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Original article

Growth hormone response to TRH in male broiler chickens selected for body weight gain or food conversion and reared at either a moderate or a high ambient temperature

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Summary — The aim of the present experiment was to study the growth hormone (GH) response upon thyrotropin releasing hormone (TRH) challenge (2 µg/kg body weight) in broiler chickens selected for body weight gain (GL line: fat line) or for feed efficiency (FC line: lean line) reared at either a moderate (33–23 °C) or high (33 °C) ambient temperature. A higher plasma GH level at 5 min after TRH administration was observed in the high temperature conditioned chickens of both lines. Also at high ambient temperature, an enhanced GH decrease between 15 min and 30 min post-injection and a higher acute elimination rate was calculated compared to moderate ambient temperature. A significantly higher GH secretory response was observed in the leaner FC line chickens, which was probably related to the more pronounced pulsatory GH secretion rate in these chickens. There was no difference in GH acute elimination rate between both lines in both environments. No interactions between line and rearing temperature for these parameters of GH dynamics were observed.

TRH administration / plasma GH elimination / ambient temperature / lean broiler / fat broiler

Résumé — Réponse de GH à une injection de TRH chez les poulets de chair sélectionnés pour leur croissance ou leur capacité de conversion alimentaire et élevés à température forte ou modérée. *Le but de cette expérience était d'étudier la réponse de l'hormone de croissance (GH) à une stimulation par l'hormone provoquant la sécrétion de thyrotropine (TRH) chez des poulets de chair. Les animaux provenaient de lignées sélectionnées en vue d'une croissance rapide (lignée GL ou lignée grasse) ou bien d'un taux élevé de conversion alimentaire (lignée FC ou lignée maigre). Ils ont été entretenus, soit à température ambiante élevée (33°C), soit à température moyenne (33°–23°C). Nous avons observé un niveau de GH plus élevé dans le plasma 5 min après l'administration*

de TRH dans les 2 lignées de poulets élevés dans un environnement chaud comparé à l'environnement moyen. De même, nos calculs ont montré un déclin du niveau de GH plus prononcé et un taux d'élimination par unité de temps plus rapide entre 15 et 30 min après l'injection chez les poulets maintenus à température élevée. La hausse de sécrétion de GH après stimulation a été significativement plus élevée chez les poussins de la lignée FC, ceux-ci montrant d'ailleurs un niveau spontané des pulsations sécrétrices de GH de plus grande amplitude que ceux de la lignée GL. Nous n'avons pas trouvé de différence dans le taux d'élimination de GH selon les lignées dans les 2 conditions d'environnement. Il n'y a pas d'interaction entre l'effet de la lignée et celui de la température d'élevage pour ces paramètres concernant la dynamique de GH.

administration de TRH / taux d'élimination de GH / température ambiante / poulet de chair maigre et gras

INTRODUCTION

It has been well established that thyrotropin releasing hormone (TRH) is a potent growth hormone (GH) secretagogue in young chickens (Harvey and Scanes, 1984) and it may well be the principal endogenous stimulatory releasing factor in chickens since passive immunization with anti-TRH suppressed GH secretion (Klandorf *et al,* 1985). The episodic nature of GH secretion has been described both in laying (Buonomo *et al,* 1984) and meat-type (Vasilatos-Younken and Leach, 1986) chickens, as well as in broiler lines divergently selected for body weight gain (GL line) or food conversion (FC line) (Leenstra and Pit, 1988) where the latter showed a peak amplitude almost twice the value of that in the GL line (Decuypere *et al,* 1991). To our knowledge, the influence of environmental temperature on GH pulses or GH response to TRH is not yet known. However, *in vivo* GH response of immature birds to TRH and GH releasing factor (GRF) was suppressed by administering T_3 while systemic administration of T_3 was followed by a downregulation of pituitary TRH binding sites (Harvey *et al,* 1991). Since temperature changes thyroid hormone levels (Decuypere and Kühn, 1988) and T_4 turnover rate (Kühn *et al,* 1984), a changed interaction of the thyrotropic-somatotropic axis may result in an altered GH response to TRH. Because the divergently selected broiler lines showed marked differences in GH levels and in GH pulse amplitudes, the effect of temperature on TRH induced GH increase was studied in both lines.

MATERIAL AND METHODS

Four-week-old male broiler chickens from the GL and FC lines were used. The history of these selection lines has been reported by Leenstra and Pit (1988) and production characteristics have been extensively described elsewhere (Leenstra *et al,* 1991). Endogenous GH was stimulated indirectly by a single injection of TRH (2 µg per kg body weight: purchased from Sigma, St Louis, MO, USA) in a brachial arterial cannula of fed conscious animals. Blood samples (0.3 ml) were collected just before TRH injection and 5, 15, 30 and 60 min thereafter. Birds of both lines were reared either in a hot (constant 33 °C) or a moderate (step-down from 33 to 23 °C over a 4-week period) environment. This resulted in a 2-factorial model (genotype–temperature) the final animal number being: GL moderate = 11; Gl hot = 10; FC moderate = 9; FC hot = 14.

GH assay

Blood samples were collected in heparinized tubes, centrifuged, and plasma stored at –18 °C

prior to cGH determination with a homologous radioimmunoassay (Berghman *et al,* 1988). Affinity-purified cGH was iodinated using the chloramine T method (Greenwood *et al,* 1963). Twenty µl sample or standard GH (2-200 ng/ml) was incubated for 24 h with 100 µl mouse monoclonal GH antibody (1/2-10^6 ascites) and 100 µl tracer. After adding 50 µl goat antimouse antiserum (1/40 dilution) a second 24-h incubation period followed. After centrifugation at 2 400 *g* for 10 min, the supernatant was aspirated and the precipitate was counted in a Q-counter. The system detection limit was 2 ng/ml and no cross-reactivity with other pituitary hormones was observed. The intra- and inter-assay variation coefficient was 4 and 15.5% respectively. Good parallelism between standard and plasma dilution curves was obtained. For determination of peak levels, dilution series were needed to meet the standard curve of the assay. Inter-dilution variability within the assay, however, was sometimes very high (up to 60%). The mean values for GH in dilution series, which generally fitted the disappearance curve more closely, were used in these cases.

Statistical analysis

Statistical differences between measured or calculated parameters were revealed by parametric (ANOVA or MANOVA) or non-parametric statistics (Bartlett test or Mann–Whitney *U*-test) where appropriate. Comparison of groups of curves from individual birds was made by analysis of variance after transformation of the time series data of individual birds into orthogonal polynomials (GLM, repeated measurements with polynomials: SAS, 1986). Alternatively, the coefficients of the curves that were fitted to the individual data were used as a new data set, which was then compared between groups by a multivariate analysis of variance (MANOVA). Visual illustration of maximal differences between groups resulted from multivariate canonical discriminant analysis (CANDISC: SAS, 1986) on the new matrix with the coefficients of the curves as new data. Although the canonical procedure is usually applied to evaluate complex multivariate data matrices, here it was only used to obtain an optimal rotation of the picture. Acute elimination rates were calculated as the differential of the curve that fitted the hormone-disappearance data. This variable represents the slope of the tangent line to the curve at each point, and thus the steepness of the direction the curve is taking. Curve fitting also followed an exponential approach ((GH(ng/ml) = $ae^{-b.time}$)) and the half-life was calculated as ln (0.5/*b*). Curve fittings and calculations used the data of individual birds as entries without correction for extrapolated baseline values.

RESULTS

TRH-induced GH release in both lines and environments is shown in figure 1.

The increase in plasma GH levels following TRH injection was characterized by the difference in GH at 5 min post-injection minus the value noted immediately before injection (table I). This increase was significantly different according to line (FC > GL; F_{40}^{1} = 6.22, P = 0.014) and the effect of temperature was even more manifest (hot > moderate; F_{40}^{1} = 8.9, P = 0.005). There was no line x temperature interaction (F_{40}^{1} = 0.47, P = 0.5), and no effect of the pre-injection value (pre-value as a covariable did not influence ANOVA results). Only 4 post-injection data points represent a rather poor picture for curve fitting and time pattern evaluation. Nevertheless, the obvious time pattern of GH disappearance

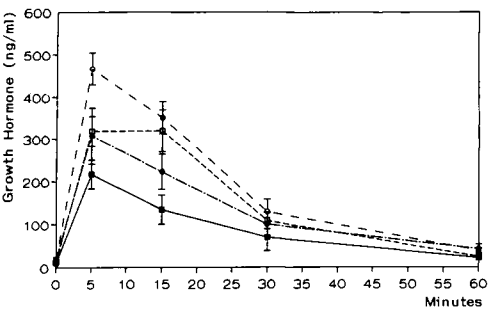

Fig 1. TRH-induced GH responses according to line (GL or FC) and temperature (GL 33 °C, □ GL 23 °C ■, FC 33 °C O, FC 23 °C ●). Time 0 = injection of TRH.

Table I. TRH induced GH (ng/ml) increases (difference between concentration before injection and 5 min after) in 2 lines of chickens at 2 temperatures.

Temperature (°C)	*GL line*	*FC line*
23	206 ± 43.2 (11)	289 ± 47.8 (9)
33	307 ± 45.4 (10)	450 ± 38.3 (14)

Values are means ± SEM. ( ) : No of chickens. Line effect $P = 0.014$; temperature effect $P = 0.005$; no interaction.

Table II. GH decrease (ng/ml) according to temperature and line between 15 and 30 min following TRH injection.

Temperature (°C)	*GL line*	*FC line*
23	64 ± 25 (11)	113 ± 26 (9)
33	207 ± 48 (10)	223 ± 42 (14)

Values are means ± SEM. ( ) : No of chickens. Temperature effect : $P = 0.002$.

was highly significant ($F_{36}^{3} = 19.8$, $P < 0.000\ 1$). There was an effect of line (ANOVA with polynomials covaried for pre-value: $F_{38}^{1} = 4.3$, $P < 0.05$), and also particularly of temperature ($F_{38}^{1} = 11.8$, $P < 0.002$). There was also a significant interaction of time x temperature ($F_{36}^{3} = 5.2$, $P < 0.005$) which represents a temperature-dependent difference in the GH disappearance pattern. The hot environment resulted in a higher response of GH, but subsequently in a much steeper decline. There was a highly significant temperature effect on the decrease in GH levels between 15 and 30 minutes post-injection (table II: 2-factor ANOVA: $F_{39}^{3} = 11$, $P = 0.002$; hot > moderate). There was no significant effect of line or of line x temperature on this aspect of GH decrease. When the 2 coefficients of the individual exponential curves (table III) were compared in a 2 x 2 factor multivariate ANOVA, similar general conclusions about the hormone disappearance were obtained. The effect of temperature was more important (Wilks' Lambda: 0.76, $F_{39}^{2} = 6.2$, $P < 0.005$) than the effect of line (Wilks' Lambda = 0.84, $F_{39}^{2} = 3.6$, $P < 0.05$) and there was no line x temperature interaction (Wilks' Lambda = 0.93, $F_{93}^{2} = 1.4$, $P = 0.3$). However, because there was a significant effect of line and temperature only on the *a*-coefficient of

Table III. Curve characteristics (mean ± SEM) of the exponential fitting ((GH(ng(ng/ml) = $ae^{-b.time}$)).

	GL line		*FC line*	
	23 °C	*33 °C*	*23 °C*	*33 °C*
n birds	11	10	9	14
a-Coefficient	307 ± 50	436 ± 50	416 ± 95	645 ± 57
b-Coefficient	−0.062 ± 0.007	−0.041 ± 0.004	−0.048 ± 0.010	−0.051 ± 0.007
r^2	0.973 ± 0.011	0.953 ± 0.013	0.977 ± 0.007	0.965 ± 0.013

Significant effect of temperature ($P < 0.005$) and line ($P < 0.05$) on *a*-coefficient but not on *b*-coefficient.

the exponential fittings, there was no significant difference according to line or temperature in the half-lives deduced from the exponentials (Kruskal–Wallis, $P = 0.13$). Acute elimination rates, calculated as the differential of the second power polynomial fitted to the hormone disappearance data of individuals, did not differ according to line (ANOVA, Wilks' Lambda = 0.90, $F_{39}^2 = 2.25$, $P = 0.12$) but showed and effect of temperature (MANOVA, Wilks' Lambda = 0.72, $F_{39}^2 = 7.7$, $P < 0.002$). There was no line x temperature interaction. These differences can be illustrated by plotting the 2 coefficients of the differentials of the individual curves (fig 2), but visualization of the significant effects can be optimized by a canonical discriminant procedure, which maximizes the differences between groups (fig 3).

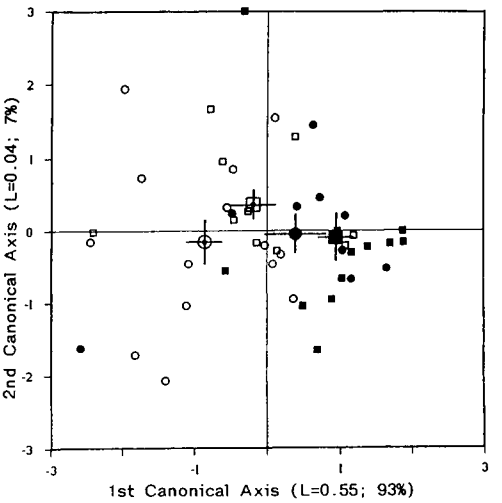

Fig 3. Optimized visual difference by canonical discrimination of the data on TRH-induced GH response. Group means (larger symbols) ± SEM and individual birds shown: GL 33 °C □ , GL 23 °C ■, FC 33 °C O, FC 23 °C ●.

DISCUSSION

Although temperature by itself hardly showed any effect on pre-injection GH values, high temperature enhanced both the GH secretory response to TRH and the GH clearance. The increased magnitude of GH peak following TRH administration at the higher temperature may be explicable in terms of the depressive effects of thyroid hormones on GH secretion (Harvey, 1983; Harvey *et al*, 1990, 1991) while the thyroid function is known to be reduced in warmer environments (Decuypere *et al*, 1981; Kühn *et al*, 1984). The reduced thyroid function may then result in an enhanced sensitivity of GH to a secretagogue challenge such as TRH (Harvey *et al*, 1990). The enhanced GH decrease between 15 and 30 minutes after a similar TRH injection in the high temperature conditioned animals should be interpreted carefully.

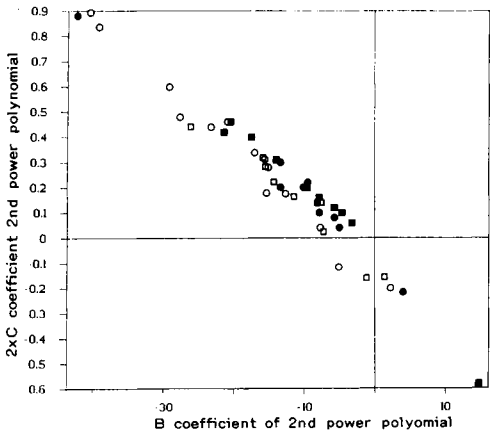

Fig 2. Characteristics of the acute elimination rate curves in individual birds (coefficients of the differential of the 2nd power polynomial) according to line (GL or FC) and temperature (GL 33 °C □, GL 23 °C ■, FC 33 °C O, FC 23 °C ●).

It may be interpreted as a shorter secretagogue challenge, due to a hypothetical shorter half-life of TRH in high tempera-

ture reared chicks, or to an increased elimination of GH. The first possibility is rather unlikely in view of the higher GH peak values obtained after TRH linked with a reduced thyroid function on a TRH challenge. From the higher decrease in GH levels at a specific time interval and the acute elimination rates calculated as the differential from the individual hormone disappearance curves, the second possibility seems valid. Nevertheless the half-life, characterized by the *b*-coefficient of the indvidual exponential curves, was not different between lines or temperature groups.

A higher GH secretory response to TRH was observed in the FC line, which may be linked to the higher endogenous pulsatility found in this line (Decuypere *et al,* 1991). Experimentally induced or natural GH pulses have been found to be related to leanness and better feed efficiency in chickens (Johnson *et al,* 1986, 1987; Vasilatos-Younken *et al,* 1988; Decuypere *et al,* 1991), while genetically lean and fat sheep differ in their GH response to GRF (Sutti *et al,* 1991). The mechanisms responsible for the differences in cGH response to TRH between lean and fat broiler lines have still to be elucidated. They may involve a lower TRH responsiveness and/or a lower availability of pituitary store of cGH in the GL line. In contrast to the higher rate of GH clearance in fat compared to lean sheep, inversely related to the GH relesed by GRF (Suttie *et al,* 1991), no differences were found either in GH decrease between both chicken lines in the interval between 15 and 30 minutes after the TRH challenge, or in the calculated acute elimination rates as can be seen in figures 2 and 3. The effect of temperature was unrelated to the line effect both as far as GH response to TRH and GH disappearance were concerned, since a line x temperature interaction was never observed. Both temperature and line data indicate the physiological unrelatedness of the amplitude of GH pulses or GH responses upon TRH with the GH disappearance or elimination rate. Our data confirm the earlier findings of Pethes *et al* (1979) in ducks showing a reduced plasma GH response to TRH in cold (10 °C) adapted animals; however, this is the first time that it has been shown in divergently selected chicken lines kept under 2 different temperature schemes.

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