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Growth Performance and Concentrations of Thyroid Hormones and Growth Hormone in Plasma of Broilers at High Temperatures

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Abstract

Responses of broiler chickens to a high ambient temperature (35°C) were measured in two experiments. In one experiment temperatures were increased abruptly from 21°C to a daily range of 21-35°C whereas, in the other, temperatures were increased more gradually over 6 days. The high temperatures were maintained for 5 h/day. In both experiments, birds exposed to the high temperatures ate less food and gained less liveweight than birds maintained at 21°C. Efficiency of food conversion to liveweight gain and body composition were not affected by high temperature but there was a tendency for thyroid weight to decrease. Overall, the plasma concentration of triiodothyronine (T_3) decreased and the plasma concentration of thyroxine (T_4) increased, resulting in a decreased T_3/T_4 molar ratio, during exposure to high temperature. The concentration of plasma growth hormone, but not plasma reverse T_3, was increased by high temperature. The initial responses to increased temperature were variable, with birds exposed more gradually adjusting relatively well until the maximum temperature was increased to 35°C. All heated birds readjusted quickly to the daily reduction in temperature to 21°C.

Introduction

High ambient temperatures reduce food intake and growth rate in chickens (Cowan and Michie 1978; Cerniglia *et al.* 1983; Sinurat and Balnave 1985) and there are indications also that thyroid function is altered at high temperatures. Plasma concentrations of triiodothyronine (plasma T_3) have decreased during exposure of chickens to high temperatures in most (May 1978; Cogburn and Harrison 1980; Klandorf *et al.* 1981; Freeman and Cogburn 1983; Williamson *et al.* 1985) but not all (Rudas and Pethes 1980, 1984) studies. Likewise, plasma concentrations of thyroxine (plasma T_4) usually (Moss and Balnave 1978; Scanes *et al.* 1980; Freeman and Cogburn 1983) but not always (Rudas and Pethes 1980; Williamson *et al.* 1985) have increased at high temperatures. These changes reflect variations in food intake (Moss and Balnave 1978; Klandorf *et al.* 1981), with energy intake appearing to be the main factor regulating plasma T_3 (Sharp and Klandorf 1985).

The inactive thyroid hormone, reverse T_3 (rT_3), can serve as a route for dissipation of T_4 in humans (Cavalieri and Rapoport 1977) but its role in the chicken remains to be defined. In particular, the effects of age (Premachandra *et al.* 1977; Thommes and Hylka 1977; Decuypere *et al.* 1980) and temperature (Rudas and Pethes 1980, 1984; May 1985) on plasma concentrations of rT_3 (plasma rT_3) are unclear.

Growth hormone (GH) may also participate in adaptations induced by heat (Riis 1983). Plasma concentrations of GH (plasma GH) are elevated at high temperatures (Harvey *et al.* 1977; Scanes *et al.* 1980) and when nutrient intake is restricted (Engster *et al.* 1979; Scanes and Harvey 1981).

The present studies were designed to examine the effects of high ambient temperatures on plasma T_3, T_4 rT_3 and GH. In one experiment birds maintained at a constant temperature of 21°C were exposed suddenly to increased ambient temperatures whereas in a second study daily temperatures were increased gradually.

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Materials and Methods

Chickens

Male broiler chickens, 25 (experiment 1) and 21 (experiment 2) days of age, from the same genetic line were allocated to cages (five birds per cage) in controlled environment rooms on the basis of similar bodyweights. They were fed a broiler finisher diet (13.68 MJ of ME and 213 g crude protein/kg) and provided with water *ad libitum*. Continuous lighting was provided from 1 day of age.

Experimental Procedures

Two separate experiments were conducted as follows:

Experiment 1

A total of 70 birds was located randomly in two rooms (seven groups per room) maintained at 21°C between 25 and 35 days of age. On day 35 (day 1), the temperature in one room (hot room) was altered abruptly, commencing at 1100 h, to give the following diurnal pattern: 1100–1600 h, 35°C; 1600–2300 h, 28°C; 2300–0700 h, 21°C; 0700–1100 h, 28°C. This pattern was maintained for 20 days in the hot room whereas in the other room (control room) the temperature was maintained at 21°C throughout the experiment.

On days 1, 2, 3, 5, 10, 15 and 20, blood samples were obtained from one group of birds in each room at 1300 h and 0100 h (viz 2 h after attainment of maximum and minimum temperatures, respectively, in the hot room). Each group of birds was bled on one day only during the experiment.

Food intakes of all birds were measured between 1100–1600 h and 2300–0700 h on each day blood was collected. Total food intake and liveweight gain were measured between 25 and 54 days of age.

Experiment 2

A total of 280 birds was located randomly in four rooms (14 groups per room) maintained at 21°C until the birds were 28 days old (day 0). Two rooms (control rooms) were maintained at 21°C for the 26 days of the experiment whereas the temperature of the other two rooms (hot rooms) was increased, according to the time cycle for experiment 1, as follows: days 1-3, 27-24-21-24°C; days 4-6, 31-26-21-26°C; days 7-26, 35-28-21-28°C.

Blood samples were obtained from three birds per group at 1300 h and 0100 h on days 0–9, 11, 16, 21 and 26 with birds from each group being bled on one day only. The groups from which blood was collected on day 21 (viz. 49 days of age) were sacrificed for measurement of weights of thyroid glands and abdominal fat on day 26 (viz. 54 days of age).

Food intakes were measured during the periods of maximum (1100–1600 h) and minimum (2300–0700 h) temperatures for all birds on the days that blood samples were collected. Total food intake and liveweight gain were measured between 21 and 49 days of age.

In both experiments, blood samples (3–5 ml), obtained by venepuncture of a brachial vein, were collected into evacuated heparinized tubes (Vacutainer, Becton-Dickinson, New Jersey). Plasma prepared soon after collection by centrifugation (1500 rpm, 612 *g*, 15 min) was stored at −20°C pending analyses for hormones.

Radioimmunoassays

All plasma samples analysed for a particular hormone from each experiment were assayed simultaneously to overcome interassay variation. For all assays, intraassay coefficients of variation were <15%. Sensitivities of assays were determined by the procedure of Burger *et al.* (1972).

Thyroid hormones

Plasma T_3 and T_4 were measured as described by Eastman *et al.* (1975). The reagents used to effect separation of hormones from binding globulins were 8-anilino-1-naphthalene sulfonic acid and sodium salicylate for T_3 and T_4, respectively. Rabbit antisera specific for respective hormones were obtained from Mr A. L. C. Wallace, Division of Animal Production, CSIRO, Prospect, N.S.W. Broiler plasma stripped of thyroid hormones with charcoal was used to adjust the protein contents of standards to approximate those of the unkown plasma samples. The ^{125}I-labelled hormones were obtained from Amersham International plc (Amersham, U.K.). Charcoal was used to separate antibody-bound and free, labelled hormone in both assays. The sensitivities of the assays of samples from experiments 1 and 2 were respectively 0.02 and 0.05 μg/l for T_3 and 0.14 and 0.16 μg/l for T_4.

A commercial radioimmunoassay kit (Dainabot Co. Ltd) was used to measure rT_3. The hormone was assayed at both sampling times on days 1, 3, 5, 10 and 15 in experiment 1 and in the samples collected at 1300 h on the first and third days after each increase in temperature in experiment 2. Stripped broiler plasma, obtained as above, was used to adjust the protein contents of standards. The sensitivity of the assay (samples from both experiments assayed simultaneously) was 3.0 ng/l.

Growth hormone

The talc radioimmunoassay procedure of Wallace and Bassett (1970) was used for measurement of plasma GH. Recombinant chicken GH (Amgen Biologicals, California) was used as standard and was labelled with ^{125}I, obtained from Amersham International plc, by the method of Hunter and Greenwood (1962). Rabbit antibody to recombinant chicken GH was obtained from Amgen Biologicals. The sensitivities of the assays for samples from experiments 1 and 2 were 0.51 and 0.80 μg GH /l respectively.

Statistical Analysis

Procedures described by Steel and Torrie (1980) were adopted. Split plot analyses of variance, with temperatures as the main plots and subplots as days of exposure and sampling times, were used. Significances of differences between means were determined by assessing least significant differences.

Results

Liveweight, Food Intake and Tissue Weights

In both experiments liveweight gain, final liveweights and food intakes of birds in the hot rooms were significantly ($P < 0.01$) lower than for birds in control rooms (Table 1). Temperature had no effect on the efficiency of utilization of food for liveweight gain in either experiment. Whereas birds in the control rooms consistently ate more food between 1100 and 1600 h (mean values of 7.5 and 7.8 g/h in experiments 1 and 2 respectively) than those in the hot rooms (3.3 and 4.6 g/h respectively), the latter birds ate more (6.8 and 6.7 g/h respectively) than the controls (6.1 and 5.9 g/h respectively) between 2300 and 0700 h.

In experiment 2, relative weights of abdominal fat at the end of the experiment (birds 54 days old) were similar in both environments (Table 1). A tendency for the relative weights of the thyroid glands to be lower in the birds in the hot rooms was not significant ($P > 0.05$).

Table 1. Performance of broilers at different ambient temperatures in both experiments

Control = constant 21°C; hot = 21°C, 2300 - 0700 h; 28°C, 0700 - 1100 h and 1600 - 2300 h; 35°C, 1100 - 1600 h. SED = standard error of difference. ** = $P < 0.01$; *** = $P < 0.001$.

	Experiment 1			Experiment 2		
Parameters	Control	Hot	SED	Control	Hot	SED
Initial liveweight[A] (g)	666	665	7.3	509	510	1.0
Final liveweight[B] (g)	2530	2323	54.5**	2243	2076	26.8***
Weight gain[C] (g)	1864	1658	49.7**	1734	1566	26.4***
Food intake[C] (g)	4210	3705	97.3***	3683	3343	32.9***
FCR[C] (g food/g gain)	2.259	2.235	0.0427	2.129	2.138	0.0275
Abdominal fat[D] (g/kg body wt)				23.1	23.8	1.90
Thyroid weight[D] (mg/kg body weight)				99.7	92.2	8.06

[A]Chickens 25 and 21 days old in experiments 1 and 2 respectively.
[B]Chickens 54 and 49 days old in experiments 1 and 2 respectively.
[C]Days 25-54 (experiment 1) and 21-49 (experiment 2).
[D]At 54 days of age, one group of five birds per treatment.

Plasma Hormones

Thyroid hormones

Plasma concentrations of thyroid hormones are given in Table 2 (experiment 1) and Table 3 (experiment 2).

In both experiments plasma T_3 was significantly ($P < 0.05$) lower for birds in the hot rooms, the effect being most marked during the periods of maximum temperature. Although in experiment 2 there was a significant ($P < 0.05$) increase in plasma T_3 in birds in the hot room on the first day of exposure to high temperature, at other times plasma T_3 was reduced by exposure to high temperature, the effect being most marked after day 7 when maximum daily

temperature was increased to 35°C. In addition, plasma T_3 decreased significantly ($P < 0.001$) with age in both experiments.

Overall, in both experiments plasma T_4 was significantly ($P < 0.05$) increased in birds in the hot rooms, the effect being most marked during the periods of maximum temperature. However, on the first day of exposure of birds to high temperature in experiment 1, plasma T_4 in birds in the hot room was lower than for birds in the control room at both sampling times. At all other times in both experiments plasma concentrations were similar or lower for birds in the control rooms. In experiment 2 the effects during the period of maximum temperature were most marked after day 7, when the maximum temperature was increased to 35°C. As birds increased in age in both experiments, plasma T_4 increased ($P < 0.001$) as did the magnitude of the response to high temperature ($P < 0.05$).

Birds in the hot rooms on the first day of exposure had higher molar T_3/T_4 ratios at both sampling times in experiment 1 and during the period of maximum temperature in experiment 2. Thereafter, in both experiments the T_3/T_4 ratio was reduced during exposure to high temperatures, the ratios being similar in hot and cold rooms when temperatures were similar (viz. at 0100 h, 21°C).

Plasma rT_3 was variable in both experiments and, in 18% of the 76 samples assayed in experiment 1 (eight for birds in hot room and six for birds in control room), concentrations were below the limit of detection of the assay. No effect of temperature on plasma rT_3 was seen in either experiment.

Table 2. Mean values for plasma triiodothyronine (T_3), thyroxine (T_4) and reverse T_3 (rT_3) concentrations and molar T_3/T_4 ratios in broilers kept at different ambient temperatures in experiment 1

Values presented are means for five birds at each time

ND = not detectable; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Days of exposure	T_3 (μg/l)		T_4 (μg/l)		rT_3 (ng/l)		100× T_3/T_4 ratio	
	Noon	Night	Noon	Night	Noon	Night	Noon	Night
			Control Room					
1	1.23	1.15	9.14	8.00	38.2	80.7	17.8	17.7
2	1.08	1.02	7.90	5.30			17.9	26.5
3	1.04	1.13	3.74	4.29	31.2	3.5	51.2	32.7
5	1.02	0.73	3.39	4.49	26.0	19.3	39.8	21.9
10	0.71	0.90	4.90	8.29	27.7	31.2	18.0	13.7
15	0.64	0.54	5.29	6.04	24.2	24.7	14.7	11.3
20	0.67	0.49	9.60	12.03			8.6	5.6
Means	0.91	0.85	6.28	6.96	29.5	31.9	24.0	18.4
			Hot Room					
1	1.13	1.26	3.77	5.17	45.0	40.0	48.7	44.6
2	0.92	1.37	7.92	6.69			13.8	25.9
3	0.97	1.13	5.86	6.44	33.3	18.8	23.1	29.5
5	0.73	0.71	7.82	4.45	ND	35.0	12.5	12.7
10	0.57	0.49	8.40	9.56	22.5	14.0	9.0	6.2
15	0.58	0.36	10.15	10.26	57.8	26.7	7.0	5.4
20	0.24	0.35	17.54	10.40			2.0	4.3
Means	0.72	0.81	8.7	7.57	30.5	26.9	16.6	19.7
	Standard Error of Difference and Level of Significance							
Temperature (*T*)	0.51*		0.595*		5.69		3.23	
Day	0.083***		1.182***		7.75**		6.58**	
Time	0.037		0.369		3.75		1.23	
T × Day	0.120		1.658*		11.33		9.20*	
T × Time	0.063*		0.700*		6.82		3.45**	
Day × Time	0.107		1.369*		9.76*		6.97*	
T × Day × Time	0.154		1.924**		14.10**		9.76**	

Table 3. Mean values for plasma triiodothyronine (T_3), thyroxine (T_4) and reverse T_3 (rT_3) concentrations and molar T_3/T_4 ratios in broilers kept at different ambient temperatures in experiment 2
Values presented are means for three birds at each time. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Days of exposure	T_3 (μg/l)		T_4 (μg/l)		rT_3 (ng/l)	100× T_3/T_4 ratio	
	Noon	Night	Noon	Night	Noon	Noon	Night
			Control Rooms				
0	2.13	2.03	8.47	7.97		31.9	34.3
1	1.37	1.37	8.34	6.80	4.0	21.2	24.8
2	2.45	2.15	7.03	5.84		45.1	44.3
3	1.93	1.77	5.28	4.86	33.9	45.9	43.1
4	1.68	1.22	5.32	4.24	107.2	40.1	35.7
5	1.74	1.36	9.54	11.87		22.1	14.2
6	1.81	1.61	7.08	6.80	78.3	30.5	28.4
7	2.32	1.71	6.45	5.98	17.5	45.6	35.0
8	1.95	1.64	5.22	5.55		47.2	36.4
9	2.17	2.31	4.56	5.69	12.0	58.9	49.5
11	1.91	1.59	5.84	7.70		40.2	25.4
16	1.61	1.65	12.37	12.42		15.7	16.2
21	1.44	1.26	11.86	9.82		14.7	16.2
26	1.54	1.46	10.75	10.36		18.3	17.5
Means	1.86	1.65	7.72	7.57	42.2	34.1	30.1
			Hot Rooms				
0	1.84	1.79	7.36	6.35		32.7	40.2
1	2.02	1.52	8.89	7.84	20.8	27.6	24.4
2	1.42	1.91	8.16	6.33		21.2	41.7
3	1.55	1.56	5.66	4.20	15.0	37.7	46.6
4	1.53	1.20	6.10	5.65	115.7	30.2	27.1
5	1.27	1.54	10.50	9.53		15.9	20.1
6	1.31	1.48	8.05	6.27	86.5	19.8	30.1
7	1.19	1.24	6.74	5.05	29.5	24.3	31.1
8	1.08	1.27	7.06	5.51		20.9	29.5
9	0.96	1.97	9.11	5.18	39.2	13.2	50.5
11	1.42	1.41	9.28	7.04		21.5	25.4
16	0.80	1.05	13.38	11.86		7.1	10.9
21	0.94	1.19	16.45	10.09		7.6	15.8
26	0.88	1.13	20.90	14.27		5.4	9.7
Means	1.30	1.45	9.83	7.51	51.1	20.4	28.8
	Standard Error of Difference and Level of Significance						
Temperature (T)	0.046*		0.123*		8.1	0.50**	
Day	0.106***		0.710***		10.8***	3.47***	
Time	0.040		0.229***			1.21	
T × Day	0.152***		0.976**		16.1	4.76*	
T × Time	0.061***		0.260***			1.31***	
Day × Time	0.150**		0.934*			4.72	
T × Day × Time	0.214		1.308			6.57*	

Plasma growth hormone

Plasma GH decreased significantly with age in both experiments (Table 4). Even though substantial variations in plasma GH occurred, significantly ($P < 0.01$) higher values were obtained from birds in the hot temperatures in experiment 1.

Discussion

High temperatures reduced liveweight gain in both experiments due principally to reduced (9–12%) food intake, since the efficiency of utilization of food for liveweight gain was not

affected. The birds in the hot rooms attempted unsuccessfully to compensate for the reduction in food intake during the hot part of the day by increasing food intake during the coolest period — a time when the food intake of control birds was reduced.

Table 4. Mean values for plasma growth hormone concentrations (μg/l) in broilers kept at different ambient temperatures in both experiments

Values are for five and three birds in experiments 1 and 2 respectively. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

	Experiment 1				Experiment 2			
Days of	Noon		Night		Noon		Night	
exposure	Control	Hot	Control	Hot	Control	Hot	Control	Hot
0					13.85	8.52	7.19	3.51
1	16.0	21.7	11.6	13.8	5.96	4.17	14.44	6.52
2	11.9	14.9	12.5	10.1	3.73	2.82	4.65	12.85
3	11.6	22.2	8.2	8.2	1.25	12.23	17.83	10.98
4					11.25	8.47	6.37	6.57
5	9.5	21.7	8.6	30.5	2.17	1.98	1.55	13.02
6					3.80	2.35	12.55	8.25
7					2.48	4.70	1.62	6.93
8					2.28	3.63	5.25	3.53
9					2.85	2.17	4.82	11.07
10	7.1	8.1	9.4	10.9				
11					2.68	1.92	3.02	0.72
15	8.0	13.6	10.8	12.9				
16					0.02	3.42	1.65	1.67
20	8.2	6.7	8.5	8.6				
21					0.15	0.42	8.45	1.93
26					0.33	3.30	0.35	0.85
Means	10.3	15.6	9.9	13.6	3.77	4.29	6.41	6.31
	Standard Error of Difference and Level of Significance							
Temperature (*T*)	0.80**				0.828			
Day	2.19***				2.174**			
Time	0.79				0.775**			
T × Day	2.98*				3.076			
T × Time	1.13				1.134			
Day × Time	2.65**				2.988*			
T × Day × Time	3.64				4.227			

Although the heat-stressed birds grew more slowly, body composition was unaffected as evidenced by the data for abdominal fat in experiment 2. However, there was an indication that endocrine adaptations occurred in response to heat stress. A tendency for decreased thyroid weight in birds in the hot rooms in experiment 2 was correlated with changes in measured thyroid hormone concentrations.

In agreement with most other reports plasma T_3 was decreased, and plasma T_4 was increased, during the period of exposure to high temperatures when food intakes were reduced to between 44 and 59% of the intakes of birds in the control rooms. The birds in both experiments quickly adjusted to changes in ambient temperature as shown by the similar concentrations of plasma T_3 and T_4 in both hot and control rooms during the period when room temperatures were similar (viz. 0100 h, 21°C) and food intakes varied by between 11 and 14%. It is apparent that chickens were able to adjust to moderate increases in ambient temperature as shown by the relatively minor changes in plasma concentrations of thyroid hormones during the first days of experiment 2. It was not until the maximum ambient temperature was increased to 35°C that marked changes were measured in this experiment.

On the first day of exposure to high temperature in both experiments the molar ratio of T_3/T_4 increased, although the cause of the change differed. In experiment 1, the principal cause of the increased T_3/T_4 was a marked reduction in plasma T_4 whereas in experiment 2 the increased ratio of T_3/T_4 was due to an increase in plasma T_3. On subsequent days a decrease in the molar ratio of T_3/T_4 occurred as a result of an increase in plasma T_4.

It is apparent that the plasma of chickens up to 54 days of age contains measurable concentrations of rT_3 and that, in spite of the increased plasma T_4 and decreased plasma T_3 in birds exposed to high temperatures, there were no changes in plasma rT_3. Accordingly, the present results do not indicate that the formation of rT_3 serves as an alternative pathway to T_3 formation in heat-stressed chickens.

Plasma GH increased during the period of exposure to maximum temperatures in both experiments. These observations are consistent with reports that plasma GH increases during summer months (Scanes *et al.* 1983) and with restriction in nutrient intakes (Scanes and Harvey 1981).

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