 to 5</th>
<th>Pregnancy</th>
<th>Before parturition</th>
<th>After parturition</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 to 5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>.26</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before parturition</td>
<td>.30</td>
<td>.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>After parturition</td>
<td>.15</td>
<td>.17</td>
<td>.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of pairs varies from 26 to 28.
<sup>b</sup> P<.05.
<sup>c</sup> P<.01.

Correlations involving RNA/DNA were often close to zero and sometimes negative. On the basis of relatively low correlation coefficients, we conclude that quantitative measures of mammary development in pubertal heifers will not be a practical means of predicting future mammary development or lactational performance.

Serum prolactin concentrations among the 28 heifers at various physiological states were not correlated with each other (P > .05), nor were there consistent patterns in the sign of coefficients ranging from -.16 to .35. In contrast, various estimates of serum GH among animals at different physiological states were positively correlated with each other and two coefficients were significant (Table 4). This more consistent pattern for GH correlations may be a reflection that serum GH concentrations are much less erratic among different physiological states than those for serum prolactin (7, 17).

Except for two coefficients associated with measurements after parturition, there were no significant correlations between prolactin and GH suggesting that the two hormones were secreted relatively independent of each other.

Correlation coefficients between hormones and mammary tissue nucleic acid content or milk yield after 60 days of lactation are summarized in Table 5. Many coefficients were negative and low between any pair of variables. The positive correlations between prolactin after parturition with milk yield agrees with observations in our laboratory (4, 6), but the number of observations was not sufficient to achieve significance.

Correlation coefficients between hormone concentration at each bleeding (rather than

Table 5. Correlations between measurements on udder-halves after 60 days of lactation and serum prolactin and growth hormone measured at various physiological states.

<table>
<thead>
<tr>
<th>After 60 days lactation</th>
<th>Milk yield</th>
<th>(0 to 30 days)</th>
<th>(31 to 60 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt</td>
<td>DNA</td>
<td>RNA</td>
<td>RNA/DNA</td>
</tr>
<tr>
<td>Prolactin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 to 5 mo</td>
<td>-.19</td>
<td>-.37</td>
<td>-.17</td>
</tr>
<tr>
<td>Cycle</td>
<td>.20</td>
<td>.15</td>
<td>.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>-.24</td>
<td>.07</td>
<td>-.15</td>
</tr>
<tr>
<td>Before parturition</td>
<td>-.01</td>
<td>-.17</td>
<td>.08</td>
</tr>
<tr>
<td>After parturition</td>
<td>-.06</td>
<td>-.03</td>
<td>-.01</td>
</tr>
</tbody>
</table>

Growth hormone

| 3 to 5 mo | -.18 | .27  | -.23    | -.30 | .06  | -.04 |
| Pregnancy | -.28 | .06  | -.26    | -.22 | .02  | -.11 |
| Before parturition | -.04 | .19  | -.08    | -.31 | -.08 | -.10 |
| After parturition | .05  | .19  | -.04    | -.15 | .14  | .06  |

<sup>a</sup> Number of pairs varies from 26 to 28.
<sup>b</sup> P<.05.
correlations based on physiological-state groupings) and milk yield were highly variable; some were positive and others negative. Koprowski and Tucker (6) observed that increased serum prolactin after milking stimulus was more positively correlated with milk yield than if basal measurements of the hormone were correlated with milk production. It would be of interest to determine if serum hormone concentrations in calves after application of a suitable uniform stimulus might be more highly related to subsequent milk yields, as compared with relationships presented in this report.

References