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Thyroid Hormones Characteristics and Hepatic Deiodinase Enzyme Activity in Broiler Lines Selected for Growth and Feed Conversion

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Abstract: The present study was carried out to investigate the influence of genotypes on endocrine parameters in broiler chickens selected for fast growth or better feed conversion rate during the early growth phase. The thyroid hormones levels, hepatic deiodinase enzymes and thyroid hormone receptor activity were analyzed in plasma and hepatic cells of broiler chickens. Age-related changes in plasma T_4 and T_3 levels were observed. The decrease of plasma T_3 levels as a function of age was correlated with relative growth rate. The positive relation between T_3 levels and relative growth rate supports the idea that the greater synthesis of this anabolic hormone may be required for the increase in efficiency of protein deposition in chickens at younger ages. In contrast to plasma T_3 concentrations, plasma T_4 levels increased gradually with age. Analysis of the T_3 concentration showed a tendency for higher plasma T_3 levels at week 4 and significantly higher at week 7 on the GL compared to the FC line. The plasma T_4 concentration in growth selected line was higher in general, and statistically significant at week 4 and 7 of age. The present data show that the decrease of T_3 as a function of age is associated with a decrease in type I deiodinase activity. Analysis of deiodinase enzymes showed a non-significantly higher type I deiodinase activity in the fast growing than in the slow growing line at week 4 and 7 of age. A significant age differences were found in type I deiodinase activity within the GL line with lower hepatic type I deiodnase activity at week 7 compared to week 4. No line differences were found in specific binding activity, binding capacity and binding affinity constant between lines, which may indicate that the receptor configuration does not differ among the lines. With this finding, it may be concluded that the hepatic T_3-receptor characteristics have not been influenced by the selection criteria that has been used to create these divergent lines.

Key words: Broilers, T_3, T_4, T_3- receptor, deiodinase enzyme activity

Introduction

It is well established that the endocrine system is a major regulator of the partition of nutrients between adipose and other tissues. Genetically fat and lean line chickens can be used as a good model in order to understand the physiological and endocrine mechanisms underlying differences in proportion of fat and protein deposition. There is abundant evidence that thyroid hormones tri-iodothyronine (T_3) and thyroxine (T_4) are very important for the hatching process in the chicken (Decuypere and Kuhn, 1988) The normal post-hatch growth in birds is positively correlated with the rising of circulating T_3 and T_4 (Decuypere *et al.,* 1991). Differences in circulating T_3 levels are also observed between fat and lean broilers divergently selected for abdominal fat weight (Decuypere *et al.,* 1994). A genetic model, using chickens carrying a sex-linked dwarf gene has shown a clear picture for the role of thyroid hormones during growth. Throughout growth and maturation the plasma concentration of T_3 in sex-linked dwarf birds is considerably lower compared to normal chickens (Scanes *et al.,* 1983; Harden and Oscar, 1993; Bartha *et al.,* 1994). In relation to body composition the hypothyroid status in chicken is associated with enhancement of fat deposition whereas in hyperthyroid status in chicken is associated with enhancement of fat deposition whereas in hyperthyroid chickens fat content is decreased (Cogburn, 1991; Decuypere *et al.,* 1987). It has been found that both T_3 and T_4 increased basal lipolytic activity and the glucagon induced lipolysis in cultured broiler adipocytes (Griffin, 1992). The increase in efficiency of protein deposition in younger birds compared to older birds was correlated with higher concentration of T_3 in younger chickens (Kühn *et al.,* 1982).

In the studies described above, attention was paid to changes in plasma concentrations and functional disappearance rate of T_4 and T_3. We should keep in mind that changes in circulating thyroid hormones are the integrated results of production, elimination, tissue utilization and peripheral conversion (Darras *et al.,* 1990). The deiodination of iodothyronines, which has been shown to take place in many tissues, can be considered an important phenomenon in peripheral metabolism. Specific enzymes called deiodinase enzymes catalyze the deiodination processes that finally result in active or inactive thyroid hormone. The hepatic type I deiodinase enzyme activity in peripheral tissue has

responsible for the conversion of prohormone T_4 into the metabolically active T_3 (Darras *et al.,* 1992; Griffin, 1992). Another type of enzyme, the type III deiodinase, brings about the conversion of T_4 to rT_3 and T_3 to T_2. (Griffin, 1992). Since the activity of deiodinase enzymes influences the thyroid hormone status of the organism, they are very important in regulating the changes in plasma T_3. Evidence accumulated from both *in vivo* (Apreiletti *et el.,* 1988; Samuel *et al.,* 1979) and *in vitro* (Oppenheimer, 1983) studies indicates that the actions of thyroid hormones are mediated by cellular receptor(s) that are located in the nucleus. It is generally believed that thyroid hormones exert their effects on various tissues by acting in concert with these nonhistone chromosome bound protein receptors to alter the expression of specific genes. Therefore another important factor which influences the effects of the hormone is the number of receptors available to respond to the hormone. It thus becomes of importance to determine whether or not the number of thyroid hormone receptors in metabolically active tissue is influenced by selection strategies. This study examined the T_3 and T_4 concentration in circulation taken together with the hepatic deiodinase enzyme activity as well as quantification of binding characteristics of the hepatic T_3 receptor. In the present study broiler chickens selected for 6 week body weight (GL) line and feed conversion (FC) line were used.

Materials and Methods

Rearing management and sample collection: Male and female broiler chickens selected for 6 week body weight (GL) line or for feed efficiency between 3 and 6 weeks of age (FC line) were used. Leenstra (1988) described the history and production traits of these lines. Chickens of both lines were reared in litter pens (3 pens with 12 chickens/sex/pen). Water and commercial pelleted broiler diet (13.2 MJ ME, 210g crude protein/kg) were provided *ad libitum*. Lighting was continuous and all environmental conditions were essentially the same for all birds. Blood samples were taken from the wing vein into heparinized syringes at 4, 5, 6 and 7 weeks of age. Plasma was stored at -20°C until assayed. At 4 and 7 weeks of age 8-9 birds per sex from each line were killed, the liver were removed and stored at -20°C until assayed were performed. T_3 and T_4 were measured in plasma and liver were used for quantification of T_3-receptor and hepatic type I and type III deiodinase activity.

Hormone analysis: Measurements of the T_3 concentrations in the plasma were performed by radioimmunoassay using a commercially available T_3 antiserum (Mallinckrodt Diagnostica) in combination with a specific tracer [^{125}] T_3 (Amersham). The intra-assay and interassay coefficients of variation were respectively 2.9% and 6.2% (Huybrechts *et al.,* 1989). T_4 concentrations in plasma were assayed by using tracer from Amersham and a laboratory raised rabbit T_4 antiserum. This T_4 antiserum had a 0.16% cross reactivity with T_3, an intra-assay coefficient of variation of 3.2% and an interassay coefficient of variation of 10.1%. T_3/T_4 determinations were performed as described by Leenstra *et al.,* (1991).

Hepatic type I and type III deiodinase assay: Microsomal fractions were prepared from liver tissue by differential centrifugation method (Bradford, 1976). Type I and type III deiodinase activity was measured following the method described by Darras *et al.,* (1992). The method is based on the measurement of radioiodine release from ^{125}I-labelled rT_3 for type I and $^{125}IT_3$ for type III deiodinase activity respectively. The details of the procedures are described by Rahimi (1996).

Hepatic nuclear T_3-receptor assay: For nuclei preparation from hepatic cells the technique of Bellabarba and Lehoux (Bellabarta and Lehoux, 1981) was applied in a slightly modifications. Cell's nuclei were purified in differential centrifugation and the sucrose buffer was used for discontinuous density gradient (Stewart *et al.,* 1984). T_3-receptor binding assay was performed as described by Dewil *et al.* (1992). Binding capacity (Bmax) and equilibrium association constants (Ka) were measured by Scatchard analysis using the LIGAND program (Kelly *et al.,* 1979; Munson and Rodbard, 1980).

Statistical analysis: Statistical analyses were performed using the statistical package GLM (General Linear Model) procedure (SAS Institute, 1986). For all datasets the fixed effect of line, sex was investigated by ANOVA. The significance of the fixed effect in the ANOVA models was assessed using F-tests for the variance ratio. If a significant effect of variables was calculated, means were contrasted by Duncan's multiple range test.

Results

The mean plasma T_3 levels for male and female broiler chickens selected for growth or feed conversion is presented in Table 1. The plasma T_3 concentrations decreased by age. A significant line ($P<0.05$) effect was observed at week 7, with higher plasma T_3 levels in the GL compared to the FC birds. Comparison between sexes shows significant sex differences ($P<0.05$) within GL line with higher plasma T_3 in females compared to males at week 4 and 7 and the opposite at week 6. Within FC line a significant sex effect ($P<0.05$) was only found at week 4, with higher plasma T_3 levels in males compared to females. The mean plasma T_4 level for male and female broiler chickens is presented in Table 2. In contrast with plasma T_3 levels, plasma T_4

Table 1: Mean plasma T_3 levels in male and female broiler chickens selected for growth and feed conversion. Values are means ± SEM

	Plasma T_3 levels (ng/ml)			
	week			
Line	4	5	6	7
GL male	2.27 ± 0.1^{b}	1.94 ± 0.1	2.56 ± 0.2^{a}	1.75 ± 0.1^{b}
GL female	2.59 ± 0.1^{a}	2.23 ± 0.2	1.88 ± 0.1^{c}	2.40 ± 0.2^{a}
FC male	2.65 ± 0.2^{a}	1.98 ± 0.1	2.13 ± 0.1^{bc}	1.59 ± 0.1^{b}
FC female	2.11 ± 0.1^{b}	1.96 ± 0.1	2.36 ± 0.1^{ab}	1.66 ± 0.2^{b}

Means with no common superscripts in columns differ significantly (P<0.05)

Table 2: Mean plasma T_4 levels in male and female broiler chickens selected for growth and feed conversion. Values are means ± SEM

	Plasma T_4 levels (ng/ml)			
	week			
Line	4	5	6	7
GL male	12.1 ± 0.9^{a}	14.70 ± 1.1^{ab}	16.7 ± 0.8	16.5 ± 0.8^{a}
GL female	9.45 ± 0.6^{b}	16.23 ± 1.1^{a}	15.8 ± 1.4	16.6 ± 0.7^{a}
FC male	8.06 ± 0.5^{b}	12.80 ± 1.0^{b}	14.3 ± 0.6	9.63 ± 1.1^{c}
FC female	8.27 ± 0.8^{b}	14.10 ± 0.8^{ab}	15.0 ± 1.0	12.2 ± 1.4^{a}

Means with no common superscripts in columns differ significantly (P<0.05)

concentrations increased gradually with age. Sex differences were hardly present if males and females were compared within lines, except at 4 week for GL and week 7 for FC birds. The calculated mean plasma T_3/T_4 ratio is presented in Table 3. No sex differences were found between males and females within lines. Within FC line the sex difference was only present at 7 weeks of age, with higher plasma T3/T4 ratio in male compared to female chickens.

Hepatic type I and type III deiodinase activity in broiler chickens from both lines are summarized in Table 4. No sex differences were found in type I and type III deiodinase activity within and between GL and FC birds. In contrast with plasma T_4 levels, both type I and type III deiodinase activity decreased with age, with higher activity at week 4 compared with week 7. A non-significantly higher type I deiodinase activity was found in GL birds compared to FC birds at week 4 of age. No line differences were found in deiodinase enzyme activity between GL and FC the FC birds at 4 or 7 weeks of age. Since the available liver samples were not large enough to determine the T_3-receptor binding characteristics in all the samples from both lines, in the FC line we only used the liver tissue of male birds for T_3-receptor assay. The results of percentage of specific binding (%SB), maximum binding capacity (Bmax) and binding affinity (Ka) of the hepatic nuclear T_3-receptor is shown in Table 5. No significant line differences were found between lines in T_3-receptor binding activity.

Discussion

The results that were obtained in the present study demonstrate a complex relationship between plasma T_4 and T_3, hepatic type I and type III deiodinase and T_3-receptor binding activity in broiler lines selected for rapid growth and better feed conversion during rapid growth phase. An age-related changes in plasma T_3 and T_4 levels were observed in the present study. The decrease of the plasma T3 levels was correlated with relative growth as a function of age, which is in accordance with earlier publications (Bobek *et al.,* 1977; Buyse *et al.,* 1991; Decuypere *et al.,* 1993; Kühn *et al.,* 1982). The positive relation between T_3 levels and relative growth as a function of age supports the idea that the greater synthesis of this anabolic hormone may be partly responsible for the increase in efficiency of protein deposition in chickens at younger ages. However, the pattern of T_4 in circulation was not parallel with T_3 which indicate a negative correlation between relative growth rate and plasma T_4 concentrations. A positive correlation between body weight and plasma T_4 levels was observed. The higher plasma T_3 levels in rapid growth compared to slow growth broiler chickens have already been reported (Lauterio *et al.,* 1986). No line differences in plasma T_3 have been reported by between chickens divergently selected for body weight gain (Dunnington and Siegel, 1985; McNabb *et al*., 1991). Mitchell (1988) found higher total T_3 levels (21%) in lean compared to fat lines selected for high or low density lipoprotein at 49 days of age (Surks *et al.,* 1973). The line differences in circulating T_4 levels between GL and FC birds, which related in significantly higher T_3/T_4 ratio in the FC line, may indicate a role for T_3/T_4 ratio in the growth regulation in FC birds.

The type I deiodinase enzyme activity in peripheral tissue is responsible for the conversion of the prohormone T_4 into the metabolically active T_3. The present data show that the decrease of T_3 as a function of age is associated

Table 3: Mean plasma T_3/T_4 levels in male and female broiler chickens selected for growth and feed conversion. Values are means ± SEM

	Plasma T_3/T_4 ratio			
	week			
Line	4	5	6	7
GL male	0.49 ± 0.9[b]	0.48 ± 0.02	0.41 ± 0.01	0.33 ± 0.01[b]
GL female	0.60 ± 0.6[ab]	0.39 ± 0.02	0.41 ± 0.04	0.40 ± 0.02[b]
FC male	0.61 ± 0.03[a]	0.42 ± 0.01	0.40 ± 0.01	0.52 ± 0.05[a]
FC female	0.60 ± 0.04[a]	0.39 ± 0.02	0.40 ± 0.02	0.36 ± 0.02[b]

Means with no common superscripts in columns differ significantly (P<0.05)

Table 4: Hepatic type I and type III deiodinase activity in male and female broiler chickens selected for growth and feed conversion at 4 and 7 weeks of age. Values are means ± SEM

	Type I		Type III	
	pmol rT_3 deiodinased/mg/min		pmol rT_3 deiodinased/mg/min	
Line	week 4	Week 7	week 4	week 7
GL male	345 ± 45[a]	266 ± 14	42 ± 11	15 ± 6
GL female	375 ± 39[a]	280 ± 29	36 ± 8	12 ± 4
FC male	291 ± 52[ab]	215 ± 15	38 ± 9	21 ± 9
FC female	297 ± 32[ab]	226 ± 12	32 ± 10	28 ± 11

Means with no common superscripts in columns differ significantly (P<0.05)

Table 5: Specific binding activity, maximum binding capacity and binding activity the hepatic nuclear T_3-receptor in broiler chickens selected for growth and feed conversion. Values are means ± SEM

	%SB	Bmax	Ka
	% binding of total radioactivity	fmol/µg DNA	l/nmol
Line	week 7	week 7	week 7
GL male	11.5±2	4.5±0.2	4.5±1
GL female	6.5±1	3.85±0.5	5.05±1
FC male	12.1±2	4.30±0.4	4.65±0.5
FC male	5.7±1	3.25±0.3	

with a decrease in type I deiodinase activity. This is in agreement with the findings of Darras *et al.* (1992) that type I deiodinase activity declines in broiler chickens after 3 weeks of age since plasma T_3 level declines whereas T_4 level increases. A significantly higher type I deiodinase activity in high body weight than in low body weight broiler lines at mature age has been reported by McNabb *et al.* (1991). Our data also show that fast growing line had a tendency for higher type I deiodinase activity compared to slow growing line at both 4 and 7 weeks of age. The opposite pattern was observed for type III deiodinase activity between GL and FC lines at 7 week of age. Lower circulating T_3 with higher hepatic type III activity have been reported in hypophysectomized chickens. (Darras *et al.*, 1992). GH injection in these hypophysectomised chickens increased plasma T_3 and decreased hepatic type III deiodinase activity (Darras *et al.*, 1992; Huybrechts *et al,* 1986). It was concluded that the high endogenous GH levels as well as GH receptor availability with a low hepatic type III activity reduce the impact of exogenously administrated GH in growing chickens. In the present study no line differences were found in type III deiodinase activity in spite of clear line differences in plasma GH levels between GL and FC birds. T_3 finally exerts its biological effects after binding to nuclear binding site.

No significant differences were found in specific binding (%SB) of T_3 to its nuclear receptor, binding capacity (Bmax) and binding affinity (Ka) between GL and FC birds. With this finding it may be stated that the hepatic T_3-receptor characteristics have not been influenced by the selection criteria that has been used to create these divergent lines. However, a significantly higher binding capacity on granulosa cells in the GL than in the FC line has already been reported (Dewil *et al.*, 1991).

With the earlier finding that FC chickens are characterized by higher plasma GH levels (Zeman *et al.*, 1994) and a more pronounced pulsatile GH secretion (Decuypere *et al.*, 1991; Leenstra *et al.*, 1991) it can be concluded that the GH axes is more important for the higher protein deposition in the FC chickens compare to their GL counterparts.

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