RESEARCH BULLETIN 1061 SEPTEMBER 1988

UNIVERSITY OF MISSOURI-COLUMBIA
COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
ROGER MITCHELL, DIRECTOR

Environmental Physiology
and Shelter Engineering
With Special Reference to Domestic Animals

LXXVI.

Short-term Heat Acclimation Effects on Hormonal Profile of Lactating Cows

Part II

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CONTENTS

Abstract

The three-day exposures to environmental heat significantly lowered plasma T_4, T_3 and milk T_3. The stage of lactation effects were not significant, however the levels of production did not vary greatly with stage. All of these animals had potential for the same general level of milk yield (greater than 20 kg/day). Plasma TSH and growth hormone (GH) significantly declined with environmental heat and stage of lactation. Plasma prolactin increased due to heat and declined with advancing stage.

In contrast, the energy metabolism and cortisol significantly increased with stage of lactation. Though the differences were not great, this may have been due to a trend with each stage for higher ad libitum feed intake. Metabolism declined significantly with heat, though cortisol and insulin increases are not significant. Frequency distribution curves for these data provide an assessment of the ranges of values at each stage and temperature condition.

The data demonstrate the short-term acclimation effects on selected temperature regulatory, metabolic and hormonal functions.

Introduction

Moderate environment heat exposure initiates thermoregulatory (Johnson, 1980; Magdub et al., 1981) hormonal (Magdub, et al., 1981; Mitra et al., 1972) and metabolic (Johnson, 1980) adjustments in cattle (Baccari et al., 1982; Collier et al., 1982). These and other acclimation responses measured over a period of a few days encompass the acute phases of the overall processes of acclimation (Prosser, 1965; Selye, 1955) to environmental heat (Johnson et al., 1967; Kibler et al., 1965). Among other functions, the pituitary, thyroid and adrenal glands are believed to play a role in the thermoregulatory and metabolic functions of lactating dairy cattle as they acclimate to moderate levels of environmental heat.

Varied heat exposure data are available on TSH function in animals, ranging from no significant effects on cattle (Konno and Morikawa, 1982; Hurley et al., 1981; Schams, et al., 1980) and humans (Weeke and Gundersen, 1983), to significant depression on humans (Koono and Morikawa, 1982; O'Malley et al., 1980) and mice (Mueller et al., 1974). GH is known to increase with acute heat stress on mice (Mueller et al., 1974) and cattle (Mitra et al., 1972), but under prolonged environmental heat data range from no effect (Schams et al., 1980; Tucker and Wettemann, 1976) to modest declines in cattle (Mitra, 1972).

Prolactin readily responds to moderate environmental heat in cattle (Mertsching, 1981; Schams et al., 1980; Tucker and Wettermann, 1976; Wettermann, et al., 1982) and in rats (Deeter and Mueller, 1981), as well as other stressors. The heat response in prolactin is different than other pituitary hormones in that an acute elevation (Mertsching, 1981) persists even during prolonged acclimation for moderate heat (Johnson, 1980).

It is well established that exposure to moderate heat of sufficient intensity to significantly raise deep body temperature (Baccari et al., 1983; Johnson, 1980; Koono, 1980; Magdub et al., 1981; Pratt and Wetterman, 1985), lowers plasma T_3 and T_4, even though some reports show T_4 and TSH to remain near normal (Koono, 1980).

Insulin changes due to heat are not clear (Mertsching, 1981). Usually no effects are observed even though voluntary decline in feed intake occurs during heat

exposure (Johnson, 1980; Magdub et al., 1981). Cortisol does not consistently increase when animals are exposed to moderate heat (Johnson, 1980). Acute heat will significantly increase cortisol (Abilay et al., 1975), whereas prolonged heat is accompanied by slight declines in plasma level of cortisol (Adeyemo et al., 1981; Alvarez and Johnson, 1973; Ingraham et al., 1979; Marple et al., 1972; Rhynes and Ewing, 1973; Vanjonack and Johnson, 1975a). Transportation stress will readily increase plasma cortisol of livestock for brief time periods (Crookshank et al., 1979).

Energy metabolism declines with moderate heat exposure over a period of two to three days (Johnson, 1980) and will continue as feed intake declines (Johnson and Hahn, 1981), while body temperatures remain elevated (Johnson, 1982). Milk yields decline as the above functions change (Johnson and Hahn, 1981).

Generally, homeothermic adjustments to moderate heat initiate a complex of changes in energy dynamics, heat regulation and neuroendocrine functions to try to restore "normal" body temperature. In cattle, these above changes will in turn alter the processes of milk synthesis resulting in lower yield (Johnson, 1980).

Upon exposure to environmental heat stress, such as a moderate three-day exposure, the hypothalamo-hypophysial axis that controls primarily the pituitary, thyroid and adrenocortical hormones and the sympathetico-adrenomedularry system becomes activated as a part of the animal's thermoregulatory adjustments (Johnson, 1980).

In turn, heat production and heat loss functions act to restore thermal balance, including reductions in feed intake and growth or milk yields. Some of the hormonal changes of the hypophysial axis will be related to the simultaneous changes in body temperature and milk yields of higher lactating cows.

Within a breed, individual animals vary in many of these above responses to the heat stressor. Stages of lactation or production level are also known to alter these responses to environmental heat. In most of these above investigations, greater numbers of animals were necessary to separate stages of lactation or assessment of individual animal heat tolerance.

Further, a relatively large number of experimental animals at a similar stage of lactation and production level exposed to a standardized heat stressor would permit a characterization of the hormonal responses to a short-term stressor and also characterize the initial heat recovery characteristics.

The measurement of a number of hormones known to be associated with environmental stress and lactation can permit an assessment of the combined hormonal responses or a profile of hormonal acclimation in lactating cows subjected to heat.

The purpose of this study was to relate the various simultaneous endocrine and metabolic changes on 51 lactating cows, each exposed at three stages of their lactation to a relatively short-term (three days) moderate heat exposure (32 degrees Celsius). The basal (pre-stress), acclimation responses during heat stress and post stress have not been previously reported on early, mid and late-stage lactating cows.

Further, the objective was to observe an array of endocrine functions during exposure to heat and post-heat on higher yielding cows (22 kg/day) and to measure the influence of stage of lactation. These hormonal functions were related to changes in rectal temperature, energy metabolism and milk yields (Johnson et al., 1987). Lastly, the effects of heat and stage of lactation on distribution curves of hormonal and related functional changes will be described.

Procedures and Methods

Hormone Assays

Plasma Thyroxine: Plasma T_4 (thyroxine) was measured by the radioimmunoassay, solid phase procedure from Micromedic Systems, Horham, PA 19044. Standard curves ranged from 0-320 ng/ml. Sample volumes of 0-10 ul were tested for linearity and found to be parallel to standard curve. Sample volumes of two ul (microliters) were used in assays. Cold recoveries of 40 and 80 ng/ml were 96.9 and 98.7%, respectively. The inter- and intra-assay variations were 6.8 and 4.6%, respectively, and nonspecific binding averaged 2.5%.

Plasma Triiodothyronine: Plasma T_3 (triiodothyronine) in plasma and milk was analyzed by radioimmunoassay, solid phase technique from Micromedic Systems, Horsham, PA. The standard curve ranged from 0-6 ng/ml. Sample volumes in range of 50 to 100 µl were linear to standard curve. Sample volumes of 100 µl were used in assays. Recoveries of 1.0 ng and 2.0 ng were 94.3 and 95.8%, respectively. The inter- and intra-assay variations were 6.3 and 5.4%, respectively. The nonspecific binding averaged 2.7%.

Milk Triiodothyronine: The milk was prepared for T_3 assay by following this extraction procedure: 250 µl of milk was added to 500 µl acidified ethanol (pH = 2.0), mixed thoroughly on a Kraft shaker (Kraft Apparatus, Inc., Menelo, TN) for ten minutes, then centrifuged at 3000 rpm for 40 minutes. Supernatant was decanted into tubes (12 X 75 mm), pH adjusted to 8.6 with a single drop of ammonium hydroxide and vortexed 10 seconds. Tubes were placed in waterbath with individual air streams and evaporated to dryness in approximately 30 minutes (37C). When tubes were completely dry, 250 µl assay buffer (barbital buffer pH 28.6) was added. One hundred microliters of extract was assayed by the same T_3 solid-phase technique, used for plasma. The recovery of labeled hormone from the extraction was 72.6%. The cold recoveries with milk pools of 1.0 ng/ml and 2.0 ng/ml were 90.9 and 87.4%, respectively.

Plasma TSH: The radioimmunoassay procedure for thyroid stimulating hormone was adapted from Diagnostic Products Corp., Los Angeles, CA. Two hundred microliters of plasma and 100 µl of TSH antiserum were added to 12 X 75 mm assay tubes, vortexed for ten seconds and incubated at 37 degrees Celsius for two hours. One hundred microliters of I^{125} TSH was added to all tubes, vortexed for ten seconds and incubated at 37 degrees Celsius for 60 minutes. One hundred microliters of second antibody (goat anti-rabbit gamma-globulin) was added to all tubes and incubated at 37 degrees Celsius for 15 minutes. Following this, 2.0 ml of cold 4% polyethylene glycol (PEG) was added to each tube and centrifuged at 3000 rpm for 30 minutes. The supernatant was aspirated and the tubes with precipitate were counted for one minute on Beckman gamma counter. The results were calculated on log logit curve and expressed as µIU/ml. Range of standard curve was 0-40 µIU/ml. Plasma sample volumes of 100-400 µl tested for linearity were parallel to standard curve within this range. The size of sample used in all assays was 200 µl. The cold recoveries of pools at 5 µIU/ml, 10 µIU/ml and 20 µIU/ml were 84.6, 88.4 and 85.2%, respectively. The nonspecific binding averaged 7.3%. The inter- and intra-assay variations were 5.0 and 9.3%, respectively. There was no cross-reactivity with LH.

Plasma Prolactin: Ten liter (0.5 µg/µl) of bovine prolactin was added to 1 mCi

of I^{125} and reacted with 10 µl chloramine T (1 µg/µl) for 30 seconds. The reaction was stopped with 10 µl (1 µg/µl) Na metabisulfite. The reaction mixture was added to sephadex G-50 column and diluted with .01M phosphate buffer saline (PBS) at ph 7.6. Fractions from first radioactive peak representing I^{125} PRL were pooled. Prior to assay, this fraction was purified on sephadex G-75 and diluted in PBS with (2.5% bovine serum albumin [BSA]) to provide 10,000 CPM per 100 µl. Iodination procedure was adapted from Becker (1985). Prolactin assay procedure was modified from Abbott Laboratories, North Chicago, IL.

To each assay tube (12 X 75 mm) 50 µl of plasma and 100 µl of prolactin I^{125} and 100 µl of first antibody (1:175,000). Antibody was kindly provided by Dr. W.R. Butler, Cornell University, NY. Tubes were vortexed for ten seconds, incubated at room temperature for 18 hours, then 100 µl of second antibody (anti-rabbit sheep gamma globulin, 1:24 dilution) was added and incubated for six hours. Cold saline (1 ml) was added, vortexed, and centrifuged for 3000 rpm for 30 minutes. Tubes were decanted and counted for one minute in Beckman Gamma Counter.

The sample volumes were tested for linearity and were found parallel to the standard curve in the range of 50-200 µl. The recovery from samples of 5.0 and 40.0 ng/ml were 94.6 and 89.9%, respectively. The inter- and intra- assay variations were 8.7 and 8.1%, respectively.

Plasma Growth Hormone: Following the procedure of Davis et al. (1979) 5 µg of bovine growth hormone (GH) (0.5 µg/µl) and 1 mCi I^{125} was reacted 30 seconds with 4 ng/ml chloramine T. The reaction was stopped by adding 100 µl sodium metabisulfite (2.5 mg/ml of sodium metabisulfite in .01 M phosphate buffer). Contents were added to sephadex column G-50 and diluted with .01M phosphate buffer (pH = 7.5). The labeled hormone bGH I^{125} was purifed on sephadex G-100 column. One hundred microliters of plasma and 100 µl of first antibody (1:400), kindly provided by Dr. S.L. Davis, were added to a 12 X 75 mm tube, incubated 24 hours in a cold room (4 degrees Celsius). Then 100 ul of GH^{125} was added, vortexed and incubated 24 hours in a cold room. Following this, 100 µl of second antibody (goat-anti-rabbit; 1:100) was added and incubated 72 hours at 4 degrees Celsius. Cold phosphate buffer (.01M) was added to tubes to separate bound from free, centrifuged 30 minutes at 3000 rpm, decanted and counted in a Beckman Counter. Standard curves were in the range of 0-80 ng/ml. Sample volumes in range of 50 to 200 µl were linear in range of standard curve. Inter- and intra-assay variability was 18 and 13%, respectively.

Plasma Cortisol: Plasma cortisol was analyzed by the solid phase procedure of Micromedic Systems, Horhsam, PA. Twenty microliters of plasma sample and 1000 µl of cortisol I^{125} was added to antibody-coated tubes, vortexed and then incubated at room temperature for 90 minutes. Samples were decanted and counted in a Beckman Gamma Counter. The standard curve ranged from 2.5 to 40 ng/ml. Samples ranging in volume from 50 µl to 200 µl were linear to standard curve. Recoveries of 10 and 20 ng/ml in pool plasma samples were 94.7 and 8.9%, respectively.

Milk Cortisol: The procedure of Butler and DesBordes (1980) was used for extraction of the milk samples and assayed by the solid phase procedure of Micromedic Systems. Two hundred microliters of milk, extracted in 4 ml of methylane chloride, were shaken vigorously 15 minutes in a shaker (Kraft Apparatus, Mineola, NY) and centrifuged at 3000 rpm, 4 degrees Celsius for 10 minutes.

Tubes are then placed in cold room at -20C for 30 minutes to freeze the milk fraction and then decant the methylene chloride fraction. Two ml of hexane and 1 ml of 70% methanol was used to further remove lipids. The lipid fraction was removed and liquid evaporated to dryness in a water bath. One hundred microliters of phosphate buffer (pH, 7.5) was used to restore volume. Extraction recoveries for 0.5, 2.5 and 5.0 ng/ml were 87.1, 83.3 and 85.3%, respectively. Two hundred microliters of extract was used for radioimmunoassay.

Plasma Insulin: Insulin in plasma was measured by the solid phase system of Micromedic Systems, Horsham, PA 19044. To each antibody-coated assay tube (12 X 75 mm) 900 ul of I^{125} insulin plus 100 µl of plasma was added. The tubes were vortexed for ten seconds and incubated for 18 hours at room temperature. The tubes were decanted and washed two times with distilled water and counted on the Beckman Counter for one minute. Sample volumes were tested for linearity and found to be parallel to standard curve in the range of 100 to 300 µIU. The recovery from samples of 20 and 80 µIU/ml were 83.1 and 82.4%, respectively. The inter- and intra-assay variations were 12.2 and 10.4%, respectively.

Plasma and Milk Sampling: All of the hormone values are based on the analysis of twice daily samples (1000 and 2200 hours) for three days at thermoneutral (TN_1; 18 degrees Celsius, 50%rh), three days at heat (H) and four days post-heat (TN_2; 32 degrees Celsius, 50%rh). A total of twice daily samples for ten days times 51 cows for each stage of lactation provided approximately 1500 samples for hormones at each stage of lactation. Milk was sampled at each milking place in plastic tubes in triplicate and frozen until analyzed. Milk T_3, milk cortisol and plasma cortisol, insulin and TSH were measured in a lesser number of samples (approximately 500 samples), as will be indicated in the data section.

Experimental Design: The major objective of the experiment was to obtain heat-induced and post-heat responses and compensatory measures to a short-term heat stressor on a large number of cows at early, mid and late stages of lactation. Six animals at midstage were used as a partial sham control for time in laboratory and heat exposure. Animals at each of the stages were tested at the various seasons of the year. The individual sensitivity or tolerance to the heat stress, recovery time and magnitude of response were also of special interest. The data were sorted into early, mid and late stage of lactation categories. Mean daily values at thermoneutral (TN_1, heat exposure (H) and post-heat stress (TN_2) were obtained for each cow at the three stages. Mean values for each stage at TN_1, heat (H) and TN_2 were tested for significance to compare the time changes or acclimation trends following heat and post-heat. The daily mean values for each stage following heat exposure (Day 3) through Day 10 were compared.

Statistical Analysis: Sample mean physiological values were compared by stage of lactation and day of trial (environmental treatments were imposed as day differences). Analysis of variance procedures with sources for "stage of lactation", "day of test" and interaction were computed and least squares means generated. Sample means were compared by day and stage to the production of days (1 - 3; TN_1). This permitted an evaluation of the effects of heat stress (Days 4-6) and recovery during the post-thermal neutral period (Days 6-10).

Data and Discussion

The data presented describes the metabolic and selected endocrine responses of

51 lactating Holstein cows at three stages of lactation. These data are regarded as relatively short-term exposure (three days) to one moderate heat condition (32 degrees Celsius, 50%rh) and the post-heat responses. The differences of all cows' average values for Day 3 of TN were compared to subsequent days to assess trends during the three-day heat exposure and the four days post-heat stress. The average of all days at each temperature treatment, TN, heat and TN_2 were presented to show the overall effects.

Plasma T_4, T_3, Milk T_3: The ANOVA Table 1 indicates the significance of stage of lactation effect for T_4 ($P<.01$), and plasma T_3 ($P<.06$). Milk T_3 was only measured in the early stage of lactation. The temperature effects were highly significant for T_4 and significant at .06 for T_3, though the stage-temperature interaction was not significant.

Table 2 presents the average daily plasma T_4 and T_3 values for all cows at each of the three stages of lactation for each day of the pre-heat (TN_1), heat and post-heat (TN_2) treatments. Milk T_3 is also presented. The last day of TN_1 on Day 3 prior to heat exposure was compared to Days 4, 5 and 6 of heat and Days 7, 8, 9 and 10 post-heat to indicate the significance for the daily changes. Within one day after heat treatment (Day 4), plasma T_4 declined significantly for all stages. Lowest plasma T_4 was on Day 6 with values ranging from 24 to 29 ng/ml and recovered significantly on Day 8 after animals were returned to the TN_2 conditions.

Plasma T_3 responded similarly to plasma T_4 with first day of heat (Day 4) being different than Day 3 of TN_1. Following heat, the recovery was significant by second day of TN_2 (Day 8). Of major significance was the similarity of milk T_3 and plasma T_3 concentrations. The milk concentrations declined similarly to the plasma concentrations during heat with recovery during post-heat TN_2. This lower excretion in the milk (associated with a lower plasma T_3) suggested a reduced T_3 synthesis by the animal. Some post-heat (TN_2) compensation in the milk T_3 is evidenced by higher average levels on Days 9 and 10 than TN_1. With the exception of early-stage cows, T_4 levels, plasma T_3 and T_4 did not show evidence of post-heat "over shoot" or compensation (Baccari et al., 1983), which was due likely to the relatively short-time three-day heat exposure. The average TN_1, heat and TN_2 period values for plasma T_4, T_3 and milk T_3 are shown in Table 3 for each stage of lactation. Heat values for T_4 were significantly lower than TN_1 and TN_2 for all stages of lactation as indicated by superscripts a, b,c. As indicated by superscripts a, b, c, the stage effects were not different though early stage tended to be higher than midstage as observed in earlier field studies (Vanjonack and Johnson, 1975a).

Plasma T^3 changes in response to heat and the post-heat effects were similar. However, the late-stage cows during TN_2 were not different than the heat values. As with T_4, the stage of lactation was not significant, though mid and late stages tended to be higher. Milk T_3 declined significantly during heat and returned to TN_1 levels after heat exposure during early stage of lactation data.

To summarize and illustrate the maximal acclimation changes and any differences in stage of lactation due to the three-day heat exposure, a comparison of average Day 3 versus Day 6 for each T_4 and T_3 at stage of lactation is shown in Figure 1. Though later stages tended to be higher, the stage effect was not significant though the heat effect was highly significant for both plasma T_4 and T_3 and milk. Heat significantly reduced plasma T_3 more in early and midstages than late stage.

Plasma TSH: The analysis of variance for plasma TSH showed the stage,

temperature and interaction to be significant (P=.004; Table 4). A comparison of daily values (Table 5) of TSH during TN_1, heat and TN_2 showed declines by second or third day (Days 5 and 6). Following heat exposure early and midstages did not regain TN_1 values. The late-stage animals regained TN_1 levels by Day 9 and 10 of TN_2. The similarity of changes in plasma TSH, T_4, T_3 and milk T_3 during heat exposure and recovery strongly indicate a decline in synthesis of thyroid hormones. For some unexplained reason, the changes in plasma TSH by stage are more pronounced ($P<.05$) than the changes in either T_4 or T_3. Most earlier evidence shows T_4 and T_3 to be slightly lower in early lactation cows.

Plasma GH and Prolactin: The effects of environmental heat and stage of lactation on GH and prolactin are also shown in Tables 4 and 5. The stage of lactation and temperature effect is significant for both GH and prolactin (Table 4). Table 5 shows GH to be higher in early-stage lactation with declines due to heat at all stages following heat exposure on Day 4. Heat significantly increased prolactin. Following heat, prolactin returned to preheat levels by Day 8.

Table 6 presents the mean levels for the average of all days at TN_1, heat and TN_2 for a comparison of stage of lactation and temperature on GH and prolactin. Growth hormone was significantly higher during the early stage at all temperature periods that the heat effects were significant at all stages. Prolactin for some unexplained reason showed midstage cows to be lower at all temperature conditions than the early or late stages, though the temperature effect was significant.

To summarize and illustrate the maximal acclimation changes and any differences in stage of lactation for plasma TSH, GH and prolactin due to the three-day heat exposure, a comparison of Day 3 versus Day 6 is shown in Figure 2. The general trend in hormone decline with advancing stage is rather clear, though not significant in all cases as discussed previously. The stage effects during heat were not so apparent in Day 3 versus Day 6, especially for prolactin.

Presumably, elevated core body temperatures are responsible for all of these hormonal and other production declines via effects directly on the secretion or indirectly via feed intake and energy metabolism changes. The casual interrelations between these functions have not been studied, with the exception of forced feeding studies to attempt to maintain milk yields and feed intake (Johnson et al., 1962) during heat stress and more recently, the use of exogenous doses of T_3 (Riviera-Lopez, 1982) and exogenous GH (Mohammed, 1985) on lactating cows subjected to heat stress. Triiodothyronine at doses used did not increase milk yields or feed intake during heat exposure, whereas GH did increase milk yield.

Growth hormone appeared to offer the most benefit in terms of milk yield. TSH has not been utilized or tested in such studies to increase milk yields, however, Vanjonack and Johnson (1975b) injected TRH in lactating cows with minimal effects on milk yield.

Insulin, Cortisol and Energy Metabolism: Table 7 summarizes the analysis of variance for plasma insulin, cortisol, milk cortisol and energy metabolism. The stage of lactation was significant for all measures. The temperature and interaction effect was significant for energy metabolism, but not for insulin or cortisol.

Table 8 provides the daily mean values for each TN_1, heat and TN_2 periods for these measures. Insulin values for early and midstages tended to decline during heat exposure, whereas, the late-stage values were the highest with no indication of

decline due to temperature. In fact, the values at all stages during post-heat stress (TN^2) were higher than during heat. This may have been due to a temporarily lowered voluntary intake of feed during heat. Neither plasma nor milk cortisol (Table 8) significantly declined due to heat. Energy metabolism declined during the first day of heat (Table 8) for early stage and continued through the third day of heat (Day 6) for the mid and late stages. Post-heat recovery was rather rapid with TN_2 values after 48 hours (Day 8), being similar to TN_1 (Day 3) for all stages. The stage of lactation effects were pronounced for insulin. The late stage being much higher than early or midstages (Table 9), cortisol was significantly lower in early stages than mid or late at all temperature conditions. Metabolism progressively increased with advancing lactation. The only ready explanation is the increasing feed intake and body weight during mid and late stages (see Johnson et al., 1980, and Part I data of this bulletin).

Figure 3 again summarizes the maximal temperature effects by the average comparison of Day 3 and 6 values. The stage effect was very apparent for cortisol and insulin, but with no significant changes due to heat. Energy metabolism did not display a significant trend with stage, but a very consistent temperature depression.

Frequency Distribution: As described previously in the procedure section, plasma T_4, T_3, GH, and metabolism was measured daily on all cows at each stage of lactation. These selected data permitted an estimation of the frequency distribution of these measured values for all experimental animals at each temperature treatment for all three stages of lactation. The individual cow means values for the TN^1 period are represented by Day 3, and for the heat period Day 6 was selected since this provided the maximal time (three days) for adjustment to the temperature stress at each stage of lactation. Figure 4 describes the distribution curves for plasma T_4 and T_3 at early, mid and late stage of lactation for cows at TN, and heat. The significance of these curves is the shift of the distribution curves to the lower levels at 32 degrees Celsius for each stage. The high temperature shifts the curves to the left (lower values). Figure 5 also shows the mean distribution values for GH and metabolism to shift to the lower levels when exposed to heat. Prolactin shifts to the right (higher mean distribution). The range of values for prolactin is much greater during heat exposure than at 18 degrees Celsius.

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Table 1. Analysis of variance for plasma T_4, T_3 and milk T_3.

	Plasma T_4				Plasma T_3			Milk T_3			
	D.F.	Sum of Squares	F	P	Sum of Squares	F	P	D.F.	Sum of Squares	F	P
Stage	2	1488.83	7.59	.0006	3.87	2.78	.0623	0	0	-	-
Temperature	2	18624.40	94.90	.0001	16.94	12.17	.0001	2	1.98	6.25	.0034
Stage x Temp	4	260.49	.66	.6175	2.37	.85	.4926	0	0	-	-

Table 2. Effect of stage of lactation and temperature on daily mean values of plasma T_4, T_3 and milk T_3.

		Plasma T_4 ng/ml						Plasma T_3 ng/ml						Milk T_3 ng/ml	
		Early		Middle		Late		Early		Middle		Late		Early	
Temp	Day	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.
TN_1	1	59.7	3.3	46.7	2.3	51.5	2.8	1.1	.11	1.21	.09	1.12	.08	1.10	.08
	2	50.9	3.3	44.2	1.6	49.9	3.1	1.1	.08	1.50	.07	1.19	.08	1.01	.21
	3	45.4	2.5[a]	44.0	1.5[a]	47.3	2.7[a]	1.10	.05[a]	1.21	.06[a]	1.18	.07[a]	1.20	.17[a]
H	4	39.1	2.3[b]	37.6	1.7[b]	41.7	2.4[b]	.93	.06[a]	1.00	.05[a]	1.21	.07[a]	.91	.06[a]
	5	31.4	1.6[b]	28.3	1.4[b]	34.5	1.3[b]	.57	.06[b]	.87	.17[a]	.74	.11[b]	.82	.09[b]
	6	28.9	1.4[b]	24.8	.8[b]	29.0	1.2[b]	.52	.05[b]	.64	.06[b]	.89	.06[b]	.60	.08[b]
TN_2	7	34.9	1.1[b]	28.3	1.5[b]	30.5	1.7[b]	.53	.04[b]	.55	.06[b]	.72	.07[b]	.63	.09[b]
	8	44.3	3.8[a]	43.2	1.8[a]	44.3	1.9[a]	.78	.07[a]	1.15	.10[a]	.90	.08[a]	.96	.09[a]
	9	51.2	2.4[b]	47.9	1.4[a]	51.2	2.0[a]	.90	.09[a]	1.12	.09[a]	1.00	.08[a]	1.22	.09[a]
	10	50.7	1.5[a]	48.4	1.7[a]	50.7	2.8[a]	1.12	.09[a]	1.49	.47[a]	1.07	.06[a]	1.41	.10[a]

[a,b]Refer to significance of difference from Day 3 ($P<.05$).

Table 3. Average value of plasma T_4, T_3 and milk T_3 during TN_1, heat and TN_2 for each stage of lactation.

Stage	Temp	Plasma T_4 ng/ml x	S.E.		Plasma T_3 ng/ml x	S.E.		Milk T_3 ng/ml x	S.E.
E	TN_1	49.9	1.8	A^a	1.09	.04	A^a	1.09	$.11^A$
	H^1	33.1	1.2	B^a	.69	.04	B^a	.78	$.05^B$
	TN_2	43.3	1.2	C^a	.84	.04	C^a	1.19	$.08^A$
M	TN_1	45.0	1.0	A^b	1.15	.04	A^a	-	-
	H^1	30.2	1.1	B^a	.84	.05	B^a	-	-
	TN_2	42.2	1.1	A^a	1.04	.14	A^b	-	-
L	TN_1	49.6	1.6	A^a	1.17	.04	A^a	-	-
	H^1	35.1	1.2	B^a	.91	.05	B^b	-	-
	TN_2	44.9	1.3	C^a	.89	.03	B^a	-	-

^{A,B,C}Refers to significance of temperature periods ($P<.05$) within stage.

^{a,b,c}Refers to significance of stages of lactation ($P<.05$) within temperature period.

Table 4. Analysis of variance for plasma TSH, prolactin and growth hormone.

	Plasma TSH				Plasma Growth Hormone				Plasma Prolactin			
	D.F.	Sum of Squares	F	P	D.F.	Sum of Squares	F	P	D.F.	Sum of Squares	F	P
Stage	2	79.72	103.39	.0001	0	597.01	17.45	.0001	0	7907.77	36.27	.0001
Temperature	2	14.47	18.77	.0001	2	172.37	2.12	.0440	2	10593.16	48.59	.0001
Stage X Temp	4	5.98	3.88	.0043	0	118.50	1.73	.0470	0	2085.77	4.78	.0011

Table 5. Effect of stage of lactation and temperature on daily mean values of plasma TSH, growth hormone and plasma prolactin.

		Plasma TSH μIU/ml						Growth Hormone ng/ml						Plasma Prolactin ng/ml					
		Early		Middle		Late		Early		Middle		Late		Early		Middle		Late	
Temp	Day	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.
TN_1	1	2.40	.39	1.75	.16	1.50	.09	8.08	1.20	6.41	1.50	5.53	1.31	15.4	1.99	6.5	1.11	7.5	2.55
	2	2.62	.15	2.01	.20	1.45	.07	8.05	1.18	5.78	1.89	5.47	.81	16.9	4.16	7.3	2.4	8.7	3.87
	3	2.86	.23[a]	1.93	.17[a]	1.39	.13[a]	8.10[a]	2.79	6.28[a]	4.66	6.38[a]	.75	18.4[a]	3.13	9.4[a]	2.13	9.4[a]	1.56
H	4	2.62	.23[a]	1.21	.14[b]	1.47	.10[a]	6.04[b]	1.84	4.33[b]	2.09	3.9[b]	.65	38.4[b]	5.09	16.5[b]	2.76	19.8[b]	2.7
	5	2.25	.21[b]	.81	.12[b]	1.31	.15[a]	6.25[b]	1.67	4.11[b]	.67	3.85[b]	.70	49.4[b]	5.4	14.3[b]	2.44	28.9[b]	4.4
	6	1.36	.17[b]	.47	.11[b]	1.06	.12[b]	6.47[b]	2.55	4.91[b]	2.48	4.42[b]	1.28	35.6[b]	6.47	16.8[b]	2.45	44.2[b]	6.7
TN_2	7	1.70	.11[b]	.70	.11[b]	1.01	.13[a]	8.38[a]	2.53	5.44[b]	1.19	3.66[b]	1.24	28.0[a]	4.36	9.0[a]	2.84	23.2[b]	4.89
	8	2.23	.12[b]	.92	.17[b]	1.27	.16[a]	6.39[b]	2.13	5.22[b]	.69	3.61[b]	1.16	18.3[a]	1.44	8.0[a]	.56	13.9[a]	4.59
	9	2.79	.27[a]	1.51	.14[a]	1.73	.14[a]	10.48[a]	0.86	5.13[b]	.76	2.85[b]	1.87	22.8[a]	5.46	6.4[a]	2.18	16.3[a]	2.90
	10	2.84	.15[a]	1.49	.13[b]	1.70	.09[a]	8.02[a]	1.56	4.78[b]	.48	3.47[b]	.48	13.5[a]	2.47	8.4[a]	1.59	15.7[a]	4.16

[a,b]Refer to significant difference from Day 3 ($P<.05$).

Table 6. Average mean values of TSH, growth hormone and prolactin for TN_1, Heat and TN_2.

Stage	Temp	TSH μIU/ml x	TSH S.E.		Growth Hormone ng/ml x	Growth Hormone S.E.		Prolactin ng/ml x	Prolactin S.E.	
E	TN_1	2.7	.13	A^a	8.0	2.3	A^a	16.9	1.9	A^a
	H	2.1	.14	B^a	6.3	1.1	B^a	41.1	3.4	B^a
	TN_2	2.5	.11	C^a	8.3	1.1	A^a	18.0	2.3	A^a
M	TN	1.8	.10	A^b	6.1	2.2	A^b	7.8	1.6	A^b
	H	.8	.08	B^b	4.3	1.1	B^b	15.8	1.9	B^b
	TN_2	1.3	.08	C^b	5.1	.5	B^b	10.6	.9	A^b
L	TN_1	1.5	.06	A^c	5.7	.5	A^b	8.8	1.5	A^b
	H	1.3	.09	B^c	4.0	.5	B^b	30.9	3.6	B^b
	TN_2	1.4	.07	A^c	3.4	.4	B^c	16.1	2.1	C^c

^{A,B,C}Refers to significance of temperature periods ($P<.05$) within stage.

^{a,b,c}Refers to significance of stages of lactation ($P<.05$) within temperature period.

Table 7. Analysis of variance for plasma, insulin, plasma and milk cortisol, and energy metabolism.

		Insulin			Plasma Cortisol			Milk Cortisol			Energy Metabolism		
	D.F.	Sum of Squares	F	P	Sum of Squares	F	P	Sum of Squares	F	P	Sum of Squares	F	P
Stage	2	3926.59	14.48	.0001	212.42	7.25	.0008	0	-	-	1.43	17.73	.0001
Temperature	2	719.09	2.65	.0733	41.36	1.41	.2453	.39	.5	.6089	4.42	54.64	.0001
Stage X Temp	4	560.05	1.03	.3919	63.31	1.08	.3662	0	-	-	0.56	3.46	.0081

Table 8. Effect of stage of lactation and temperature on daily mean values of plasma insulin, plasma and milk cortisol and metabolism.

		Insulin μIU/ml						Plasma Cortisol ng/ml					
		Early		Middle		Late		Early		Middle		Late	
Temp	Day	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.	x	S.E.
TN_1	1	8.1	2.1	9.6	2.4	22.6	6.02	6.5	1.0	8.6	1.1	6.0	1.3
	2	9.3	3.4	9.5	1.4	27.8	6.66	6.2	1.3	8.5	1.5	6.9	1.3
	3	10.8	5.6[a]	9.4	.88[a]	22.34	3.56[a]	4.9	.8[a]	8.3	1.4[a]	7.7	1.2[a]
H	4	5.9	1.4[a]	10.7	1.7[a]	19.5	7.12[a]	5.8	.9[a]	8.6	1.2[a]	7.6	1.4[a]
	5	9.7	2.1[a]	14.8	6.6[a]	17.11	7.60[a]	6.9	.7[a]	8.7	1.0[a]	7.6	1.0[a]
	6	14.5	4.6[a]	13.4	4.7[a]	21.62	6.52[a]	5.6	.4[a]	9.0	1.2[a]	10.7	2.2[a]
TN_2	7	17.9	2.9[a]	18.9	6.4[a]	29.42	5.82[a]	5.4	.6[a]	10.7	1.7[a]	6.6	.5[a]
	8	21.2	3.3[b]	27.0	5.8[b]	23.06	4.98[a]	8.0	1.4[a]	7.43	1.2[a]	6.6	1.0[a]
	9	17.0	2.5[a]	20.9	4.5[b]	30.65	5.05[b]	6.2	.6[a]	7.43	.9[a]	7.5	1.4[a]
	10	10.3	1.9[a]	20.3	4.2[b]	18.64	5.67[a]	6.1	1.5[a]	6.26	.9[a]	5.1	.9[a]

		Milk Cortisol ng/ml		Metabolism kwatts/hr					
		Early		Early		Middle		Late	
Temp	Day	x	S.E.	x	S.E.	x	S.E.	x	S.E.
TN_1	1	1.49	.42	.90	.03	.95	.05	.93	.04
	2	1.13	.11	.87	.02	.94	.03	.91	.03
	3	.73	.09[a]	.86	.02[a]	.93	.03[a]	.89	.02[a]
H	4	1.05	.36[a]	.82	.02[b]	.82	.03[a]	.87	.02[a]
	5	1.26	.21[a]	.73	.02[b]	.77	.03[a]	.82	.02[a]
	6	1.22	.30[a]	.63	.03[b]	.76	.03[b]	.70	.02[b]
TN_2	7	1.60	.46[b]	.62	.02[b]	.71	.02[b]	.81	.04[b]
	8	1.15	.32[a]	.71	.03[a]	.83	.03[a]	.83	.03[a]
	9	1.09	.12[a]	.75	.04[a]	.80	.02[a]	.82	.02[a]
	10	.99	.14[a]	.79	.02[a]	.84	.02[a]	.98	.09[a]

[a,b]Refer to significant difference from Day 3 ($P<.05$).

Table 9. Mean values of TN_1, heat and TN_2 for each stage of lactation.

Stage	Temp	Insulin μIU/ml x	Insulin μIU/ml S.E.		Plasma Cortisol ng/ml x	Plasma Cortisol ng/ml S.E.		Milk Cortisol ng/ml x	Milk Cortisol ng/ml S.E.		Metabolism k watts/hr x	Metabolism k watts/hr S.E.	
E	TN_1	9.5	2.8	A^a	5.6	.78	A^a	1.0	.10	A	.87	.01	A^a
	H_2	10.1	1.8	A^a	6.1	.41	A^a	1.2	.16	A	.73	.01	B^a
	TN_2	13.1	1.7	B^a	6.1	.54	A^a	1.2	.15	A	.72	.01	B^a
M	TN_1	9.5	.9	A^a	8.5	.99	A^b	-	-		.94	.02	A^b
	H	13.3	2.8	A^a	8.6	.66	A^b	-	-		.78	.02	B^b
	TN_2	17.9	2.5	B^b	8.2	.64	A^b	-	-		.79	.01	B^b
L	TN_1	24.1	2.9	A^b	6.8	.88	A^b	-	-		.91	.01	A^b
	H_2	19.4	3.7	B^b	8.8	.92	A^b	-	-		.79	.01	B^b
	TN_2	22.8	2.7	A^c	6.5	.51	B^b	-	-		.86	.02	C^c

^{a,b,c}Refer to significance of stages of lactation ($P<.05$) within-temperature period.

^{A,B,C}Refer to significance of temperature periods ($P<.05$) within-stage.

Table 10. Comparison of Day 3 and Day 6 for Rectal Temperatures*, Milk Yields*, Feed Intake*, Energy Metabolism* and Plasma Hormonal Data of 51 cows at each stage of lactation.

	Early				Middle				Late			
	Day 3	Day 6	Diff	Sig	Day 3	Day 6	Diff	Sig	Day 3	Day 6	Diff	Sig
Plasma T_4, ng/ml	45.4	28.9	16.5	.0001	44.0	24.8	19.2	.0001	47.3	29.0	18.3	.0001
Plasma T_3, ng/ml	1.1	0.52	.58	.0006	1.2	.64	.57	.0013	1.2	.89	.29	.0073
Plasma TSH (μIU/ml)	2.8	1.3	1.5	.0001	1.9	.47	1.46	.0001	1.4	1.1	.33	.20
Plasma GH, ng/ml	8.1	6.5	1.63	.041	6.3	4.9	1.39	.01	6.4	4.4	1.9	.015
Plasma Prolactin, (ng/ml)	18.4	35.6	-17.2	.0042	9.4	16.8	-7.4	.041	9.4	44.2	-34.2	.0001
Plasma Insulin (μIU/ml)	10.8	14.5	-3.7	.6	9.4	13.4	-4.03	.6	22.3	21.6	.73	.9
Plasma Cortisol, (ng/ml)	4.9	5.6	-.7	.6	8.3	9.0	-.70	.6	7.7	10.7	-3.0	.08
Energy Metab., (kwatts/hr)	.86	.66	.20	.0001	.93	.76	.17	.0001	.89	.70	.19	.0001
Rectal Temp °C	38.8	40.6	-1.8	.0001	38.8	40.0	-1.2	.0001	38.7	40.3	-1.6	.0001
Milk Production, (kg/day)	23.4	19.9	3.5	.0001	19.7	16.4	3.3	.0001	16.7	13.6	3.1	.0002
Feed Intake, (MCal/day)	27.9	19.1	8.8	.0001	28.6	18.2	10.4	.0001	19.7	18.8	10.9	.0001

*See Research Bulletin - Part I for further information.

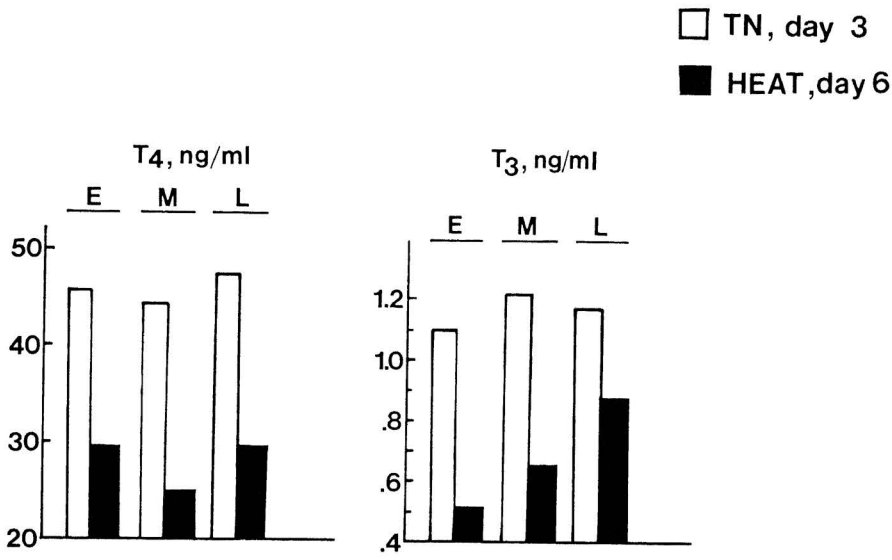


Figure 1. Comparison of 51 cow average for TN Day 3 and heat for Day 6, values for Plasma T_4 and T_3 for Early (E) Mid (M) and Late (L) stage of lactation.

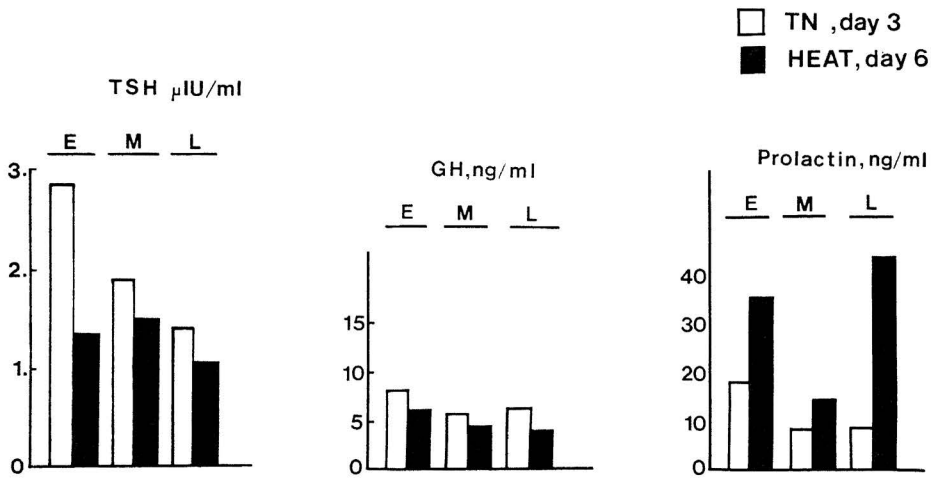


Figure 2. Comparison of 51 cow average for TN Day 3 and Heat Day 6 values for Plasma TSH, GH and Prolactin for Early (E), Mid (M) and Late (L) stage of lactation.

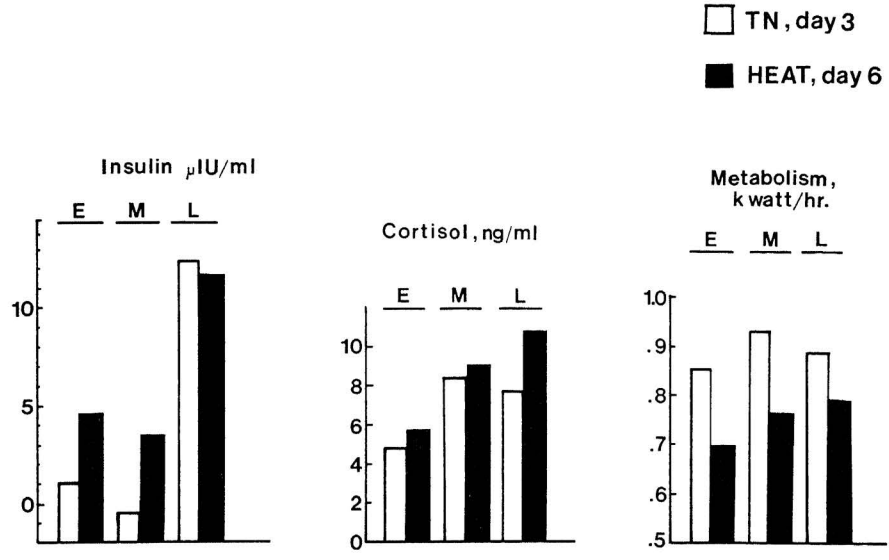


Figure 3. Comparison of 51 cow average Plasma, Insulin, Cortisol and Energy Metabolism for TN_1 Day 3 and Heat Day 6 for Early (E), Mid (M) and Late (L) stage of lactation.

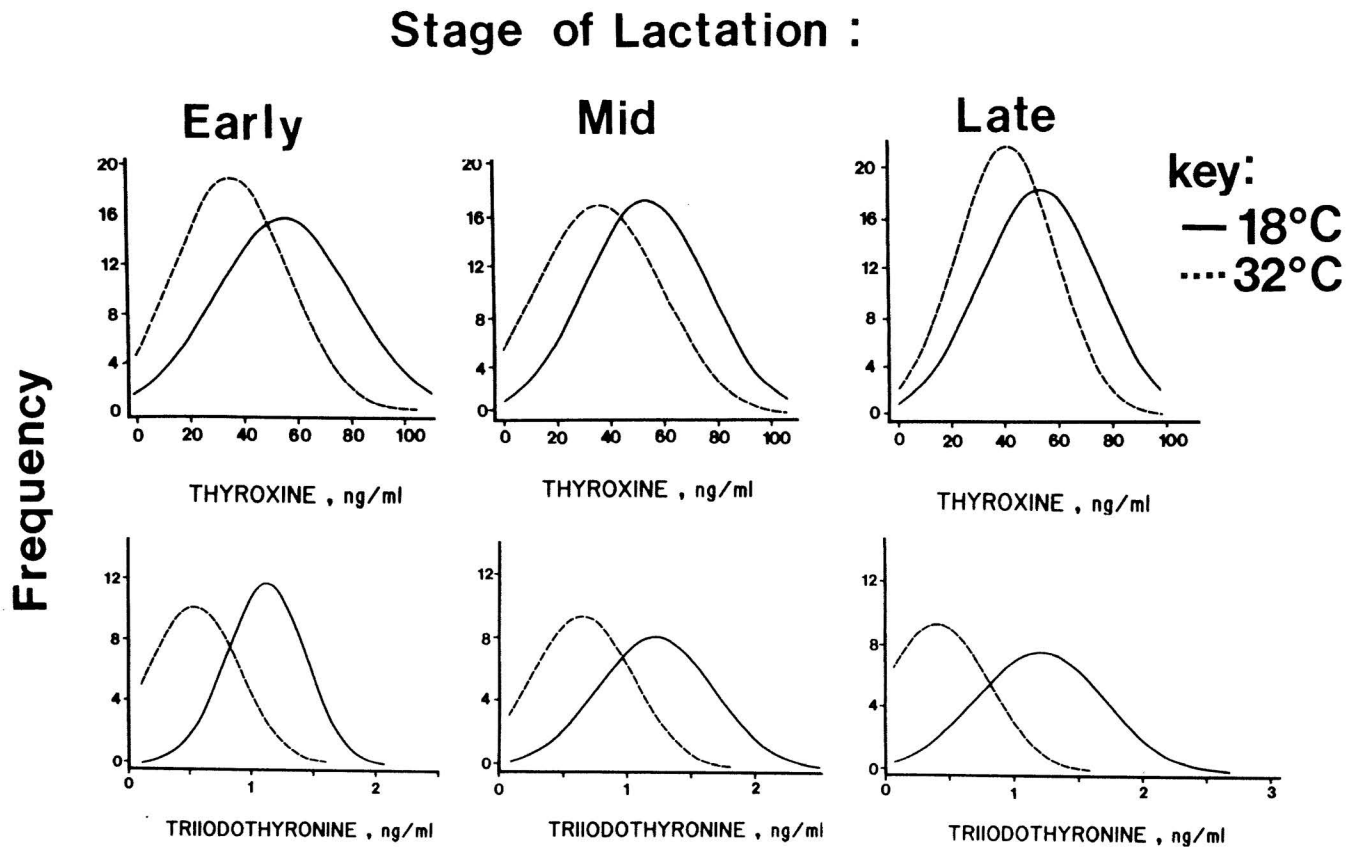


Figure 4. Mean distribution curves for Plasma T_4 and T_3 at each stage of lactation, when subjected for 3 days to 18°C and 32°C.

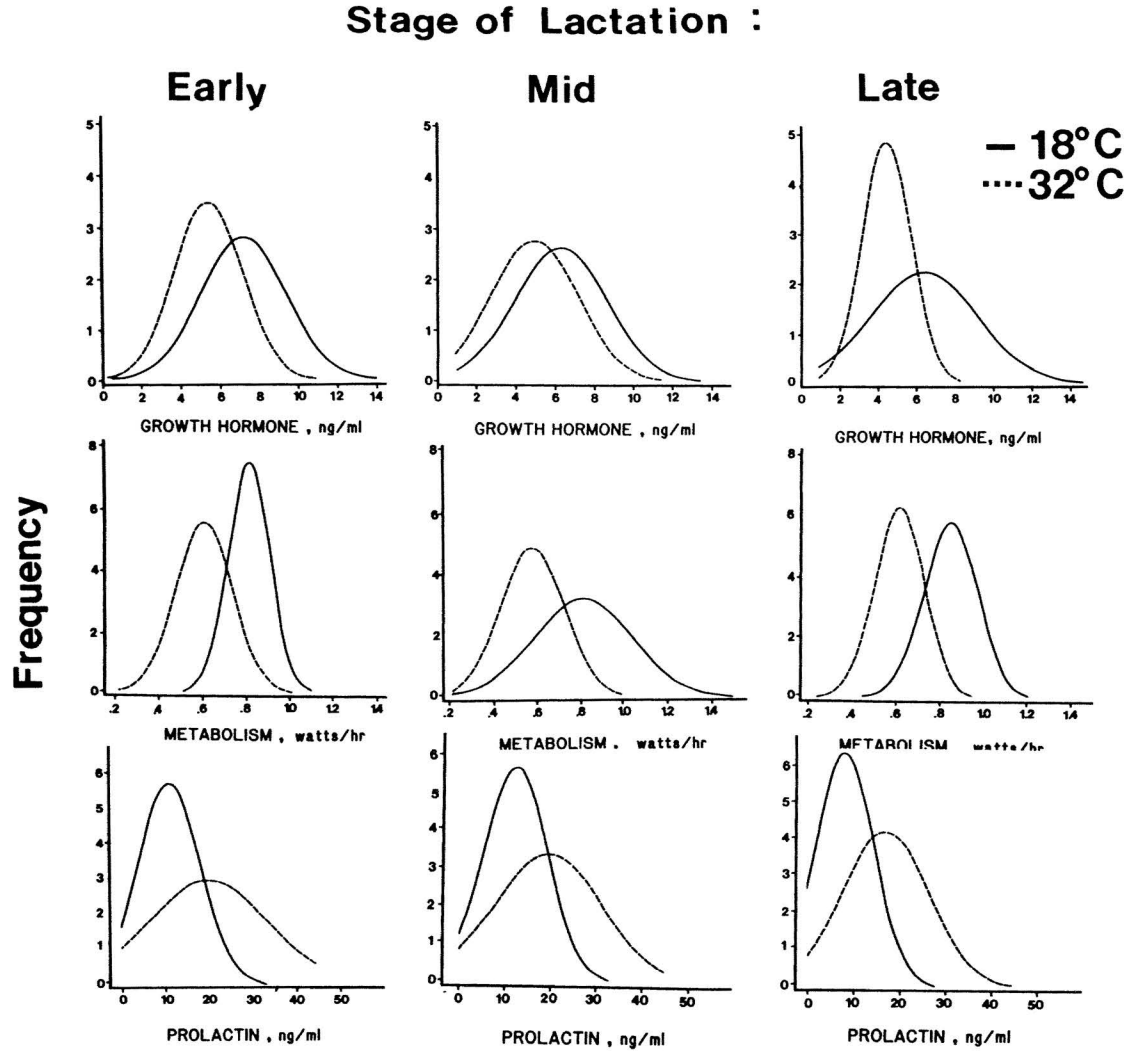
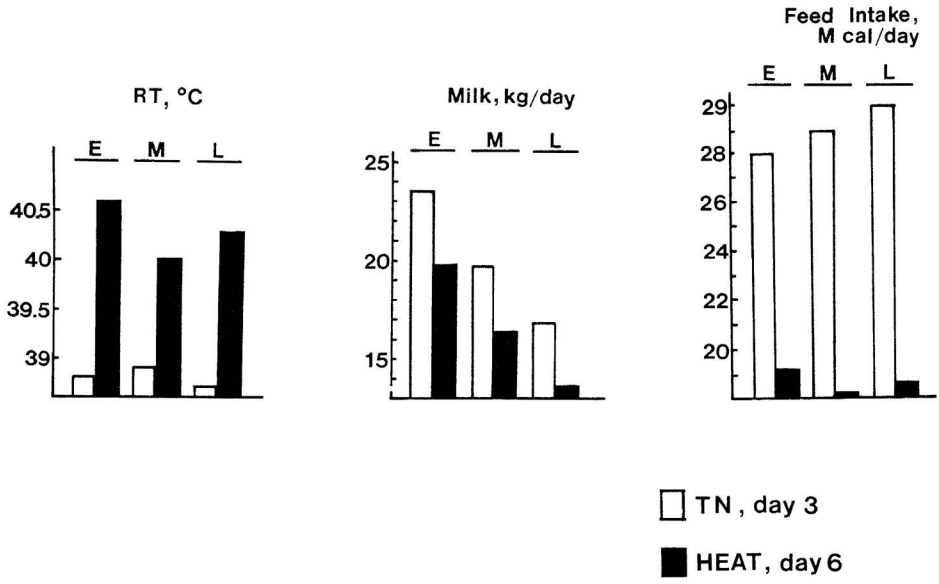


Figure 5. Mean distribution curves for Growth Hormone, Metabolism and Prolactin at each stage of lactation, when subjected for 3 days to 18°C and 32°C.



Appendix, Figure 6.
Comparison of 51-cow average for TN, day 3 and Heat, day 6 for Rectal Temperature (RT) Milk Yield and Feed Intake at Early (E), Mid (M) and Late (L) stage of lactation.