Effects of Somatotropin on Milk Yield and Physiological Responses
During Summer Farm and Hot Laboratory Conditions

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ABSTRACT

The effects of bST on performance and physiological responses of lactating cows was studied under farm summer and laboratory heat conditions. Twelve cows, 90 to 50 d postpartum, were injected with either bST or vehicle solution for 30 d under farm summer and 10 d under either laboratory thermoneutral or heat conditions. Somatotropin increased milk yield by 6.1 (21%), 8.1 (32%), and 7.3 kg (35%) under the farm summer, laboratory thermoneutral, and heat conditions, respectively. Somatotropin also increased milk fat by 15 and 19% and dry matter intake by 16 and 18% under laboratory thermoneutral and heat conditions, respectively. Somatotropin increased the efficiency of feed conversion into milk without any significant changes in body weight and temperatures. Somatotropin increased the plasma concentrations of triiodothyronine and cortisol and had no effect on plasma prolactin and insulin concentrations. Somatotropin did not increase water intake; however, hematocrit was decreased. The results suggest that stimulatory effects of bST on milk production are still observed on heat-stressed cows without any significant indications of additional heat stress.

(Key words: heat stress, somatotropin, milk yield)

Abbreviation key: PRL = prolactin, T₃ = triiodothyronine, T₄ = thyroxine, THI = temperature-humidity index, TN = thermoneutral, WBC = white blood cells.

INTRODUCTION

Bovine somatotropin, purified or recombinant, increases milk yield (15, 19, 20) and feed efficiency of lactating dairy cattle (26) in thermoneutral conditions. In theory, if the environmental temperature increases above the upper critical temperature, an animal's effort to maintain heat balance or homeothermy takes priority over production processes. Lactating cows in hot environments in the field and laboratory reduced milk yield and feed intake (11) and induced hormonal changes (10) in an attempt to attain homeothermy. During the summer in a subtropical environment (Florida), slight increases in milk yield and 3.5% FCM production have been observed in bST-treated cows (25, 30); however, such increases were not accompanied by changes in feed intake and milk fat percentage. In controlled laboratory conditions (18), heat-stressed lactating cows injected with purified bST also showed increases in milk yield without significant changes in feed intake. Several studies reported that somatotropin supplementation under heat conditions did not affect plasma concentrations of triiodothyronine (T₃), thyroxine (T₄), cortisol, prolactin (PRL), and insulin (18, 30), although the responses of heat-stressed cows to bST treatment varied among reports. Somatotropin injec-
SOMATOTROPIN EFFECTS IN HOT ENVIRONMENTS

Experimental Design and Protocol

Eighteen lactating Holstein cows were selected from the university dairy herd for 1) milk production of 25 kg/d or greater, 2) 90 to 150 d postpartum, 3) sound health, and 4) low scores for mastitis as determined by the California mastitis test. Cows were designated for either control or recombinant-bST treatment. The experiment consisted of two periods: farm summer and laboratory conditions with intervening adjustments. During period 1, cows were injected with either vehicle or bST under seasonal farm summer conditions for 30 d. During period 2, cows were divided into two groups (each group consisted of 3 bST- and 3 vehicle-injected cows) and exposed to either laboratory heat or TN for 10 d with a single-reversal design. The detailed protocol and environmental conditions are presented in Table 1. Data were obtained from all cows during farm summer period to provide assurance of adequate cow numbers; however, the analyzed data from the farm included only cows that were used in the subsequent laboratory study. Cows remaining at the farm were held for replacement of any animals that might have been withdrawn from the laboratory for health or injury reasons. One cow was substituted during the initial acclimation to laboratory conditions.

TABLE 1. Protocol summary describing the environmental temperature and humidity, time, and duration of each environmental condition.

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<td>Laboratory simulated hot or TN</td>
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<td>24-35</td>
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1Each group consisted of 3 control and 3 bST-treated cows.
2Dates of farm period are from June 29 to July 27, 1987.
3Thermonutral conditions.
Environmental Conditions

Farm. Temperature and humidity were recorded continuously by a hygrothermograph (Cole-Parmer, Chicago, IL) maintained in a weather station adjacent to the loose housing lot, and results are presented in Figure 1.

Climatology Laboratory. The Missouri Brody Animal Climatology Laboratory, located in the Animal Science Research Center at the University of Missouri-Columbia, consists of four individually controlled chambers (7.3 × 9.1 m) with supporting milk, feed, sample, and animal preparation facilities. The air-handling system is computer controlled within the following ranges: temperature (−15 to 45°C), humidity (10 to 100%), air flow (0.5 to 8 m/s), and photoperiod as required. In three chambers, standard air flow is 549 m³ with ability to increase air flow to 792 m³ with air changes every 4 min. The fourth chamber is specifically designed for cold environmental conditions. Only fresh air (not recycled) is utilized for each chamber. Refrigeration capacity for the laboratory is 250 tons, with a heating capacity of 250,000 BTU. Humidity is controlled by silica gel dehydrators and steam. Temperature and humidity (Figure 1) were recorded continuously by a hygrothermograph (Cole-Parmer, Chicago, IL) placed in each chamber. In this study, one chamber was specified for TN conditions, a second for hot conditions.

Injection Preparations and Administration

One hundred fourteen milligrams of bST (Sometribove; Monsanto Co., St. Louis, MO, Lot M-91-07266, CP 104301) were dissolved...
in 11.4 ml of sterile water prior to injection. Each cow was injected i.m. with 2.5 ml of either bST (equivalent to 25 mg bST) or control vehicle (sodium bicarbonate) once daily in one of four alternating locations on the cow’s hind-quarters.

Animal Management

Period 1. During period 1, cows were maintained at the University Dairy Farm under standard management. Cows were housed in open free stall housing and fed a complete ration. Ration composition and nutrient analysis are shown in Table 2 and Table 3, respectively. Water was available for ad libitum intake. Cows were milked with the herd at 0200 and 1300 h. California mastitis tests were performed immediately after milking at 1300 h. At the farm, injections were given immediately following 1300 h milking.

Period 2. Twelve cows from the summer farm experiment were transported approximately 20 km to the Brody Animal Climatology Laboratory after milking on d 30 of period 1. While in the environmental chambers, cows were housed individually in stanchions provided with rubber comfort mats. Cows were milked at 0500 and 1700 h and individually fed for ad libitum intake with the same ration composition used during period 1. Water was available for ad libitum intake from individual water cups. Injections were administered daily at 0830 h. A 14-h light period was maintained from 0500 to 1900 h using an automatic timer (Intermatic, Timers Inc., Chicago, IL). Chambers were cleaned twice daily.

Blood, Milk, and Feed Sampling and Measurements

Period 1. Blood samples were obtained by tail venipuncture once a week after milking at 1300 h and prior to injections. Blood was transported to laboratory for immediate centrifugation at 2000 rpm for 20 min, and the plasma was removed and frozen at −5°C until further hormone analyses. Milk yield was measured daily, but no milk sample was taken during this period. There was no feed and water intake measurement in period 1. Rectal temperatures were measured by inserting a thermistor probe into the rectum for 1 min, and the readings were recorded from electric digital thermometer (Fisher Scientific, St. Louis, MO) prior to blood sampling. Cows were weighed every week directly after p.m. milking.

Period 2. Blood samples were obtained by tail venipuncture at 0830 h prior to injection. Blood samples were taken on d 1, 2, 4, 6, 8, and 10. White blood cells (WBC) and hematocrit were determined immediately, as explained in sample analyses. The remaining blood was centrifuged at 2000 rpm for 20 min. Plasma was harvested and stored at −5°C for later hormone analyses. Milk yield was measured twice daily at 0500 (a.m. or night milk) and 1700 h (p.m. or day milk), and 10-ml composite samples were taken for fat, protein, and SCC determinations. Individual water intake

Sample Analyses

**Triiodothyronine**. Concentrations of plasma T₃ were determined by solid phase radioimmunoassay (Diagnostic Products, Los Angeles, CA). The standard curve for plasma T₃ ranged from 0 to 6.0 ng/ml. A sample volume of 100 μl was used. Samples ranging from 50 to 200 μl were linear to the standard curve. Recoveries from samples with known T₃ concentrations of 1.0, 2.0, and 6.0 ng were 92.9, 92.2, and 95.1%, respectively. The interassay and intraassay coefficient of variations were 6.7 and 7.7%, respectively.

**Thyroxine**. Concentrations of plasma T₄ were determined by solid phase radioimmunoassay (Diagnostic Products, Los Angeles, CA). The standard curve ranged from 0 to 240 ng/ml. Samples ranging from 15 to 30 μl were linear to the standard curve. Sample volume of 25 μl was used for assay. Recoveries from samples with known T₄ concentrations of 40, 100, 160, and 250 ng were 95.4, 97.7, 97.2, and 99.9%, respectively. The interassay and intraassay coefficient of variations were 5.6 and 4.2%, respectively.

**Cortisol**. Plasma cortisol concentrations were determined by solid phase radioimmunoassay (Diagnostic Products, Los Angeles, CA), and standards were diluted to accommodate the lower plasma cortisol concentrations. A sample volume of 50 μl was used. Recoveries from samples with known cortisol concentrations of 10, 20 and 50 ng were 93.5, 97.2, and 95.9%, respectively. The interassay and intraassay coefficient of variations were 8.4 and 7.9%, respectively.

**Prolactin**. Concentrations of plasma PRL were determined by double antibody radioimmunoassay (4). Twelve micrograms of PRL (NIADDH-oPRL-I-2[AFP-7150B]) were used for ¹²⁵I-iodination (Amersham Corporation, Arlington Heights, IL). Prolactin antiserum (NIADDK-anti-oPRL, AFP-973269) was used at a final dilution of 1:250,000. The second antibody was developed in goat anti-rabbit gamma globulin (Behring Diagnostics, La Jolla, CA). Plasma volumes of 50 μl were used in the assay. The recoveries of known PRL concentrations of 20, 40, 80, 160, and 200 ng were 97, 97.1, 96.4, 99.4, and 96.4%, respectively. The interassay and intraassay coefficient of variations were 6.1 and 4.6%, respectively.

**Insulin**. Plasma insulin was measured by radioimmunoassay procedure (Diagnostic Products Corporation, Los Angeles, CA). The standard curve ranged from 0 to 400 μIU/ml. Sample volume of 200 μl was used. Samples ranging from 150 to 300 μl were linear to the standard curve. Recoveries of known insulin concentrations of 15, 50, and 100 μIU were 93.4, 98.0, and 98.9%, respectively. The interassay and intraassay variations were 6.9 and 7.7%, respectively.

**White Blood Cells and Hematocrit**. White blood cell counts and hematocrit were determined by using a Coulter Counter (Model ZBI; Coulter Electronics, Inc., Hialeah, FL).

**Feed Analyses**. Feed energy was estimated by adiabatic bomb calorimeter. Composition of nutrients (Table 3) was analyzed by University of Missouri Nutrition Laboratory Services.

**Milk Fat, Protein, and Somatic Cell Counts**. Milk fat and protein were determined by Missouri-DHIA Testing Center (Springfield, MO).
using the multispec infrared milk analyzer (Hollywood, CA). The somatic cells were counted by milk cell Coulter Counter (Model 62; Coulter Diagnostics, Hialeah, FL). Milk energy was derived using the equations of Tyrrell and Reid (27).

Statistical Analysis. Data were analyzed with the general linear models procedures (24) for main effects of bST, temperature, and their respective interactions by using cow(treatment x group) as the error term. Data from period 1 were analyzed only for the bST main effects. Least square means were compared using the F test.

RESULTS

Diurnal cycles of temperature and humidity during the course of the experiment were averaged and are presented in Figure 1. The farm summer conditions represent typical hot and humid summer conditions in Missouri. The temperature and humidity ranges are presented in Table 1. The laboratory heat conditions were similar to conditions at the farm during July, except that the night temperatures were set at 24°C. Figure 2 illustrates a diurnal cycle of the temperature-humidity index (THI) during the experiment. The upper critical THI for lactating Holstein cows (11) is also indicated.

The average rectal temperatures and THI are shown (Figure 3) to provide an easy assessment of the stress effects of environment on the cows. Temperature and humidity were very high during the last few days of the farm conditions, which created an average THI near 80 that was reflected in increased body temperatures.
The profile of milk yields during the course of the experiment is presented in Figure 4. For comparison, average milk yields prior to the treatment are also shown. Milk yield increased within 2 to 3 d from approximately 29 to 38 kg/d by d 7 under farm conditions following initiation of bST treatment. The average daily milk yields under TN or heat are also presented in Figure 4. Bovine somatotropin increased \( P < .01 \) milk yields, 3.5% FCM, milk fat, and milk energy secretion (Table 4). Somatotropin increased milk yields by 21 (28.8 vs. 34.9 kg), 32 (24.8 vs. 32.9 kg), and 35% (21.0 vs. 28.3 kg) under farm, laboratory TN, and heat, respectively. After correction to 3.5% fat, the increase in 3.5% FCM due to bST treatment was even higher by 42 (21.4 vs. 30.4 kg) and 48% (17.9 vs. 26.5 kg) during laboratory TN and heat conditions, respectively. Milk fat increased by 15 (2.6 vs. 3.0%) and 19% (2.6 vs. 3.1%) under bST treatment during laboratory TN and heat conditions, respectively. Somatotropin treatment tended to increase \( P = .06 \) milk protein under both laboratory TN and heat conditions. Overall milk energy secretion was increased by 40 (14.8 vs. 20.8 Mcal) and 48% (12.5 vs. 18.5 Mcal) due to bST treatment at TN and heat conditions, respectively. High temperature reduced \( P < .05 \) milk yield of control and bST-treated cows. Milk energy secretion declined similarly for both control and bST cows during heat environmental conditions. There were no interaction effects between bST treatment and temperature on milk yields.

Somatotropin treatment increased \( P < .01 \) DM and energy intake. Treatment with bST increased DMI by 16 (18.5 vs. 21.5 kg) and 19% (14.5 vs. 17.2 kg) under laboratory TN and heat conditions, respectively. Environmental heat lowered \( P < .01 \) DMI and energy intake. The decline in energy intake under the hot environment was similar for both control and bST-treated cows (Table 4).

Bovine somatotropin increased \( P < .01 \) the efficiency of feed energy utilization to milk energy secretion. Each megacalorie of milk energy secreted required 5.7 and 4.6 and 5.2 and 4.1 Mcal of feed energy for both control and

![Figure 4](https://example.com/figure4.png)

Figure 4. Average daily milk yields for control (○) and bST-treated (●) cows prior to experiment and during summer farm, laboratory thermoneutral (TN), and laboratory heat conditions. Initiation of bST injection (June 29, 1987) is indicated by arrow.

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<th>Parameter</th>
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<th>bST</th>
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<td>Milk energy (MB), Mcal/d</td>
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*Significant due to heat conditions ($P < .05$).

$^A$,$B$ Significant due to bST treatment ($P < .05$).

$^1$Preceded by means and SE of 6 cows for each treatment.

$^2$No milk and feed samples analyses were conducted in the farm period. ND = not determined.
bST-treated cows under TN and heat, respectively. Heat conditions decreased \( (P < .01) \) the ratio for both control and bST-treated cows.

Somatotropin treatment did not increase water intake \( (P > .05) \); however, heat conditions tended to increase water intake \( (P = .07) \). There was no significant effect of bST treatment or of the hot environment on the average body weights. There was no significant effect of bST on body temperature under either TN or heat conditions; however, heat conditions increased \( (P < .01) \) this parameter. Somatic cell counts were not affected \( (P > .05) \) by the bST treatment or the environmental conditions.

The day and night milk yields and feed and water intake were based on the afternoon and morning measures, respectively. Milk yields were not different \( (P > .05) \) between day or night under heat, yet milk yields tended to be higher during the cooler night than during the warmer day conditions for both treatments (Figure 5). Feed intake was higher \( (P < .01) \) during night than day conditions. Water intake was lower \( (P < .01) \) during the night for both control and bST-treated cows under both TN and heat conditions. The cows drank more and ate less during the day even under TN conditions. Rectal temperatures were greater \( (P < .01) \) during the day only under heat conditions.

Bovine somatotropin decreased plasma concentrations of T3 \( (P < .05) \) and cortisol \( (P < .01) \). There was no significant effect of bST treatment on plasma T4 under laboratory conditions. Laboratory heat reduced \( (P < .01) \) both plasma T3 and T4. There was no effect of temperature on plasma cortisol concentrations.

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**Figure 5.** Day (D, p.m. measures) versus night (N, a.m. measures) milk yields, water intake, feed intake, and rectal temperatures for control (○) and bST-treated (■) cows during laboratory thermoneutral (TN) and heat conditions.
The bST treatment did not affect \((P > .05)\) plasma PRL concentrations during any period (Table 5). Laboratory heat and summer farm heat tended to increase \((P = .06)\) plasma PRL concentrations. Bovine somatotropin did not affect plasma insulin concentrations under farm summer conditions. Plasma insulin concentrations at the farm were generally lower than those found under laboratory conditions. Plasma insulin tended to increase \((P = .05)\) 30 d after initiation of bST injection. No significant effects of temperature on plasma insulin concentrations were found.

White blood cell counts and hematocrit were lower \((P < .01)\) for bST-treated cows than for control cows during farm summer conditions, laboratory TN, and heat conditions. Hot temperature decreased WBC and hematocrit \((P < .05)\).

**DISCUSSION**

Daily administration of bST to lactating dairy cows increased milk yields 21, 32, and 35% over those of the control cows during summer farm conditions, laboratory TN, and heat conditions, respectively. The increased milk yields in bST-treated cows in the TN environment were consistent with other reports (2, 3, 5). In this study, the increased milk yields in response to bST treatment under hot conditions were greater than those reported previously (18, 25, 30). Staples et al. (25) reported a 9.3% increase in milk yields in cows receiving purified bST under Florida summer heat. Zoamae et al. (30) reported no change in milk yields, but there was a 9.4% increase in 3.5% FCM in cows receiving purified bST under shade or no shade hot environmental conditions. A report from the Missouri laboratory (18) using purified bST showed a similar response. The greater responses in milk yield to bST treatment in the cyclic hot Missouri laboratory conditions were partly due to the bST dosage and to the duration of the bST treatment. A study with a longer period of bST injection under warm season showed a higher response in milk production (7). Of particular interest in our studies were the responses of both control and bST-treated cows during the last week of the summer trial when THI dramatically increased near 80. In response to these extreme conditions, milk yield dropped
dramatically in all cows; however, bST-treated cows still produced more milk than did the control cows. Thus, based on milk yield and supported by rectal temperature data, we conclude that cows treated with bST maintained the increased milk production attributed to bST despite thermoregulatory demands imposed by the hot environment.

The increase in milk fat observed during TN has been reported by others (8, 28). Increases in milk fat in bST-treated cows during heat were also observed by Mohammed and Johnson (18). However, other investigators reported no change in milk fat percentage even though there was an increase in total fat yield (7, 25, 30). Milk protein did not significantly increase in this study, unlike other reports (3, 14). The increase in milk fat in this study was possibly due to the increased pool of precursors for fat synthesis due to increased feed intake as suggested by Chilliard (5). The variability in milk composition response to bST treatment under TN conditions was also observed (5). Somatic cell counts in the milk did not significantly change as reported by others (5, 7). In contrast, bST-treated cows under hot conditions had lower SCC than control cows.

The increases in feed intake have been reported (2) in cows under long-term bST treatment under TN conditions. In contrast, long-term (7) or short-term experiments under hot climate or heat stress (18, 25, 30) did not show significant increases in feed intake due to bST. Despite the observed higher feed intake reported in this study, the ratio of milk yield to feed intake was much higher for bST-treated cows than for control cows, with this ratio being relatively greater under hot conditions. These data and others (7, 18, 25, 30) support the homeothermic concept (1) that bST increases the diversion of food nutrients for milk synthesis in the lactating dairy cow under heat conditions despite the thermal burden imposed by the heat stress. Possibly, the increase in mammary blood flow (6) could make a significant contribution to the availability of milk precursors, thus leading to enhanced milk synthesis.

The mechanism by which bST increases milk production has not been clearly delineated; however, the data here suggest that the mechanism remains the same regardless of environment. The increased milk yields of bST-treated cows remained greater than the milk yields of the control cows, even when the THI rose to 80. Furthermore, treatment with bST increased heat production in lactating cows in both TN and hot environments; however, the cows increased evaporative heat loss, thereby maintaining homeothermy similar to the untreated cows (16). The cumulative data suggest that bST acts as a partitioning agent for production without disturbing thermal balance. Most importantly, however, the partitioning of energy intake for milk production and the thermoregulatory functions appear to act in concert, and no interaction occurs under demands of environmental stress that produces thermal imbalance.

Research has shown that cows treated with bST have an increase in metabolism (26). Kronfeld (13) hypothesized that bST supplementation of lactating cows will cause thermal imbalance as shown by other reports (7, 25, 30). However, this study, along with a previous report (18) that was also conducted under short-term exposures to heat stress, showed that the bST-treated cows maintained thermal balance similar to that of control cows as indicated by body temperatures. These results were confirmed by a report (16) that bST-treated cows appear to dissipate more heat by evaporation in order to maintain thermal balance.

The natural fluctuating conditions during the farm summer conditions and the simulated cyclic conditions of the laboratory heat allowed the cows to respond to the continuous changes throughout the day. To provide a greater understanding of these cyclic changes, several performance parameters were illustrated by comparisons between day and night. Feed intake was greater during the night for both control and bST-treated cows. Body temperature and water intake were much higher during the day for both control and bST-treated cows. Despite the differences in those parameters, no differences between day and night were found in milk yield. Such results present several interesting questions about cyclic relationships between energy utilization, nutrient metabolism, and lactation that merit further investigation.

Endocrine changes associated with bST treatment varied in previous reports (20, 30) and in this study. Reduced plasma T3 concentrations under bST treatment were probably a consequence of increased heat production (16). Such effects were not found on plasma T4.
concentrations. In contrast, heat conditions significantly reduced both plasma T₃ and T₄ concentrations, which resulted in a higher T₄:T₃ ratio. The greater depression of plasma T₃ versus plasma T₄ is expected as a negative feedback of increased heat production on bST treatment or increased heat gain under heat stress, because T₃ is the hormone that directly induces calorigenesis. Such a reduction in the presence of calorigenic effects of T₃ may assist the animals to control body temperature better to maintain homeothermy.

Plasma cortisol concentrations were lowered due to bST treatment at all temperature conditions. Because increased glucose production may cause a decrease in plasma cortisol, these reduced concentrations of cortisol were probably due to glucose-sparing effect of bST (29). Others have shown that bST has no apparent effect on the serum concentration of cortisol (21, 22).

Bovine somatotropin did not increase plasma PRL during TN or heat, which agrees with previous reports by Mohammed and Johnson (18) and Peel et al. (21, 22). In contrast to results reported by Igono et al. (10) and Mohammed and Johnson (18), environmental heat in this study did not increase plasma PRL (P < .06). This is probably due to the short duration of the heat exposure that was not long enough to show a significant increase in PRL. Because PRL is a reliable indicator of thermal stress (10), it is important to observe that the bST-treated cows under heat had similar PRL values to the control cows, suggesting no additional increased thermal stress in the bST-treated cows. The similarities in plasma PRL concentrations in control and bST-treated cows, along with the similarities in body temperatures, add further support that bST-treated cows are able to maintain the relatively greater production under hot environmental conditions with no more thermal stress than controls.

Plasma insulin was moderately increased in bST cows at both laboratory TN and heat conditions. Some investigators reported no apparent changes in serum insulin concentrations (21, 22, 30), although an increase has been found in some studies (9, 17). The significantly lower concentrations for all cows at the farm were probably due to sampling time with respect to feeding as suspected by Prosser and Mepharm (23). Cows in the laboratory were fed for ad libitum intake with fresh feed offered twice daily. At the farm, cows were group fed, with fresh feed offered after milking. Blood sampling at the farm occurred prior to milking and at the laboratory after milking and feeding.

In summary, bST administration markedly increased milk yield in lactating dairy cows regardless of the limitations of hot environment on production. Bovine somatotropin, however, still increased feed intake and feed efficiency along with increased milk yields with no significant changes in body weight. The significant findings of this study were that bST treatment induced increases in feed intake and milk yields under a hot environment without any significant thermal stress effects.

REFERENCES