ABSTRACT
Circulating thyroxine, triiodothyronine, and prolactin were analyzed by radioimmunoassay from two groups of pregnant heifers representing genetic populations that differed by 685 kg milk in their first lactation. At 100 days of gestation, blood samples were taken every 15 min for prolactin assay and every 2 h to determine variations of thyroid hormone serum concentration from 0800 to 2000. Basal serum prolactin concentrations in the two genetic groups were not significantly different. A pattern was consistent for both triiodothyronine and thyroxine; concentrations were lower in the morning and higher during the afternoon. Serum triiodothyronine means were not different in the high-producing (1.84 ng/ml) and low-producing heifers (1.86 ng/ml). Serum thyroxine concentrations were significantly higher in the low-producing heifers (67.84 ng/ml) than in high-producing heifers (59.18 ng/ml). The higher thyroxine concentration in pregnant heifers with lower producing ability suggests a negative relationship of thyroxine to milk yield potential.

INTRODUCTION
Milk production in dairy cows depends upon many complex interacting factors. Adequate milk production depends upon proper development and growth of the mammary gland, initiation of milk secretion, and maintenance of milk synthesis. Hormones are necessary for lactation in all mammals and are the primary physiological factors controlling milk production (29).

The involvement of the thyroid in lactation has been recognized for almost a century. Feeding cows dried thyroid gland or thyroxine increased milk yields (11), and conversely, removal of the thyroid gland reduced milk yields (12). Increased milk yields also were obtained by iodinated proteins (4, 20, 23). The thyroid hormones, thyroxine (T4) and triiodothyronine (T3), regulate basic metabolism of all body tissues and organs. Because milk yields were increased by administration of thyroid hormones, these findings suggest that lack of thyroid hormones keeps mammary gland cellular machinery from functioning at maximum during normal lactation, i.e., that the lactating cow is in a functional hypothyroid state (28). A negative relationship exists between milk yield and thyroid hormones in lactating cows (6, 8, 14, 30, 33).

Selection of cows at early ages for increased milk production has been an important goal of dairy geneticists (5). Study of endocrine factors involved in the genetic improvement of milk production has been a difficult research area because of the complexity of the endocrine system (5, 10, 29). At Beltsville, a selection project was initiated in 1970 (21). This experiment was designed to determine whether higher net profitability of offspring could be achieved if sire selection was based solely on milk yield of daughters during the first lactation (Yield group) or whether higher profitability would be achieved from sires selected by milk yield, fat percent, and an udder index based on udder type (Merit group). Results showed Yield heifers produced 685 kg more milk and 51 kg more solids-not-fat than Merit heifers during first lactation (21). Physiological and hormonal bases for the genetically increased milk yield in these cows, however, are unknown. Because there appears to be a relationship between

Received November 22, 1983.

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thyroid hormones and milk yield, the purpose of the present experiment was to investigate whether thyroid hormone or prolactin differences exist in Holstein heifers selected for potential milk production. Because much of the growth of the mammary gland occurs during pregnancy, hormones during gestation may have a profound influence on subsequent milk production. The involvement of prolactin has been studied extensively in the last 25 yr, but its role in mammary gland growth in ruminants has not been elucidated (29). In the current study we have focused on these two endocrine influences and studied circulating prolactin and the thyroid hormones, T4 and T3.

MATERIALS AND METHODS

Heifers were a subsample of animals from the Beltsville genetic selection project (21). Estrus was synchronized to minimize the potential for environmental and seasonal factors to mask differences in hormone concentrations between groups and as an aid to allow blood sampling on the same day during gestation. Fifteen Merit and 15 Yield heifers (at least 14 mo old raised under standard dairy heifer management procedures) were treated with prostaglandin F2 to synchronize estrus (18). When the heifers came into heat following treatment, they were inseminated with semen from unrelated sires (not Yield or Merit sires). Heifers subsequently diagnosed as pregnant (12 Merit; 4 Yield) were sampled at 100 days of gestation (mid-December). On day 99, a polyvinyl cannula (.48 m in length) was placed into a jugular vein of each animal in the direction of the heart to a depth of .32 m. Blood samples were taken every 15 min for prolactin assay and were taken every 2 h for variations of thyroid hormone secretion from 0800 to 2000 h. Blood was collected on ice, stored at 4°C overnight, and sera were prepared by centrifugation the following morning. Sera samples were stored at -20°C until assayed.

Triiodothyronine and T4 concentrations were evaluated statistically by ANCOVA and heterogeneity of slopes/elevations analyses (35).

Prolactin was determined by radioimmunoassay according to methods described (1). Within-assay coefficients of variation averaged 6.6%; between-assay coefficients of variation were 6.8%.

RESULTS AND DISCUSSION

Serum T4 concentrations showed a consistent pattern between 0800 and 2000 h: concentrations were lower in the morning and higher in the afternoon (Figure 1). The concentrations for Yield heifers were significantly lower than for Merit heifers. Statistical analyses by ANCOVA and heterogeneity of elevations/slopes indicated that elevations of regression lines were different (P<.05), but slopes were not different (35). Mean T4 concentrations for all blood samples were 59 ng/ml for Yield heifers and 68 ng/ml for the Merit (Table 1). These differences in circulating T4 concentration trends at 100 days of pregnancy may be useful in differentiating high and low potential for milk production.

Serum T3 in these nonlactating Holstein heifers at 100 days of gestation are in Figure 2. A pattern similar to T4 was observed; morning was lower than afternoon. However, the mean T3 of 1.84 ng/ml in Yield heifers was not different from the mean T3 of 1.86 ng/ml in Merit heifers.

Triiodothyronine and T4 concentrations were evaluated statistically by ANCOVA and heterogeneity of slopes/elevations analyses (35).

Prolactin was determined by radioimmunoassay according to methods described (1). Within-assay coefficients of variation averaged 6.6%; between-assay coefficients of variation were 6.8%.
TABLE 1. Serum thyroxine (T\textsubscript{4}) and triiodothyronine (T\textsubscript{3}) in merit and yield heifers at 100 days of gestation.

<table>
<thead>
<tr>
<th>Genetic group</th>
<th>n</th>
<th>T\textsubscript{4}</th>
<th>T\textsubscript{3}</th>
<th>Prolactin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\bar{X}) SE</td>
<td>(\bar{X}) SE</td>
<td>(\bar{X}) SE</td>
</tr>
<tr>
<td>Merit</td>
<td>12</td>
<td>68 2</td>
<td>1.86 .14</td>
<td>7 1</td>
</tr>
<tr>
<td>Yield</td>
<td>4</td>
<td>59* 4</td>
<td>1.84 .10</td>
<td>7 1</td>
</tr>
</tbody>
</table>

\footnotesize{1Means SE based on seven serum samples taken from each animal in each group between 0800 to 2000 h. *Significantly different (P<.05).}

The higher T\textsubscript{4} in pregnant heifers from lower-producing cows suggests a negative relationship to milk yield potential. This observation agrees with results in lactating cows where a correlation appears negative between serum T\textsubscript{4} and milk yield. During lactation in the cow, serum thyroid hormones were reduced in cows producing higher yields of milk (8). In rats, there was an inverse relationship between lactational intensity and serum T\textsubscript{4} (17). In two studies in cattle involving 170 Holstein cows at Missouri (30) and in 86 Holsteins in Alberta, Canada (33), negative correlations also were between milk yield and serum T\textsubscript{4}, leading to the conclusion that high-producing cows had lower T\textsubscript{4} than low-producing cows. Hart et al. (14) in England also reported that plasma T\textsubscript{4} was higher in low-yielding cows and that T\textsubscript{4} was negatively related to milk yield. Recently, Blum et al. (6) studied serum T\textsubscript{3} and T\textsubscript{4} in 36 lactating cows in Switzerland and also found negative correlation with milk yield during lactation. However, there were conflicting data.

Anderson (2) found a positive relationship between thyroid hormone secretion rates and milk production, but the correlation coefficient was statistically nonsignificant. Swanson (25) also found that T\textsubscript{4} secretion rates increased slightly as lactation progressed from 3 to 6 months.

Several factors could be involved in the negative relationship between milk yield and thyroid hormones. Differences in plasma thyroid hormone between high- and low-producing cows could be due to a differential loss of thyroid hormones into milk. The high-producing cows could secrete more total thyroid hormones in their milk, and this would result in lower circulating hormones. Such a mechanism was supported by data in lactating rats (9). The differences in T\textsubscript{4} in the pregnant heifers in the present experiment, however, suggest that they are due to genetic factors because the negative relation of thyroid hormones to milk production potential was revealed in nonlactating animals.

We are not aware of evidence that variation in circulating T\textsubscript{4} concentrations during pregnancy alter mammary development of ruminants. Mittra (19) reported that following thyroidectomy the mammary gland of estrogen-treated rats undergoes extensive growth because of increased sensitivity to prolactin. In contrast, Vonderhaar and Greco (32) found that decreased concentrations of serum thyroid hormones at weaning were associated with decreased mammary growth in mature mice. Thus, conflicting data showing both positive and negative effects are apparent (27, for discussion). Others (24, 27) have observed that lobulo-alveolar development in vitro can be modified markedly.
by changing the ratio of prolactin to T₄ in culture medium. Also T₃ acts directly on mouse mammary tissue to enhance prolactin-induced synthesis of alpha-lactalbumin (31), and prolactin binding is reduced in thyroidectomized rats (32). Thus, the physiological significance of lower serum T₄ concentrations in pregnant heifers from genetically superior dams remains to be determined.

The lower T₄ and T₃ concentrations measured in the morning and higher in the afternoon suggests a time dependent rhythm in thyroid hormone secretion. The sparse literature in cattle is not consistent on this subject. Yousef and Johnson (34) estimated the disappearance rate of radiolabeled thyroxine by measuring radioactivity in plasma at 3-h intervals. Because I¹³¹ decreased at a constant percentage they concluded that this indicated the absence of any diurnal rhythm. However, clearance of T₄ was measured only by disappearance of radioactivity from blood. Loss of radiiodine from the T₄ molecule or conversion into other forms was not monitored by this methodology. Protein-bound iodine, which includes T₄, T₃, thyroglobulin, and other iodine proteins, was also determined as 3-h intervals, and no differences were found. The iodine proteins, in some cases, greatly may exceed T₄. The specific radioimmunoassays for T₄ and T₃ largely have replaced protein-bound iodine as a measure of thyroid function.

Refsal et al. (22) investigated whether serum T₃ and T₄ exhibited diurnal variations in lactating dairy cows. A diurnal pattern was observed for T₃, characterized by a low baseline from 0400 to 1000 h and a rise to an elevated plateau from noon to 2200 h. Thyroxine changes paralleled T₃ but with smaller relative changes. They concluded diurnal variations existed in serum T₃ and T₄ in lactating dairy cows, and failure to standardize time of sampling may affect experimental results.

We analyzed plasma T₄ and T₃ every 2 h in steers over 48 h (13). Harmonic time series analysis indicated trends for T₄ toward periodicity corresponding to feeding period (12 h) and daylength (24 h). Lower concentrations were in the morning and increased beginning at or shortly after feeding. The only apparent cycle for T₃ was at 28 h, suggesting that no biologically significant cycles of 24 h or less exist for T₃. The diurnal rhythm in these steers was consistent with the data of Refsal et al (22) and with data of this report.

Patterns of serum prolactin concentrations (at 15-min intervals) showed a decline in the early afternoon from morning concentrations, followed by generally higher concentrations in the late afternoon and evening (Figure 3). Several erratic peaks were unexplained in morning samples (Figure 3). Basal prolactin in the two groups of pregnant heifers were not significantly different (Table 1). Thus,

Figure 3. Serum prolactin variations in nonlactating Holstein heifers at 100 days of pregnancy. Overall standard errors were: Merit .62, Yield .66.
there does not appear to be a difference in circulating hormone related to the selection in this experiment.

Several studies also have demonstrated genetic relationships in hormone or metabolite concentrations in cattle. Joakimsen et al. (15) reported a genetic correlation between thyroxine degradation and milk yield of .42. Edfors-Lilja et al. (7) also found genetic relationships between growth and T₄ degradation in two breeds of Danish bulls. In England, Tilakaratne et al. (26) examined several metabolites in young Friesian cattle of high or low genetic merit for milk production. Two blood metabolites, free fatty acids and urea, varied during imposition of a physiological stress, fasting. They concluded that expression of genes affecting milk yield may be measured physiologically in young animals and that fasting may induce expression of this variation. Blood concentrations of prolactin, growth hormone, insulin, and glucagon were reported to respond to selection for milk in two groups of Holstein calves (3).

REFERENCES

TECHNICAL NOTE


