Bovine Serum Thyroxine, Prolactin, Growth Hormone, Glucocorticoid, and Thyroxine Binding Globulin Response to Thyroprotein

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ABSTRACT

Twenty-one lactating Holstein cows were assigned randomly to groups of seven and received: (1) no treatment; (2) 15 g thyroprotein daily for 5 wk; or (3) 15 g thyroprotein daily for 13 wk. Combined averages for serum thyroxine of cows in thyroprotein groups increased linearly from a baseline of 54 ng/ml to a peak of 135 ng/ml at 6 days after thyroprotein feeding was begun. Serum thyroxine then decreased to approximately 80 ng/ml at 23 days of thyroprotein feeding which was maintained during the remainder of the feeding period. Following thyroprotein withdrawal from the diet serum thyroxine concentration decreased to 24 ng/ml at 6 days. Neither serum prolactin, growth hormone, nor total glucocorticoids were affected by thyroprotein feeding or withdrawal.

Average milk production of cows in the control group decreased linearly with time. Milk production of cows fed thyroprotein for 5 wk averaged 2.2 to 3.3 kg/day more than cows in the control group and that of cows fed thyroprotein for 13 wk averaged .95 to 2.5 kg/day more than controls for 60 days, but between 60 and 91 days it was .9 kg/day less than controls.

INTRODUCTION

Thyroprotein, an iodinated casein containing about 1% thyroxine (15) has been fed to dairy cows for about 30 yr to increase milk yield (1, 25). However, there has been no direct determination of changes in serum thyroxine concentration resulting from its feeding. In addition, the mechanism by which thyroprotein exerts its galactopoietic effect is unknown. Perhaps it acts indirectly via pituitary hormones since alterations in thyroid function have profound effects on pituitary structure and hormone content (2, 8, 17). Our study was designed to evaluate changes in serum concentration of thyroxine, thyroxine binding globulin (TBG), prolactin, growth hormone, and total glucocorticoids in cows fed thyroprotein.

MATERIALS AND METHODS

Twenty-one lactating Holstein cows ranging in age from 3 to 7 yr were assigned randomly to groups of seven and received: (1) no treatment (NT); (2) 15 g thyroprotein daily for 5 wk (T5); or (3) 15 g thyroprotein daily for 13 wk (T13). The experiment was begun on September 28, 1971, and completed on February 28, 1972. Thyroprotein was mixed in .453 kg grain concentrate and fed at 0700 h prior to other feeds included in the diet to insure complete thyroprotein ingestion. All cows received alfalfa haylage (4.5 kg), corn silage to appetite (16 to 45 kg/cow per day) and .45 kg grain per kg milk daily. Relative to initiation of thyroprotein feeding (day 0), coccygeal venous or arterial blood was collected from all cows on days -6 and -3, then daily for the 1st wk, weekly during wk 2 to 5, and daily during wk...
Thereafter cows in groups NT and T 13 were bled weekly during wk 7 through 13 and daily during wk 14. Blood was drawn between 1500 and 1700 h and maintained at room temperature for 2 to 3 h then at 4 °C to allow clot retraction. Serum was collected after centrifugation and stored at −20 °C until used for hormone assay. All serum samples were assayed individually for thyroxine by the Tetrasorb-125 method³ and for prolactin (27) and growth hormone (20) by specific double antibody radioimmunoassays. Total glucocorticoid concentration was determined in all serum samples from cows in groups NT and T 13 by competitive protein binding assay as previously described (24). The extent of binding of serum thyroxine to TBG was determined in an aliquot of serum and exogenous thyroxine plus tracer ¹³¹I thyroxine was added to achieve a thyroxine concentration which exceeded the binding capacity of the TBG. Unbound thyroxine was absorbed on a resin-impregnated sponge to separate it from that bound to carrier protein. From the total amount of thyroxine added to the sample and the dilution of tracer thyroxine, the amount of endogenous thyroxine bound to TBG was determined.

RESULTS

The change in average serum thyroxine concentration y (ng/ml) with time X (days) in cows not receiving thyroprotein is best described by the linear equation

\[ y = .1 X + 54.3 \]

i.e., serum thyroxine increased .1 ng/ml per day during this experiment (P < .05; Fig. 1). Average serum thyroxine changes, during comparable periods after initiation of thyroprotein feeding and following thyroprotein withdrawal, were not different (P > .05) between groups receiving thyroprotein for 5 or 13 wk (Fig. 1). Therefore, comparable periods for each response curve were combined for statistical analysis. Thyroprotein feeding increased serum thyroxine concentration linearly (P < .01) during the 1st wk. An increase (P < .01) in serum thyroxine was demonstrable in the first serum sample which was taken at 12 h after initial thyroprotein feeding when the combined average was 76.5 ± 3.7 as compared with 61.4 ± 3 ng/ml for controls. Serum thyroxine reached a peak of 135.2 ± 4.5 ng/ml at 6 days after thyroprotein feeding was initiated; the average rate of increase from day 1 to 6 was 10 ng thyroxine/ml serum per day. Thereafter serum thyroxine decreased gradually to 79 ± 7 ng/ml at day 23 establishing a somewhat erratic plateau thereafter. The period between thyroxine stabilization and thyroprotein withdrawal was too short in cows in group T 5 to be definitive concerning stabilization of serum thyroxine. However, in cows fed thyroprotein for 13 wk, serum thyroxine fluctuated between 68 and 121 ng/ml from day 23 to 91. Regression analysis revealed that the change in serum thyroxine (ng/ml) during this period fit a linear (P < .01) regression (y = .2 X + 85) with a slope not significantly different from the slope for the control group but having a y intercept 30 ng/ml greater.

Following withdrawal of thyroprotein from the diet, serum thyroxine concentration for cows fed thyroprotein for 5 or 13 wk responded similarly. For combined averages, serum thyroxine concentration averaged 94.1 ± 9.0 ng/ml on the last day of thyroprotein feeding and was not reduced significantly by 36 h following thyroprotein withdrawal (80.4 ± 6.4). From day 1 to 6 following thyroprotein withdrawal serum thyroxine decreased linearly (P < .01) reaching a nadir of 24 ± 4.2 ng/ml, which was approximately 30 ng/ml lower than the
basal concentration in the control group. Thereafter average serum thyroxine gradually increased, approaching concentrations not different (P > .05) from those of controls by 2 wk after withdrawal of thyroprotein.

The fraction of serum thyroxine bound to TBG (Fig. 2) is expressed as the saturation index. This is the ratio of total serum thyroxine to the calculated TBG capacity. Average saturation index for serum from cows in the control group remained at .7 throughout the sampling period. In contrast, the saturation index of serum from cows in the group fed thyroprotein for 5 wk increased (P < .01) rapidly to a peak of 1.6 at 6 days after thyroprotein feeding was initiated, then decreased (P < .01) concomitantly with the decrease in serum thyroxine to approximately 1.0 after 29 days of thyroprotein feeding. Following withdrawal of thyroprotein from the feed, the saturation index decreased to less than half that of comparable values for cows in the control group.

Serum prolactin concentration was not affected by thyroprotein feeding for 5 or 13 wk. However, serum prolactin concentration in controls and cows fed thyroprotein for 13 wk decreased linearly (P < .01) during the 16 wk experimental period (Fig. 3), but differences between slopes were not significant (P > .05). Serum growth hormone concentration in cows fed thyroprotein for 13 wk averaged 1.9 ± .8 ng/ml, which was less (P < .001) than the comparable control value of 2.7 ± 1.4 ng/ml (Fig. 4). However, this difference was not likely due to thyroprotein feeding since cows assigned to the control group had greater serum growth hormone concentrations before thyroprotein feeding was initiated. On days -6 and -3 serum growth hormone concentrations (ng/ml) averaged 4.2 ± .3 and 4.1 ± .6 for cows in the control group and 1.8 ± .3 and 1.7 ± .1 for cows to be fed thyroprotein (Fig. 4). This idea is also supported by failure of thyroprotein feeding to change serum growth hormone concentration in cows in the T 5 group particularly since the greatest difference in growth hormone concentration between controls and cows in group T 13 occurred during the first 6 wk of the experiment.

Total serum corticoid concentration in cows fed thyroprotein for 13 wk did not differ (P > .05) from comparable values for cows in the control group (Fig. 5).

Average milk production of cows in the control group decreased linearly (P < .001) with time over this experiment (y kg = - .06 X days + 19.1 kg). Milk production of cows in group T 5 during thyroprotein feeding, averaged 2.2 to 3.3 kg/day more than cows in the control groups during the comparable period. When thyroprotein feeding was stopped, milk production of cows in group T 5 decreased to that of control cows, and remained similar thereafter. Average milk production of group T 13 was greater than that of control cows by .95 to 2.5 kg/day for 60 days, but between 60 and 91 days it was .9 kg/day less than controls. After thyroprotein feeding ceased, milk production decreased abruptly in T 13.

**DISCUSSION**

Recent evidence suggests that serum thyroxine concentration is related inversely to lactational intensity. Thus, in rats, as litter size increased, serum thyroxine concentration decreased (11). In addition, serum thyroxine at peak lactation in cattle is 54% that of nonlactating cows and is increased to concentrations comparable to nonlactating cows by the end of lactation (10). The increase in serum thyroxine concentration with advancing lactation, in control cows in our experiment, is
consistent with results of these reports. The possibility that changes in thyroxine with time in this experiment may be attributable to environmental changes is not likely since these cows were maintained indoors and, therefore, were not exposed to marked environmental temperature variation. In addition, thyroid hormone secretion rates in goats were comparable in fall, winter, and spring (7); our experiment began in fall and was completed in winter.
The progressive increase in serum thyroxine following initiation of thyroprotein feeding probably reflects increasing absorption of thyroxine from the gut as it accumulates with continuing administration. The decrease in serum thyroxine concentration, from a maximum at 7 days after beginning thyroprotein feeding, may be due to accelerated thyroxine clearance rate. Indirect evidence for this is seen in that there was a progressive decrease in the rate of increase in average serum thyroxine concentration as it approached maximum. In addition, Premachandra and Turner (19) showed that in normal cattle half-life of thyroxine was 2.3 days but was decreased to 1.1 and .88 days when cows were injected with thyroxine at doses 50 or 100% greater than their thyroxine secretion rate. High concentrations of serum thyroxine also would be expected to reduce thyroid stimulating hormone release via negative feedback on the pituitary (21) which would markedly reduce endogenous thyroxine secretion. Increased clearance at high serum thyroxine also would be enhanced by the fact that thyroxine binding globulin is soon saturated and cannot bind the additional thyroxine.

The plateau established by feeding 15 g thyroprotein was considerably less than that predicted by Pipes et al. (18). These authors calculated that feeding 15 g thyroprotein/day would increase total body pool of thyroxine from 5.1 to 38.6 mg; thus, with a constant distribution volume, serum thyroxine would increase approximately sevenfold. In fact serum thyroxine only increased 64%. The decline in serum thyroxine which followed withdrawal of thyroprotein from the diet probably reflects rapid withdrawal of exogenous thyroxine and gradual resumption of endogenous TSH-thyroxine secretion. However, the thyroid is able to resume full function within 10 to 14 days even after prolonged suppression by exogenous thyroxine.

After initiation of thyroprotein feeding the greatest serum thyroxine preceded greatest milk production by a few days in this and other experiments (25, 26). Rapid or gradual withdrawal of thyroprotein after feeding for 30 days or longer resulted in an abrupt and large decrease in milk production (25, 26). Serum thyroxine behaved similarly, and the relationship is more than coincidental.

This investigation does not support the view that the galactopoietic effect of thyroprotein in cattle is due to enhanced serum prolactin, growth hormone, or corticoid concentration. Previous workers have reported that pituitary prolactin concentration and content are increased in hyperthyroid rats (4), decreased in hypothyroid rats (12, 13, 17), and that thyroid hormones can act directly on the rat pituitary in vitro to stimulate prolactin secretion (16). However, concomitant shifts in serum prolactin concentrations were not observed in experimentally induced hyper- or hypothyroidism in our study. Although, pituitary prolactin secretion may be altered with changes in thyroid function, compensating changes in metabolic clearance rate (MCR) of the hormone may result in a relatively stable serum hormone concentration. Prolactin MCR increased in hypothyroid sheep (5), which supports this possibility. Serum prolactin concentration in cows is highest in the summer and lowest in winter (9, 23). The period of this experiment represents a transition period from maximum to minimum serum prolactin. Thus, the linear decrease in serum prolactin concentration between September and February may reflect changes attributable to season.

Altered thyroid function has affected growth hormone secretion. Thus, pituitary (2, 8, 17) and serum (17) growth hormone concentrations are reduced in chronically hypothyroid rats. However, serum growth hormone concentration following thyroidectomy in sheep was unchanged (5). Although growth hormone is galactopoietic in cows (3), the galactopoietic effect of thyroprotein apparently is not attribu-
table to enhanced serum growth hormone concentration. Meites (14) reached a similar conclusion on his observation that milk production in goats, which had been optimally stimulated with thyroprotein, could be further enhanced by exogenous growth hormone.

The adrenal and thyroid glands seem to be interrelated closely, but the extent of their interdependence and the mechanism of their interaction are equivocal. Increased adrenal glucocorticoid secretion in hyperthyroidism is due in part to a direct stimulatory action of thyroxine on the cortical cells and in part to counteract the antiglucocorticoid actions of thyroxine. However, with serum thyroxine in this study effects on serum glucocorticoids were not demonstrable.

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REFERENCES