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Effect of High Ambient Temperature (35°), and Feed Intake on Plasma T_4 Levels in Sheep[1,2] (41341)

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Abstract. An experiment was designed to determine the relative effects of environmental heat stress and accompanying decline in voluntary feed intake on plasma thyroxine. The sheep were divided into three groups of four animals each. The control animals and feed-restricted group were maintained at thermoneutrality (22°, 50% RH). The heat group was maintained at 35° and 67% RH. All groups were fed *ad libitum* except the feed-restricted group which was fed at the level of the heat-stressed group. Results indicated that environmental heat has both a direct temperature and an indirect temperature (feed) effect on plasma thyroxine (T_4). The feed effect was determined by comparing the plasma T_4 of the feed-restricted group at 22° to the high-temperature (35°) group. Both groups consumed similar levels of feed. The heat-stressed group with elevated temperature and feed intake similar to feed-restricted group at thermoneutral showed significantly greater reduction of plasma T_4. Therefore the temperature effect was additional to the feed effect and the heat-stressed group displayed a significantly greater decline in plasma T_4.

Thyroid function of domestic animals is known to be altered by many environmental factors. Special attention has been given to the effect of ambient temperature (1–4) and feed intake (5–7) on thyroid activity. These studies have shown that thyroid function is depressed by both high ambient temperature and reduced feed intake. The independent effects of these two factors have not been thoroughly studied, however.

The present study was designed to quantify the relative effects of high ambient temperature and of heat-associated voluntary feed restriction effects on plasma thyroxine (T_4) levels in sheep.

Materials and Methods. Twelve castrated cross-bred western male sheep averaging 6 months of age were placed in the Missouri Climatic Laboratory. The animals were divided into three groups of four animals each, on the basis of similar weight animals in each group. The three groups were as follows: control (C) group at thermoneutral; heat-stressed (HS) group at 30°; and feed-restricted (FR) group at thermoneutral. The study consisted of three periods, after a 1-week adjustment to the laboratory conditions. During the 3-week pretreatment period, the animals in all three groups were maintained at thermoneutral (TN) conditions (22°, 50% RH) and fed *ad libitum*. For the next 4 weeks (actually 30 days) lambs in control group continued feeding *ad libitum* under thermoneutral conditions, while HS group lambs were fed *ad libitum* but subjected to a high ambient temperature treatment (35°, 50% RH) and FR group lambs were maintained at thermoneutral conditions, but were fed according to the level of voluntary intake of the heat-stressed animals (HS). Feed provided to the FR group was adapted daily to the average intake of the HS group. A 4-week treatment period was selected to be more assured of acclimation to the two treatments. During a 3-week post-treatment period (actually 24 days) all animals were again at thermoneutral conditions with *ad libitum* feeding. The ration consisted of a 28.48% cottonseed hulls; 4.99% alfalfa

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meal; 10.00% molasses; 39.70% cracked corn; 14.99% SBOM; and 1.47% minerals and vitamins (A and D).

Measurements were taken daily on feed intake and rectal temperature. Body weights were taken on Monday, Wednesday, and Friday. Blood samples were collected via jugular puncture in heparanized vacutainers 4 days prior to the initiation of the 4-week treatment period and every Monday, Wednesday, and Friday thereafter at 1000 hr. Samples were kept on ice until separated by centrifugation (886g for 30 min at 4°). Plasma was then kept at −20° until assayed for T_4 using the technique of Endocrine Sciences (Tarzana, Calif.) as adapted in this laboratory for bovine plasma analysis. The intra- and interassay variabilities were 6.1 and 9.3%, respectively.

Two lambs died during the course of the study due to causes not related to the treatment. The statistical analysis has, therefore, been performed on the remaining 10 animals by means of a three-way analysis of variance (8). The data were statistically analyzed as ratios of values of treatment and post-treatment to pretreatment data. The statistical model is indicated in Table I for the three indices.

Results and Discussion. The animals in the FR group had a significantly higher ($P < 0.05$) T_4 pretreatment level (59.49 ± 1.30 ng/ml) than the lambs in group C (47.52 ± 1.97 ng/ml) and HS group (49.09 ± 2.06 ng/ml), so all data have been analyzed as a ratio to pretreatment levels, arbitrarily considered as 100% (Table I). The treatment and post-treatment data were subdivided into two periods each for the statistical analysis, due to differences in response observed within the periods of the treatment groups. The treatment was divided into two periods for analysis. By Day 18 minimal values were observed on both HS and FR groups of animals. This time (0–18 days) was arbitrarily designated as period 1, the remainder of the treatment was designated period 2. The post-treatment data (periods 3 and 4) were divided at the time when all values had returned to pretreatment levels (ratio 1.0 or more).

Figure 1 illustrates the trend effects for all measurements and Table II provides data on the response of T_4 for the animals in all three groups. Both high ambient temperature (HS) and reduced feed intake (FR) produced a significant decrease ($P < 0.05$) in plasma T_4 during the first 15-day period (period 1) of the treatment. The HS group decline was significantly greater than the FR group, indicating the additional effect of heat on the animals. Both HS and FR groups consumed similar levels of feed. Both groups were significantly different ($P < 0.05$) than the control. During period 2 of the treatment both groups were significantly ($P < 0.05$) lower than the controls, but the HS group had recovered more from the initial days of heat exposure than had the FR group to the restriction of feed intake, and thus no difference between groups. The initial depression of T_4 (period 1) and the T_4 recovery trend period during the HS and FR treatments were significantly lower than pretreatment or control

TABLE I. ANALYSIS OF VARIANCE FOR PLASMA THYROXINE (T_4), RECTAL TEMPERATURE (RT), AND FEED INTAKE (FI) EXPRESSED AS RATIO TO PRETREATMENT LEVELS

Source	*df*	T_4 ratio mean square	RT ratio mean square	FI ratio
Group	2	0.00314[a]	0.00122**	1.04394[a]
Lamb:group	7	0.06320	0.00012	1.70223
Period	3	0.17712**	0.001176*	1.26010**
Group × period	6	0.06643**	0.000489*	0.30472**
Error	201	0.00366	0.00003563	0.04362

[a] $P > 0.05$ (not significant).
* $P \leq 0.05$.
** $P \leq 0.01$.

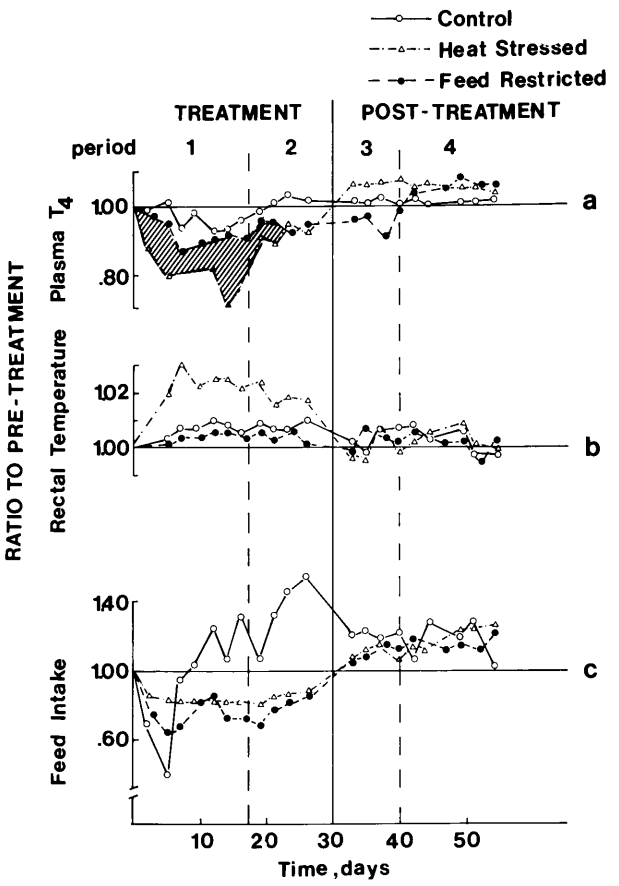

FIG. 1. Responses in T_4 (a), rectal temperature (b), and feed intake (c) in control, heat-stressed, and feed-restricted lambs, during treatment (periods 1 and 2) and post-treatment (periods 3 and 4). Values are expressed as ratios of pretreatment levels.

group (C). These results suggest a certain degree of acclimation to the hot condition.

The response in T_4 during the post-treatment period differed between the treatment groups (Fig. 1a, Table II). The heat-stressed animals showed significant increase ($P < 0.05$) in plasma T_4 within a few days after removal from the hot environ-

TABLE II. T_4 LEVELS EXPRESSED AS RATIOS OF PRETREATMENT (PERIOD 0) DURING TREATMENT (PERIODS 1 AND 2) AND POST-TREATMENT (PERIODS 3 AND 4), IN CONTROL (C), HEAT-STRESSED (HS), AND FEED-RESTRICTED (FR) ANIMALS

Period (days)		Group		
		C	HS	FR
0	−4 to 0	$1.0^{a,A}$	$1.0^{a,A}$	$1.0^{a,A}$
1	0 to 18	$0.96 \pm 0.02^{a,A}$	$0.828 \pm 0.021^{b,B}$	$0.939 \pm 0.012^{b,A}$
2	19 to 30	$0.99 \pm 0.03^{a,A}$	$0.927 \pm 0.035^{c,B}$	$0.944 \pm 0.015^{b,B}$
3	31 to 41	$1.00 \pm 0.03^{a,A}$	$1.066 \pm 0.022^{d,B}$	$0.978 \pm 0.015^{c,C}$
4	42 to 54	$0.97 \pm 0.02^{a,A}$	$1.053 \pm 0.015^{d,B}$	$1.050 \pm 0.011^{d,B}$

Note. Values are mean ± SE.

^{a,b,c,d} Values in the same column bearing different letters are significantly different ($P < 0.05$).

^{A,B,C} Values in the same row bearing different letters are significantly different.

ment. The lambs in the feed-restricted group, however, had a slower recovery. Their T_4 concentrations remained low during the first 10 days (period 3) of the post-treatment period although their feed consumption had already recovered. During period 4 of the post-treatment period both groups were near or above pretreatment levels in plasma T_4. Both groups appeared to display some compensation in plasma T_4 levels during period 4 when compared to the 30-day treatment values.

The 35° (HS) treatment produced a significant increase ($P < 0.05$) in rectal temperature (Fig. 1b, Table III). During period 2 of the treatment, the heat-stressed lambs showed a slight decrease in their rectal temperature, but remained significantly higher than the pretreatment level, and higher than the level observed for both the control and feed-restricted animals.

Figure 1c and Table IV show the changes in feed intake. The animals in the heat-stressed group had a significant decrease ($P < 0.05$) in feed intake upon exposure to heat. This response was expected, since the depressive effect of high ambient temperature on feed intake has been reported (9–11). The feed-restricted lambs were fed the level of intake of the heat-stressed group; however, occasionally the FR group did not consume all the feed they were offered which caused some variation, though differences were not significant. During the post-treatment, both groups showed a significant ($P < 0.05$) increase in feed intake, reaching levels comparable to the control toward the end of the study.

Fox *et al.* (12) found that the thyroid secretion rate (TSR) was lower in steers during a feed restriction period and during the first part of a refeeding period when the animals exhibited compensatory growth. The TSR increased to or above levels of controls as the refeeding progressed. The present results show that plasma T_4 responded in the same way. This suggested parallel between TSR and T_4 concentration agrees with the results of Magdub (13), who found a positive and significant correlation between TSR and plasma T_4 in cows.

The present study suggests that environmental temperature has a direct effect on plasma T_4. Furthermore, it appears to be the major factor determining the decrease in T_4 concentration. The increased rectal temperatures would logically increase the temperature of the "heat-loss center" in the preoptic region of the hypothalamus as demonstrated earlier by Andersson *et al.* (14, 15). They showed the increased temperature markedly inhibited plasma PBI. D'Angelo *et al.* (16) and Fortier (17) observed similar decreases in thyroid function. Data in this report clearly show this direct effect of temperature on radioassayable (RIA) plasma thyroxine. The voluntary depression of feed intake by the HS group may occur by a coupling between the thyroid regulatory system and that controlling caloric homeostasis via a neuroendocrine reflex arc involving thermoreceptors in skin or via thermosensitive neurons in the hypothalamus (18).

The feed-restricted group at TN with a similar level of feed intake as the HS group

TABLE III. RECTAL TEMPERATURES EXPRESSED AS RATIO OF PRETREATMENT (PERIOD 0), DURING TREATMENT (PERIODS 1 AND 2), AND POST-TREATMENT (PERIODS 3 AND 4), IN CONTROL (C), HEAT-STRESSED (HS), AND FEED-RESTRICTED (FR) ANIMALS

Period (days)		Group		
		C	HS	FR
0	−4 to 0	$1.00 \pm 0.017^{a,A}$	$1.00^{a,A}$	$1.00^{a,A}$
1	0 to 18	$1.00 \pm 0.017^{a,A}$	$1.021 \pm 0.011^{b,B}$	$0.974 \pm 0.008^{a,A}$
2	19 to 30	$1.00 \pm 0.015^{a,A}$	$1.014 \pm 0.012^{b,B}$	$1.00 \pm 0.013^{a,A}$
3	31 to 41	$1.00 \pm 0.026^{a,A}$	$1.002 \pm 0.018^{a,A}$	$1.00 \pm 0.014^{a,A}$
4	42 to 54	$0.998 \pm 0.017^{a,A}$	$1.001 \pm 0.014^{a,A}$	$1.00 \pm 0.019^{a,A}$

Note. Values are mean ± SE.

[a,b] Values in the same column bearing different letters are significantly different ($P < 0.05$).

[A,B] Values in the same row bearing different letters are significantly different ($P < 0.05$).

TABLE IV. FEED INTAKE EXPRESSED AS RATIO OF PRETREATMENT (PERIOD 0), DURING TREATMENT (PERIODS 1 AND 2), AND POST-TREATMENT (PERIODS 3 AND 4), IN CONTROL (C), HEAT-STRESSED (HS), AND FEED-RESTRICTED (FR) ANIMALS

Period (days)		Group		
		C	HS	FR
0	−4 to 0	1.0^{a}	1.0^{a}	1.0^{a}
1	0 to 18	$1.058 \pm 0.13^{a,A}$	$0.828 \pm 0.028^{b,B}$	$0.849 \pm 0.031^{b,B}$
2	19 to 30	$1.371 \pm 0.19^{b,A}$	$0.853 \pm 0.036^{b,B}$	$0.761 \pm 0.028^{b,B}$
3	31 to 41	$1.215 \pm 0.11^{c,A}$	$1.083 \pm 0.065^{c,B}$	$1.126 \pm 0.078^{c,B}$
4	42 to 54	$1.227 \pm 0.11^{c,A}$	$1.247 \pm 0.050^{d,A}$	$1.245 \pm 0.071^{c,A}$

Note. Values are mean ± SE.
^{a,b,c} Values in the same column bearing different letters are significantly different ($P < 0.05$).
^{A,B} Values in the same row bearing different letters are significantly different ($P < 0.05$).

showed a decreased level of T_4 during the treatment, though not as much as the HS group. It is conceivable this difference is due in some manner to the heat sensitivity of "thyroid releasing hormone (TRH) center" in the hypothalamus. Rousset and Cure (19) have *in vitro* assay evidence of the heat-depressing effects on plasma TRH. The cross-hatched area (Fig. 1a) can be generally interpreted as the effect of heat, and is supported by the force-feeding study on cattle exposed to high environmental temperature (20). They found the thyroid activity to be affected mainly by temperature when comparing the TSR and the measure of the plasma protein-bound iodine (PBI) of two groups of cows upon exposure to 30°. One group was fed *ad libitum* while the other was force fed via rumen fistual, according to the level of intake observed during the pretreatment temperature at 18°. Even the force-fed animals showed a significant decrease in thyroid activity when subjected to the heat treatment. This study merits reconfirmation with newer RIA assay procedures. The mechanism of how temperature affects thyroid function or feed intake, and further, determining whether the heat depression effect on feed intake is due to the reduction in T_4, require further experimentation. It is suggested that *ad libitum* animals under moderate heat stress sufficient to elevate deep body temperatures be repeatedly injected or infused with T_4 or T_3 during heat stress to determine if a TN level of T_4 will prevent or lessen the decline in feed intake. As indicated earlier Reichlin (21) reported that areas in the bovine brain controlling body temperature are close to those affecting thyroid function. Rousset and Cure (19) observed a decrease in hypothalamic thyrotropin releasing hormone (TRH) in rats upon exposure to heat and DiStephano (18) postulated a neuroendocrine arc involved in caloric homeostasis. With this background and the results of this report one may suggest that the effect of temperature on thyroid function is initiated at the hypothalamic level. The present study suggests that the voluntary restriction of feed intake accounts for much of the depressive effect on plasma T_4 concentration, but temperature per se appears to provide an additional depression in the decrease of plasma T_4 levels in sheep subjected to heat.

1. Hoersch TM, Reineke EP, Henneman, HA. Effect of artificial light and ambient temperature on the thyroid secretion rate and other metabolic measures in sheep. J Anim Sci 20:358, 1961.
2. Thompson RD, Johnston JE, Breidenstein CP, Baberjee MR, Burnett WT. Effect of hot conditions on adrenal-cortical, thyroideal and other metabolic responses of dairy heifers. J Dairy Sci 46:227, 1973.
3. Sutherland RL, Irvine CHG. Effect of season and pregnancy on total plasma thyroxine concentration in sheep. Amer J Vet Res 35:311, 1974.
4. Evans SE, Ingram DL. The effect of ambient temperature upon the secretion of thyroxine in the young pig. J Physiol 264:511, 1977.
5. Meites J, Wolterink LF. Uptake of radioactive iodine by the thyroids of underfed rats. Science 111:175, 1950.
6. Grossie J, Turner CW. Thyroxine secretion rates during food deprivation in rats. Proc Soc Exp Biol Med 110:631, 1962.

7. Singh DV, Anderson RR, Turner CW. Effect of decreased dietary protein on the rate of thyroid hormone secretion and food consumption of rats. J Endocrinol **50**:445, 1971.
8. Gill JT, Hafs HD. Analysis of repeated measurements of animals. J Anim Sci **33**:331, 1971.
9. Gasnier A, Mayer A. Recherches sur la regulation de la nutrition. III. Mecanismes de la nutrition chez le lapin domestique. Ann Physiol Physiochim Biol **15**:186, 1939.
10. Brody S, Ragsdale AC, Yeck RG, Worstell D. Environmental physiology and shelter engineering with special reference to domestic animals. XXXIII. Milk production, feed and water consumption, and body weight of Jersey and Holstein cows in relation to several diurnal temperature rhythms. Mo. Agric Exp. Sta. Res. Bull. 578, 1955.
11. Morrison, SR, Mount LE. Adaption of growing pigs to changes in environmental temperature. Anim Prod **13**:51, 1971.
12. Fox DG, Preston RL, Senft B, Johnston RR. Plasma growth hormone levels and thyroid secretion rates during compensatory growth in beef cattle. J Anim Sci **38**:437, 1974.
13. Magdub AB, Johnson HD. Estimation of thyroid function in relation to the milk production by measures of plasma thyroxine and thyroxine turnover. J Dairy Sci (Suppl 1) **60**:106, 1977.
14. Andersson B, Ekman L, Gale CC, Sundsten JW. Blocking of the thyroid response to cold by local warming of the preoptic region. Acta Physiol Scand **56**:94, 1962.
15. Andersson B, Ekman L, Gale CC, Sundsten JW. Control of thyrotrophic hormone (TSH) secretion by the "heat loss centre." Acta Physiol Scand **59**:12, 1963.
16. D'Angelo SA, Snyder J, Grodin JM. Electrical stimulation of the hypothalamus: Simultaneous effects on the pituitary-adrenal and thyroid systems of the rat. Endocrinology **75**:417, 1964.
17. Fortier C. Hypothalamic control of anterior pituitary. von Euler C, Heller H, eds. Comparative Endocrinology. London, Academic Press, Vol 1:p2, 1963.
18. DiStephano JJ. A model of the regulation of circulating thyroxine unbound and bound to plasma proteins and its response to pregnancy, drugs, long acting thyroid stimulator and temperature stress. Math Biosci **4**:137, 1969.
19. Rousset B, Cure M. Variation of rat thyroid activity during exposure to high environmental temperature (34 C). Relation between hypothalamic, pituitary and thyroid levels. Pflügers Arch **354**:101, 1975.
20. Yousef MK, Johnson HD. Blood thyroxine degradation rate of cattle as influenced by temperature and feed intake. Life Sci **5**:1349, 1966.
21. Reichlin S. Thyroid function, body temperature regulation and growth in rats with hypothalamic lesions. Endocrinology **66**:340, 1960.

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