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Genetics of heat tolerance and thyroid function in Athens-Canadian randombred chickens *

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Summary. Five experiments were conducted to assess the genetic variation in thyroid function (T_3, T_4), body weight and heat stress survival time in chickens. Thyroxine (T_4) levels were found to be elevated in response to 4 and 8 µg bovine thyroid stimulating hormone (TSH) in experiment I. In experiment II, 4 µg of TSH was injected into chickens from 30 sire families of the Athens-Canadian Randombred population. The heritability of T_4 levels after TSH injection was high. In experiment III, families identified as having innate high or low T_4 levels after TSH injection and a group of control birds were subjected to a heat stressor of 50 °C for up to 240 min at six weeks of age and heat stress survival time was studied. The groups did not differ from each other in heat stress survival time. Experiment IV was similar to experiment I except triiodothyronine (T_3) was also measured after TSH injection. Both T_4 and T_3 levels after TSH injection were moderately heritable. In experiment V birds were reared to six weeks of age and heritability calculated for body weight, T_4, T_3, and heat stress survival time. Heritabilities were high for body weight, moderate for T_4 and heat stress survival time, and low for T_3. Phenotypic correlations were significant and negative for heat stress survival time with body weight and T_4, and for body weight with T_3 after TSH. Significant positive correlations were found for T_4 with T_3 after TSH and also T_4 and body weight. Analysis of genetic correlations suggested that none of the traits studied would be an adequate selection parameter for achieving heat tolerance without reducing body weight.

Key words: Genetics – Heat tolerance – Thyroid function – Chickens

Introduction

The susceptibility of chickens to extreme heat results in a problem of economic importance to the poultry industry. Because little can be done to improve heat tolerance in chickens by management, the genetic development of chickens with innate heat tolerance would be of value.

Bowen and Washburn (1984) reported a low to moderate heritability for heat stress survival time (HSST) in Japanese quail and found that heat stress survival time had a negative genetic correlation with body weight. Because of the economic importance of large body size in poultry, they suggested that selection for longer heat stress survival time without consideration of body weight would not be an advisable means of achieving heat tolerance. They suggested, however, that attempts should be made to identify other physiological selection parameters that might result in improved heat tolerance without reducing body weight.

Several studies have suggested a relationship between thyroid status and heat stress survival time. Bowen et al. (1984) reported an increased survival time for either radiothyroidectomized or thiouracil treated broiler chickens exposed to 50 °C for up to 4 h; May (1982a) and Bowen et al. (1984) reported a decrease in heat stress survival time in chickens treated with triiodothyronine (T_3) and thyroxine (T_4). A similar study in laying hens showed a decrease in heat stress survival time following T_4 injections and prolonged heat stress survival time following thiouracil feeding (Fox 1980). None of these pharmacological means of reducing thyroid function have practical application, but if thyroid function could be genetically reduced the heat tolerance of commercial chickens might be improved without decreasing body weight.

Limited studies suggest that genetic changes altering thyroid function may be possible. Cole (1966) developed an obese strain of Leghorns that has a high incidence of autoimmune thyroiditis. Several single genes have also been reported to affect thyroid function. These include the gene for TSH responsiveness (Fodor and Pethes 1974), the frizzling

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gene (Landauer and Aberle 1935), the sex-linked dwarfing gene (Scanes et al. 1982; Stewart and Washburn 1982) and the autosomal dwarfing gene (Scanes et al. 1982). Line or strain differences in thyroid function in avian species have also been reported (Howarth and Marks 1972, 1973; May and Marks 1983; Burke and Marks 1984). No data are available, however, to describe additive genetic variation in thyroid hormone levels in avian populations.

MacKenzie (1981) has described a bioassay for TSH using one to three day old Leghorn cockerels and a radioimmunoassay for T_4 as the end point. Standard responses were estimated by injections of differing dosages of bovine TSH. It was hypothesized that the T_3 and T_4 levels after injection of a single fixed dose of bovine TSH could be a means of assaying genetic variability in chickens.

The objectives of these studies were to describe for a randombred broiler-type population of chickens: 1) the quantitative genetics of heat stress survival time, body weight, and four indicators of thyroid function and 2) the relationships between these traits. Indicators of thyroid function include T_4 and T_3 levels after TSH injection in three-day-old chicks and basal T_4 and T_3 levels in six-week-old chickens. Each of these traits was considered as a possible selection parameter by which heat tolerance in chickens could be improved without reducing body weight.

Materials and methods

Experimental procedures

Experiment I: MacKenzie assay in broiler chickens

Experiment I was conducted to evaluate the suitability of using broiler cockerels in MacKenzie's (1981) bioassay for TSH. Fifty-four newly hatched male commercial broiler chickens from a local hatchery were subjected to TSH treatment according to MacKenzie (1981). The birds were divided into three equal groups of 18 and given a total dosage of 0, 4 or 8 µg bovine TSH (Sigma Chemical Company, Stock No. TS-10, Lot Not. 61F-0320). Half of the total dosage of TSH was administered at 24 h after hatching (morning) and the second half 48 h after hatching. The TSH was dissolved in saline and injected subcutaneously in the neck in a volume of 100 µl. Five hours after the second TSH injection, birds were bled by decapitation. Serum thyroxine levels were determined by radioimmunoassay.

Experiment II: genetic variation in T_4 levels after TSH injection in a broiler chickens population

The MacKenzie (1981) bioassay for TSH was used to assess the genetic variability of T_4 levels after TSH injection using three-day-old chickens from a randombred population of broiler-type chickens. Eight hundred-fifty chickens from 30 sire families of the Athens Canadian Randombred population (Hess 1962) pedigreed for sire and dam were used. The MacKenzie (1981) assay was modified from experiment I so that all chicks received 4 µg TSH in two injections. Bleeding by decapitation was done by families between 5 and 9 h after the second injection. Approximately half of the chicks were sexed so that the effect of sex on T_4 levels after TSH could be analyzed.

Experiment III: T_4 levels after TSH in neonates and for six week HSST

The relationships of four-week body weight and six-week heat stress survival time (HSST) to T_4 levels after TSH injections in day-old chickens were determined. Chickens from a second hatch of the same population as those in experiment II were wingbanded and reared in floor pens by standard growing procedures. All birds were weighed at four weeks of age. At six weeks of age, groups of sire families identified in experiment II as having offspring with either high or low (six families each) T_4 levels after TSH injection and six control groups from the intermediate families were heat stressed. Seventy-five chickens from each group and 35 chickens from the control group were heat stressed intermingled in four replicates.

Experiment IV: genetic variation in T_3 and T_4 levels after TSH injection in three-day old chickens

In experiment IV variability in T_3 and T_4 levels was assessed after injection with TSH. It was a repeat of experiment II with slight modifications. First, 30 sire families of the Athens Canadian Randombred population different from those used in experiment II were studied. Secondly, chicks were individually randomized so that genetic variation would not be biased by the order of bleeding. Also, all birds were sexed and the sex effect accounted for in the genetic analysis. Finally T_3 levels as well as T_4 levels were measured by radioimmunoassay.

Experiment V: genetic variation in T_3 and T_4 levels, body weight and heat stress survival time

The objectives of experiment V were: 1) to assess the genetic variability in T_3 and T_4 levels, body weight, and heat stress survival time at six weeks of age, 2) to determine the genotypic and phenotypic relationships between these traits, and 3) to correlate these traits to those from experiment IV. Pedigreed offspring from the same 30 sire families used in experiment IV were reared by standard procedures to six weeks of age. At this time heat stress survival time, body weight, and plasma T_4 and T_3 levels before heat stress were determined.

Hormone assays. Thyroxine (T_4) and triiodothyronine (T_3) were determined using double antibody type radioimmunoassays. Rabbit γ-globulin and goat antirabbit γ-globulin were obtained from Antibodies, Inc., Davis, CA. ^{125}I-labeled T_4 and T_3 were obtained from New England Nuclear. The primary antibody for the T_4 assay was from Antibodies, Inc. and it was used according to their procedures. For the T_3 assay the primary antibody of May (1978) was used following his procedure as modified by Stewart and Washburn (1984).

Heat stress survival time determination. Chickens to be heat stressed were placed in either a forced draft heat chamber with the temperature maintained at 50 ± 1 °C (Experiment III) or in a larger chamber where temperature was maintained at 48 ± 2 °C (Experiment V). Heat stress survival time was defined as the amount of time elapsed from the time chicks were placed in the chamber until they could no longer stand erect. All birds were removed at 240 min.

Statistical and genetic analyses. Analysis of variance and correlations were conducted using the procedures of the Statistical Analysis System (SAS) (SAS Institute Inc. 1982). Treatment effects were analyzed using the General Linear Models (GLM) procedure of SAS along with Duncan's multiple range test to separate means (Duncan 1955). Variance and covariance components for genetic analysis were also obtained with the GLM procedures of SAS as described by Scott and Washburn (1984). For experiment II the model used was:

$$Y_{ij} = S_i + e_{ij}$$

where Y_{ij} is the T_4 level of the j_{th} offspring of the i_{th} sire. For experiments IV and V, the sex of all birds was known so that the sex effect was also analyzed using the model:

$$Y_{ijk} = S_i + S_{ij} + e_{ijk}$$

where Y_{ijk} is the record of the k_{th} offspring of the j_{th} sire belonging to the i_{th} sex.

Different offspring from the same sire and dam families were used in experiments IV and V so phenotypic correlation between those traits measured in experiment IV (T_3 and T_4 after TSH injection) and those measured in experiment V (T_3, T_4, HSST, BW) were estimated using dam family means instead of individual records. Covariance components for estimating genetic correlations were obtained using the model:

$$Y_{ij} = S_i + D_{ij}$$

where Y_{ij} is the mean of the offspring from the j_{th} dam mated to the i_{th} sire.

From the components of variance and covariance described above heritability (h^2) estimates and their standard errors and genotypic correlations (r_g) were determined using the procedures of Becker (1975).

Table 1. Thyroxine (T_4) levels of neonatal broiler chickens after injection of bovine thyroid stimulating hormone (experiment I)

TSH dose (μg)	T_4 (ng/ml)
	$\bar{X} \pm SE$
0	43.2 ± 3.2^a
4	80.9 ± 8.2^b
8	170.8 ± 17.3^c

[a, b, c] Means within the same column with different superscripts are significantly different ($P \leq 0.05$)

Results and discussion

Application of TSH bioassay to broiler chicks

The T_4 levels of broiler chicks after injection of 0, 4 or 8 μg of TSH were 43 ng/ml, 81 ng/ml, and 171 ng/ml respectively [experiment I (Table 1)]. These data indicate that neonatal broiler-type chicks exhibit a dose-related elevation of T_4 levels after TSH injections as did the Leghorn chicks used by MacKenzie (1981) and may be used as assay animals in a TSH bioassay.

Values measured and sex effects

Thyroid hormone levels after TSH injection in neonatal chickens sorted by sex are given in Table 2. Thyroxine values of birds treated with TSH in experiment IV (74 and 87 ng/ml for males and females, respectively) are higher than those in experiment II (38 and 42 ng/ml for both sexes). This difference may be due to interassay difference in T_4 estimation or differing TSH solutions used. In both experiments there was a significant sex effect on T_4 and T_3 levels. Thyroxine levels for males were 88 and 85% as high as those for females in experiment II and IV, respectively. In experiment IV mean T_3 levels after TSH treatment for males (4.3 ng/ml) were 95% those of females (4.5 ng/ml). This sex difference should be considered in the application of the MacKenzie (1981) assay. In six-week-old chickens T_4 levels were slightly higher in females (44 ng/ml vs. 42 ng/ml) while the mean T_3 level for both sexes was 2.3 ng/ml (Table 3). Mean body weight of 439 g for males was significantly higher than the mean body weight of 384 g for females as expected. In agreement with the work of Reece et al. (1972) heat stress survival time was longer in females (145 min) than in males (132 min).

Heat tolerance in families differing in thyroid function

Chickens from families previously identified as having high or low T_4 levels did not differ in heat stress

Table 2. Mean ($\bar{X}$), standard errors (SE) and heritabilities (h^2) of thyroid hormone levels by sex after TSH injection in neonatal chickens (experiment II and IV)

	Experiment II			Experiment IV					
	T_4 (ng/ml)			T_4 (ng/ml)			T_3 (ng/ml)		
	n	$\bar{X} \pm SE$	$h^2 \pm SE$	*n*	$\bar{X} \pm SE$	$h^2 \pm SE$	*n*	$\bar{X} \pm SE$	$h^2 \pm SE$
Male	208	38 ± 1.3^a		293	74 ± 1.8^b		304	4.3 ± 0.08^b	
Female	196	42 ± 1.3^b		299	87 ± 3.9^a		307	4.5 ± 0.09^a	
Combined	819*	41 ± 1.3	0.63	562**	81 ± 2.6	0.12 ± 0.08	581	4.4 ± 0.08	0.28 ± 0.12

[a, b] Means within the same column with the same superscript do not differ significantly ($P \leq 0.05$)

n represents the number of observations per group

* All chicks were not sexed in experiment 2

** h^2 was calculated on fewer than the total number of animals because of missing pedigree data

Table 3. Means ($\bar{X}$), standard errors (SE) and heritabilities (h^2) of body weight (BW), heat stress survival time (HSST), plasma thyroxine (T_4) and plasma triiodothyrodine (T_3) in 6 week old chickens by sex (experiment 5)

Sex	T_4 (ng/ml)			T_3 (ng/ml)			Heat stress survival time (min)			Body wt (g)		
	n	$\bar{X}\pm$SE	$h^2\pm$SE	*n*	$\bar{X}\pm$SE	$h^2\pm$SE	*n*	$\bar{X}\pm$SE	$h^2\pm$SE	*n*	$\bar{X}\pm$SE	h±SE
Male	320	42 ± 0.6^a	–	297	2.3 ± 0.06^a	–	396	132 ± 3^b	–	398	439 ± 4^a	–
Female	290	44 ± 0.7^a	–	276	2.3 ± 0.07^a	–	391	145 ± 3^a	–	387	384 ± 4^b	–
Combined	570*	43 ± 0.6^a	0.22 ± 0.10	532*	2.3 ± 0.06^a	-0.02 ± 0.08	756	138 ± 3^a	0.37 ± 12	754	412 ± 4^a	0.72 ± 0.19

[a,b] Means within the same column with the same superscript do not differ significantly

n represent the number of observations per treatment group

* h^2 was calculated on fewer than the total number of animals because of missing pedigree data

survival time (Table 4). The body weight was similar for the two groups, both of which had lower body weight than controls. Since those families with differences in T_4 levels after TSH injections did not differ in heat tolerance in this study, one would not expect to genetically improve heat tolerance in growing chickens by selecting for lower T_4 levels after TSH injection.

Heritability estimates

A measure of genetic variability for T_4 and T_3 after TSH injection in neonates (Table 2) and T_4 and T_3 levels, body weight, and heat stress survival time at six weeks (Table 3) is given by heritability estimates (h^2). The h^2 estimate for T_4 levels after TSH injection was very high in experiment II (0.63) but relatively low in experiment IV (0.12). Several factors may have contributed to these differences. In experiment II, the birds were bled by families and the bleeding lasted for approximately 4 h. The mean T_4 levels were 46.5, 40.8 and 35.6 ng/ml for the first, second and final ten families bled indicating that variation between time and families was confounded to some extent. In experiment IV chicks were randomized before bleeding which subsequently took less than 2 h. Also, the effect of sex was removed when calculating the variance component due to sire family in experiment IV. Since T_4 levels were higher in experiment IV than experiment II, the hormone values in experiment IV may have approached a plateau reducing the variation in this trait and therefore the h^2 estimate.

Heritability for T_3 levels after TSH in neonates was moderate (Table 2) while T_3 levels without TSH priming in six-week-old broilers did not appear to be heritable (Table 3). A moderate heritability was estimated for T_4 levels at six weeks of age. It appears that there is additive genetic variation in T_4 levels but not for T_3 levels. In establishing experimental lines of chickens with genetically altered thyroid function, it may be

Table 4. Heat stress survival time and body weight in families with high or low plasma thyroxine levels after TSH injection into neonates (experiment III)

Group*	T_4 ng/ml	Body wt (g)		Heat stress survival time (min)	
		n	$\bar{X}\pm$SE	*n*	$\bar{X}\pm$SE
High	54	117	$210^a\pm5$	80	192 ± 4^a
Low	29	104	$202^{a,b}\pm5$	80	185 ± 4^a
Controls	41	158	$196^b\pm1$	40	184 ± 4^a

[a, b] Means within the same column with the same superscript do not differ significantly

n represents the number of observations per treatment group

* T_4 levels of these families were determined in experiment II

more feasible to select divergently for T_4 levels in six-week-old chickens than the other traits studied. The coefficients of variation were considerably higher for T_3 than for T_4. This suggest that to obtain reliable estimates for heritability and correlations with the T_3 RIA used, a much larger number of individuals should be used.

The heritability estimate for body weight in this study was high and in agreement with previous reports (Kinney 1969; Siegel 1962). The heritability estimate for heat stress survival time was moderate and comparable to those of Saegusa et al. (1976) and Bowen and Washburn (1984) for quail and Wilson et al. (1966) for chickens.

Phenotypic correlations

There were significant phenotypic correlations for several of the traits (Table 5). Most important are the correlations between heat stress survival time and the other traits measured. Heat stress survival time had a significant negative correlation of –0.15 with T_4 and –0.42 with body weight, while heat stress survival time was not significantly correlated with T_3. Neither of the estimated correlations between heat stress survival time and T_4 or T_3 levels after TSH was significant. The negative correlation between heat stress survival time and T_4 levels is in agreement with previous reports that birds with experimentally lowered thyroid status had improved heat tolerance while those birds with increased thyroid status were less heat tolerant (Bowen et al. 1984; May 1982a; Fox 1980). The observation of a significant correlation between heat stress survival time and T_4 with no correlation between heat stress survival time and T_3 raises questions concerning the physiological activity of these two thyroid hormones relative to each other. Chandola and Bhatt (1982) suggest that in birds T_4 has physiological effects independent of its extrathyroidal deiodination to T_3.

The correlation of –0.42 between heat stress survival time and body weight is comparable to the reports of Washburn et al. (1980) for chickens and Bowen and Washburn (1984) for quail. There was a significant correlation of –0.23 between body weight at six weeks of age and T_3 after TSH injection in neonates and a correlation of –0.16 for body weight and T_4 after TSH injection suggesting a relationship between thyroid function early in life and growth. However, the magnitude of the correlations in this experiment indicates this association is slight.

Body weight was significantly correlated with T_4 levels (0.18) at six weeks suggesting a relationship between body weight and T_4 levels. In earlier work in which the effects of thyroid hormone on body weight was studied, iodinated casein gave little or no growth response (Irwin et al. 1943; Parker 1943; Newcomer 1976). These results suggest a lack of thyroid hormone effect on growth but these results must be interpreted with caution since it is not known whether increased plasma levels of T_3 and T_4 were elicited by iodinated casein. May (1980, 1982b) reported no growth response to dietary T_4, but a growth depression with one ppm T_3, even though plasma hormone levels were elevated. The data in the present study indicate that within a physiological range there is a positive relationship between plasma T_4 and body weight in six-week-old chickens.

Estimated correlation between T_3 and T_4 after TSH injection was low but significant (0.15) suggesting some relationship between the levels of the two hormones after TSH injection.

Table 5. Phenotypic correlations (r_p) for thyroid hormones (T_3, T_4), heat stress survival time (HSST) and body weight (BW) in chickens (experiments IV and V)

Trait	Experiment 4 (neonatal)				Experiment 5 (six weeks old)					
	T_4 after TSH		T_3 after TSH		T_4		T_3		Body wt	
	n^b	r_p^a	n	r_p	n	r_p	n	r_p	n	r_p
Neonatal										
T_3 after TSH	546	0.15*								
Six weeks old										
T_3	121	0.05	122	0.10	544	0.05				
Body wt	122	−0.16	123	−0.23*	592	0.18*	555	−0.02		
Heat stress survival time	122	−0.05	123	0.15	591	−0.15,	554	−0.02	775	−0.42*

[a] r_p between T_4 after TSH and T_3 after TSH is based on individual observations but r_p between each of these traits and all other raits is based on correlation of dam family means

[b] n represents the number of observations or means used in calculating r_p

* Indicates that r_p is significantly different from 0 at $\alpha = 0.05$ level of significance

Table 6. Genotypic correlations (r_g) for thyroid hormones (T_3, T_4), heat stress survival time (HSST) and six week body weight (BW) in chickens (experiments IV and V)

Trait	Experiment 4 (neonatal)				Experiment 5 (six weeks old)					
	T_4 after TSH[a]		T_3 after TSH		T_4		T_3		BW	
	n[b]	r_g	n	r_g	n	r_g	n	r_g	n	r_g
Neonatal										
T_3 after TSH	514	−0.24								
Six weeks old										
T_4	120	0.80	120	0.28						
Body wt	120	−0.75	120	−0.35	481	0.41	481	0.72		
Heat stress survival time	120	−0.02	120	0.17	481	−0.07	481	0.25	481	−0.71

[a] r_g between T_4 after TSH and T_3 after TSH is based on individual observations but r_g between each of these traits and all other traits is based on covariance components of dam means within sire families

[b] n represents the number of observations or means used in calculating r_g

Genetic correlations

In agreement with the work of Bowen and Washburn (1984) on quail, there was a negative genetic correlation between body weight and heat stress survival time ($r_g = -0.71$) (Table 6). Thyroxine, after TSH, had a very low genetic correlation to heat stress survival time (−0.02) while it was highly negatively correlated to body weight. Thus, selecting for low T_4 levels after TSH in neonates may improve growth but not heat tolerance. However, a weight difference was not observed in experiment III where chicks from families high and low for T_4 after TSH were weighed at four weeks of age (Table 4). T_4 levels at six weeks of age is a heritable trait ($h^2 = 0.22$) and has a low negative genetic correlation with heat stress survival time (−0.07) and a high positive genetic correlation (0.41) with body weight. Further study using divergent selection for six week T_4 levels is needed to describe the genetic relationship between thyroid function, body weight, and heat tolerance.

Although genetic correlations involving T_3 were obtained the trait appears to be practically nonheritable (Table 3) and the unrealistic correlation values obtained for T_3 with other traits suggest that the data were inadequate to estimate correlation of T_3 with other traits.

Conclusions

Even though heat stress survival time is a heritable trait in chickens, commercial breeders should not select for this trait without considering body weight. Such a selection would likely result in decreased body weight. None of the indicators of thyroid function tested as selection parameters for heat tolerance would be an ideal selection parameter. The results of this study indicate, however, selection for high T_4 levels would yield increased body weight with little reduction in heat stress survival time.

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