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Effect of Thyroid Hormones on the Redox Balance of Broiler Chickens

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ABSTRACT : In the present study, two trials were conducted to evaluate the effects of hyper- and hypothyroid status on the redox balance of broiler chickens. In Trial 1, 3 groups of broiler chickens were randomly subjected to one of the three treatments: subcutaneous administration of triiodothyronine (T_3, 150 μg/kg BW), methimazole (MMI, 150 mg/kg BW), or saline. The blood, liver and heart were sampled at 3 h after injection. In Trial 2, three groups of 20 broiler chickens were randomly fed with one of the three diets: control, dietary supplementation of T_3 (1.5 mg/kg diet) or MMI (1 g/kg diet) for 7 days. In trial 1, the plasma concentrations of T_3 and T_3 to thyronine ratio (T_3/T_4) were significantly increased by T_3 injection. Plasma levels of thiobarbituric acid reacting substances (TBARS) tended to be increased ($p = 0.067$) by both T_3 and MMI treatments while the ferric reduced/antioxidant capacity (FRAP) was increased only by MMI treatment. Acute T_3 treatment had no significant effect on the activities of superoxide dismutase (SOD) and the concentrations of FRAP and TBARS in either liver or heart tissue. In contrast, the hepatic activities of SOD were decreased ($p<0.05$) while the cardiac levels of FRAP were significantly increased ($p<0.0001$) by MMI treatment. In chronic treatments, the rectal temperature of chickens was significantly decreased ($p<0.05$) by MMI treatment. The circulating T_3 levels were significantly increased ($p<0.05$) by long-term T_3 treatment, and showed a trend to decrease in MMI treatment. The plasma concentrations of TBARS were significantly ($p<0.05$) increased by MMI treatment. All the redox parameters measured in either liver or heart were not significantly altered by either long-term T_3 or MMI treatment except that the hepatic SOD activities were significantly augmented by T_3 treatment. The result showed that neither acute nor long-term elevation of circulating T_3 levels induced lipid peroxidation in broiler chickens. The enhanced enzymatic antioxidant system (SOD in cardiac tissue) may be involved in the protection of the bird to increased oxidative challenge. The responses of redox balance to changed thyroid state seem to be tissue specific. (**Key Words :** Thyroid Hormone, Broiler Chickens, Lipid Peroxidation)

INTRODUCTION

Thyroid hormones are known to regulate the energy metabolism of most tissues including liver, kidney, heart, and skeletal muscles. It is well established that thyroid hormones accelerate the basal metabolic rate and oxidative metabolism by causing an increase in the mitochondria mass, mitochondrial cytochrome content and respiratory rate. In chickens, the plasma levels of thyroid hormones are changed with ambient temperature, age, feeding status and pathophysiologic status (Decuypere and Kühn, 1984, 1988; Lin et al., 2000; Stojevic et al., 2000; Luger et al., 2002). One the other hand, the reactive oxygen species (ROS) are generated as by-products of oxidative metabolism in mitochondria.

In chickens, there is limited information about the association of thyroid status with the induction of oxidative stress. In mammals, it has been suggested that the susceptibility of mitochondria to oxidative stress is increased by hyperthyroidism (Das and Chainy, 2001; Venditti et al., 2003) and decreased by hypothyroidism (Venditti et al., 2003). The administration of triiodothyronine (T_3) to hypothyroid rats results in a significant augmentation of oxidative stress parameters (Das and Chainy, 2001). In hyperthyroidism rats, enhanced oxidative stress parameters are observed in the heart (Asayama et al., 1987; Zation et al., 1993; Civelek et al., 2001), soleus muscles (Asayama et al., 1987; Zation et al., 1993), and liver (Fernandez and Videla, 1996; Venditti et al., 1997). Hypothyroidism does not modify or even decrease lipid peroxidation and oxidative damages (Venditti et al., 1997; Gredilla et al., 2001a). However, hypothyroid female patients were found to have significantly higher level of plasma TBARS, compared to healthy ones (Konukoglu et

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al., 2002). The enzymatic and not-enzymatic antioxidant systems are involving in the enhanced oxidative stress in hypothyroid patients (Adali et al., 1999; Resch et al., 2002).

Broiler chickens are more susceptible to environmental challenge and the lipid peroxidation is one of the undesired results. For example, exogenous glucocorticoid administration (Lin et al., 2004 a, b) and heat stress (Lin et al., 2000; Shim et al., 2006) resulted in lipid peroxidation in broiler chickens, which should be at least partially responsible for the reduced zootechnical performance. Moreover, the maintenance of redox balance has been found to be important for the health (Bottje et al., 1998; Iqbal et al., 2002) and meat quality of broiler chickens (Jiang et al., 2007). In modern strains of broiler chickens, the selection for high feed conversion ratio could result in functional hypothyroidism by either decreased thyroid hormone production or peripheral deiodination of thyroxine, which is believed to be linked with the susceptible of broiler chickens to ascites (Decuypere et al., 2003). The impaired redox balance is involved in the development of ascites (Iqbal et al., 2002; Han et al., 2005). Hence, we hypothesize that the changed thyroid status may play a role in redox balance of broiler chickens. Moreover, as chickens have quite different metabolic characteristics such as high body temperature and metabolic rates and enhanced antioxidant system comparing to mammals, we speculated that chickens may response to hypo- and hyperthyroid status in a different way as that of mammals.

In the present study, two trials were conducted to evaluate the involvement of changed thyroid status in the redox balance in broiler chickens. The hyperthyroidism or hypothyroidism status was respectively mimicked by dietary and subcutaneous injection of T_3 or methimazole (MMI). MMI can block thyroid hormone biosynthesis by inhibiting thyroid peroxidase, a key enzyme of thyroid hormone biosynthesis, and has been used as an antithyroid drug (Cooper, 1984).

MATERIALS AND METHODS

Chickens and diets

Commercial broiler chickens (Cobb) were obtained from local hatchery at one day of age and reared in an environmentally controlled room. The brooding temperature was maintained at 35°C for the first 2 d, and then decreased gradually to 21°C (45% RH) at 28 d and maintained as such thereafter. The broilers received a commercial starter diet (12.10 MJ ME/kg and 22.2% CP) until 7 d of age, after which a commercial grower diet (12.68 MJ/kg, 21.4% crude protein) was provided until the end of the experiment. For a detailed description of both diets, see Buyse et al. (2001). The light regime was 23 L:1 D. The birds had free access to feed and water during rearing period.

Trial 1: Acute subcutaneous administration

Eighteen 32-d-old broiler chickens of both sexes with similar body weight were randomly divided into three groups of 6 chickens and subjected randomly to the three treatments: subcutaneous administration of T_3 (150 μg/kg BW, Sigma Chemical), MMI (150 mg/kg BW, Sigma Chemical) or saline (serving as control). A blood sample of each chicken was respectively obtained before and at 3 h after injection from a wing vein with a heparinized syringe, and collected in iced tubes. At the same time, the body weight (BW) loss was recorded and rectal temperature was determined with a digital thermometer (±0.1°C, Huger Electronics GmbH, Germany). During the 3 h experimental period, feed was withdrawn and experimental chickens had free access to water.

Immediately after the second blood sample was obtained, all chickens were sacrificed by cervical dislocation and the liver and heart were dissected immediately. After washing with cold saline (4°C, 0.95%) and removed of blood, the tissues were frozen in liquid nitrogen and stored at -76°C until analysis. Plasma was obtained after centrifugation at 3,000 rpm for 10 min at 4°C and was stored below -20°C for further analysis.

Trial 2: Dietary treatment

Sixty chickens of 28-d of age were randomly assigned to three groups of 20 chickens and fed with one of the three diets: control, diet supplemented with T_3 (1.5 mg/kg diet) or MMI (1 g/kg diet), from 31 to 38 days of age. At 38 days of age, rectal temperature was determined with a digital thermometer for each chicken. Thereafter, 7 birds of each group were sacrificed by cervical dislocation after a blood sample was drawn from a wing vein with heparinized syringe and the liver and heart were dissected immediately. After washing with cold saline (4°C, 0.95%) and removing blood, the tissues were frozen in liquid nitrogen and stored at -76°C until analysis. During experimental period, all the chickens had free access to feed and water.

Plasma parameters

Commercial colorimetric diagnostic kits were used to measure plasma glucose (IL Test™ kit, No. 182508-00), urate (IL Test™ kit, No. 181685-00) and creatine kinase (CK) (IL Test™ kit, No. 181605-90), using the Monarch™ 2000 Chemistry system Model 760 (Monarch Chemistry System, Instrumentation Laboratories, B-1930, Zaventem, Belgium). Corticosterone (CORT) was measured using a sensitive and highly specific radioimmunoassay kit (IDS, Inc., Boldon, NE35 9PD, UK), with a sensitivity of 0.39 ng/ml, and low cross reactions with aldosterone (0.20%), cortisol (0.40%) and deoxycorticosterone (3.30%). The intra assay variability was 3.8%. Before assayed, plasma samples were heated at 80°C for 10 minutes to inactivate

Table 1. Effects of acute (3 h) subcutaneous injection of triiodothyronine (T_3, 150 μg T_3/kg BW) or methimazole (MMI, 150 mg/kg BW) or saline (control treatment) on plasma parameters of broiler chickens in Trial 1

	T_3	MMI	Control
T_3 (ng/ml)	150.1±20.9 ^{x}	4.84±1.29 ^{xy}	3.52±0.88 ^{y}
T_4 (ng/ml)	16.7±3.5	10.5±2.4	12.1±1.6
T_3/T_4 ratio	12.8±4.3 ^{x}	0.65±0.3 ^{y}	0.37±0.1 ^{y}
CORT (ng/ml)	16.2±4.0	26.6±3.4	24.7±8.3
TBARS (nmol/ml)	0.53±0.05	0.55±0.05	0.38±0.05
FRAP (μmol/ml)	807±45 ^{y}	2,353±117 ^{x}	715±54 ^{y}
CK (IU/L)	5,927±1,443	7,219±1,665	4,684±795
Urate (mg/d)	2.3±0.3	2.7±0.3	2.1±0.4
Glucose (mg/dl)	235±5	230±9	238±6

Values are means±SEM (n = 6).

$^{x-y}$ Means with different superscript within the same row differ significantly ($p<0.05$).

CK = Creatine kinase; CORT = Corticosterone; FRAP = Ferric reducing/antioxidant power.

T_3 = Triiodothyronine; T_4 = Thyroxine; TBARS = Thiobarbituric acid reacting substances.

corticosterone-binding proteins.

Thyroid hormones were measured by radioimmunoassay according to the method described by Darras et al. (1996). Briefly, T_3 measurements were performed using a commercial available T_3 antiserum from Byk-Sangtec (Byc-Sangtec Diagnostica GmbH, Dietzenbach, Germany), in combination with a specific tracer (Amersham International, Slough, England). The intra-assay coefficient of variation was 4.5%. T_4 concentrations were assayed using tracer from Amersham (Byc-Sangtec Diagnostica GmbH, Dietzenbach, Germany) and a rabbit T_4 antiserum (Mallinckrodt Diagnostica, Dietzenbach, Germany). This T_4 antiserum had 0.16% cross-reactivity with T_3. The intra-assay coefficient of variation was 3.2%.

Plasma lipid peroxidation was estimated by spectrophotometric determination of thiobarbituric acid reacting substances (TBARS) with a method of Lin et al (2004a). TBARS were expressed as nmoles of malondialdehyde (MDA) per ml plasma. Total plasma antioxidant activity was determined by the ferric reducing/antioxidant power (FRAP) assay as described by Benzie and Strain (1996, 1999). The measurement was conducted at room temperature and a 5-min time window was used.

Tissue parameters

Tissue samples were homogenised in 9 volumes of 10 mM sodium phosphate buffer (pH 7.4) containing 1.15% potassium chloride. TBARS of tissue homogenates was determined spectrophotometrically according to Lin et al. (2004a, b). TBARS were expressed as nmoles of MDA per gram of wet tissue. After the homogenates were centrifuged (4,000 rpm, for 10 min at 4°C), the supernatant was used for the measurement of superoxide dismutase (SOD) and FRAP. The SOD activity was measured with the commericial kit (Dojindo Inc.) using a microplate reader (Titertek Multiskan®, MCC/340).

All samples were run in the same assay to avoid inter-assay variability.

Statistical analysis

Data in Trial 1 were subjected to Repeated Measures ANOVA by SAS for windows (version 8e, SAS Institute Inc.) to evaluate the effects of treatment and time, as well as the interaction between treatment and time. In Trial 2, a one-way model of ANOVA was used to estimate the main effects of treatment. Multiple comparisons were performed between treatments means using Ducan test. Means were considered significantly different at $p<0.05$.

RESULTS

In trial 1, at 3 h after treatment, the BW and RT of experimental chickens were all decreased in all treatments but there was no significant difference in BW loss (T_3, 17.2±3.0 g; MMI, 8.3±13.1 g; Control, 20.2±5.1 g) and RT reduction (T_3, -0.4±0.1°C; MMI, -0.4±0.1°C; Control, -0.3±0.1°C) between treatments ($p>0.05$). The effects of treatment were significant ($p<0.05$) for the plasma concentrations of FRAP and T_3 and T_3/T_4 ratio, but not for plasma levels of T_4, TBARS, glucose, urate and CORT, and activity of CK ($p>0.05$) (Table 1). Compared to control group, the plasma concentrations of T_3 and T_3/T_4 ratio were significantly increased by T_3 treatment but were not affected by MMI treatment. The plasma concentrations of TBARS tended to be increased ($p = 0.067$) by both T_3 and MMI treatments, while the plasma levels of FRAP were significantly increased ($p<0.05$) by MMI treatment. In liver, the activities of SOD were significantly lower ($p<0.05$) in MMI chickens, compared to control or T_3 chickens, while the concentrations of TBARS and FRAP were not significantly ($p>0.05$) changed (Table 2). Neither the activities of SOD nor the concentrations of TBARS in heart were significantly affected by treatments ($p>0.05$). In

Table 2. Effects of acute (3 h) subcutaneous injection of triiodothyronine (T_3, 150 μg T_3/kg BW) or methimazole (MMI, 150 mg/kg BW) or saline (control treatment) on the biomarkers of oxidative stress in liver and heart tissues of broiler chickens in Trial 1

	T_3	MMI	Control
Liver			
SOD (U/mg protein)	13.33±1.17 ^{xy}	11.47±0.26 ^{y}	15.10±0.61 ^{x}
TBARS (nmol/mg protein)	0.28±0.04	0.25±0.00	0.28±0.01
FRAP (μmol/mg protein)	131.4±6.7	126.2±4.0	129.5±6.4
Heart			
SOD (U/mg protein)	3.53±0.28	3.08±0.19	2.71±0.40
TBARS (nmol/mg protein)	0.27±0.01	0.23±0.01	0.25±0.02
FRAP (μmol/mg protein)	50.8±2.4 ^{y}	68.2±1.5 ^{x}	51.5±2.9 ^{y}

Values are means±SEM (n = 6).

$^{x-y}$ Means with different superscript within the same row differ significantly ($p<0.05$).

SOD = Superoxide dismutase; FRAP = Ferric reducing/antioxidant power; TBARS = Thiobarbituric acid reacting substances.

Table 3. Effects of dietary supplementation of triiodothyronine (T_3, 1.5 mg T_3/kg diet) or methimazole (MMI, 1 g/kg diet) on rectal temperature and plasma parameters of broiler chickens in Trial 2

	T_3	MMI	Control
Rectal temperature (°C)	42.5±0.1 ^{x}	42.0±0.1 ^{y}	42.5±0.1 ^{x}
T_3 (ng/ml)	9.64±4.57 ^{x}	1.72±0.42 ^{y}	2.08±0.33 ^{y}
T_4 (ng/ml)	4.47±0.55	5.53±0.94	7.67±1.30
T_3/T_4	2.65±1.46	0.37±0.09	0.30±0.09
CORT (ng/ml)	14.2±4.2	18.20±4.48	15.11±3.33
TBARS (nmol/ml)	0.63±0.06 ^{y}	0.84±0.09 ^{x}	0.61±0.06 ^{y}
FRAP (μmol/ml)	817±55	961±31	829±49
CK (IU/L)	4,677±843	3,337±281	5,283±1,624
Urate (mg/dl)	2.96±0.34	3.3±0.2	3.2±0.4
Glucose (mg/dl)	255±7	264±5	262±6

Values are means±SEM (n = 7).

$^{x-y}$ Means with different superscript within the same row differ significantly ($p<0.05$).

CK = Creatine kinase; CORT = Corticosterone; FRAP = Ferric reducing/antioxidant power.

T_3 = Triiodothyronine; T_4 = Thyroxine; TBARS = Thiobarbituric acid reacting substances.

contrast, the cardiac FRAP levels were significantly increased ($p<0.0001$) by MMI treatment compared to T_3 and control treatments.

After the chronic dietary treatment, the RT was significantly decreased ($p<0.05$) by MMI treatment (Table 3). The circulating T_3 levels were significantly increased ($p<0.05$) by dietary supplementation of T_3, and showed a trend to decrease in MMI treatment. The plasma concentrations of TBARS were significantly increased by MMI treatment ($p<0.05$) compared to other two treatments. The plasma levels of FRAP showed a trend to be increased by dietary supplementation of MMI ($p = 0.073$) compared to other treatments. The plasma levels of T_4, CK, glucose, urate, CORT and T_3/T_4 ratio were not significantly changed ($p>0.05$) by dietary treatment (Table 3). Dietary treatments had a significant effect on SOD activity in liver ($p<0.02$) but not in heart tissue ($p>0.05$). Hepatic SOD activities were significantly ($p<0.05$) increased by dietary T_3 supplementation (Table 4). The concentrations of TBARS and FRAP in both heart and liver tissues were not significantly changed by different dietary treatments ($p>0.05$).

DISCUSSION

Hyperthyroidism has been proven to link with the occurrence of oxidative stress in mammals (Fernandez et al., 2005; Venditti and Di Meo, 2006). In the present study, T_3 administration was used to induce hyperthyroidism of broiler chickens (Decuypere et al., 1987) and the elevated circulating levels of T_3 were observed in Trial 1 of the present study. In Trial 1, the plasma concentrations of TBARS, as a biomarker of oxidative injury, showed a trend to be increased by T_3 administration at 3-h later, with no obvious changes in all the other blood parameters, such as the levels of total antioxidant capacity (FRAP), urate and CORT. Moreover, the unobviously changed levels of TBARS and FRAP and activity of SOD in liver and heart indicated the unaffected redox balance in these tissues, suggesting that acute upregulation of thyroid hormones may not induce oxidative injury within a short period (<3 h).

The long-term effect of hyperthyroidism was further explored in Trial 2 of this study. In line with the previous report (Decuypere et al., 1987), dietary T_3 supplementation significantly increased the plasma levels of T_3. In mammals,

Table 4. Effects of dietary supplementation of triiodothyronine (T_3, 1.5 mg T_3/kg diet) methimazole (MMI, 1 g/kg diet) on the biomarkers of oxidative stress in liver and heart tissues of broiler chickens in Trial 2

	T_3	MMI	Control
Heart			
SOD (U/mg protein)	4.48±0.21	4.57±0.18	4.13±0.35
TBARS (nmol/mg protein)	0.28±0.01	0.28±0.03	0.31±0.02
FRAP (μmol/mg protein)	48.0±1.3	52.1±1.8	51.8±2.0
Liver			
SOD (U/mg protein)	15.0±1.34 ^{x}	12.9±0.87 ^{xy}	9.95±0.96 ^{y}
TBARS (nmol/mg protein)	0.27±0.02	0.39±0.07	0.30±0.03
FRAP (μmol/mg protein)	112±7	115±8	125±5

Values are means±SEM (n = 7).

$^{x-y}$ Means with different superscript within the same row differ significantly ($p<0.05$).

SOD = Superoxide dismutase; TBARS = Thiobarbituric acid reacting substances; FRAP = Ferric reducing/antioxidant power.

it has been suggested that the susceptibility of tissues to oxidative stress is increased by hyperthyroidism. The augmented lipid peroxidation and changed levels of antioxidant substances and activities of enzymes were observed in hyperthyroid rats (Asayama et al., 1987; Zation et al., 1993; Fernandez and Videla, 1996; Venditti et al., 1997; Civelek et al., 2001). In contrast with these studies in mammals, lipid peroxidation was not obviously altered in hyperthyroid chickens in the present study. The oxidative damage, however, is not necessarily linked to increased reactive oxygen species (ROS) due to the altered thyroid state, as the antioxidant systems could respond to changes in the production of ROS (Mano et al., 1995b; Civelek et al., 2001). The significantly increased hepatic SOD activities together with the lack of obvious change of TBARS implies that the enhanced enzymatic scavenge capacity may compensate for the changes induced by hyperthyroidism. In addition, as these phenomena were not observed in heart, this influence seems to be tissue specific, which was found in mammals as well (Venditti et al., 1997).

On the other hand, the induction of lipid peroxidation by hyperthyroidism depends not only on the production of ROS, but also on the susceptibility of fatty acids to oxidative attack. The endogenous lipid peroxidation, sensitivity to lipid peroxidation and fatty acid unsaturation in liver were decreased in mouse treatment with T_4, indicating that the lipid changes observed in hyperthyroid animals can protect them against an increased oxidative attack to tissue lipids (Guerrero et al., 1999). Compared to mammals of similar body weight, birds have significantly lower degree of fatty acid unsaturation, which in turn leads to a lower sensitivity to lipid peroxidation (Pamplona et al., 1999a, b). The low rates of mitochondrial free radical production and relative lower degree of fatty acid unsaturation of avian tissue cellular membranes may protect them better from oxidative stress compared to mammals (Barja, 2002). Therefore, these results may infer that lower susceptibility of avian species to hyperthyroid state-induced oxidative stress.

Methimazole (MMI) can block thyroid hormone biosynthesis by inhibiting thyroid peroxidase (Cooper, 1984). In the present study, plasma levels of thyroid hormones were not significantly affected by a single injection of MMI (150 mg/kg BW), indicating the unsuccessful induction of hypothyroidism in chickens. The increased total antioxidant capacities in MMI chickens may be related to the inherent antioxidant characteristics of MMI, which has already been proven *in vitro* (Asmus et al., 1996). Methimazole could inhibit the biosynthesis of thyroid hormone by impairing the formation of H_2O_2 or scavenging H_2O_2 directly as an antioxidant *in vivo*, which is a limiting step in thyroid hormone biosynthesis (Kim et al., 2001; Ferreira et al., 2003).

In trial 2 of this study, MMI was supplemented in the diet for one week to induce hypothyroidism. The relatively lower plasma levels of T_3 and the significantly decreased rectal temperature in MMI supplemental chickens are indication of their hypothyroid status. In line with the observation in hypothyroid patients (Konukoglu et al., 2002), the significantly augmented plasma lipid peroxidation in MMI chickens indicated the oxidative stress is induced by MMI treatment. In other studies with mammals, however, hypothyroidism does not modify or even decreases lipid peroxidation and oxidative damage (Venditti et al., 1997; Gredilla et al., 2001a), which may be related to the altered defence systems of antioxidants (Mano et al., 1995b; Gredilla et al., 2001b; Resch et al., 2002; Konukoglu et al., 2002; Brzezinska-Slebodzinska, 2003; Choudhury et al., 2003). However, the lack of obvious changes of TBARS levels, SOD activity and total antioxidant capacity in the liver and heart of MMI chickens compared to control group, indicate that redox status in these tissues were not affected by hypothyroidism. Therefore, whether the augmented lipid peroxidation should be ascribed to the hypothyroidism or MMI per se should be investigated further.

In conclusion, neither acute nor long-term elevation of circulating T_3 induces lipid peroxidation in broiler chickens. The enhanced enzymatic antioxidant system (e.g. in cardiac tissue) may be involved in the protection of birds from the

increased oxidative challenge induced by altered thyroid status.

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