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Comparison of the Effects of Thyroxine and Triiodothyronine on Heat Production and Skeletal Muscle Protein Breakdown in Chicken

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Effects of exogenous thyroxine (T4) and triiodothyronine (T3) were compared on heat production and skeletal muscle protein breakdown in broiler chickens aged from 15 to 27 d. T4 and T3 were mixed in the basal diet at concentrations of 1.2, 3.6 and 10.8 mg/kg and 0.3, 0.9 and 2.7 mg/kg, respectively. Plasma T4 and T3 concentrations were increased dose-dependently by dietary T4 and T3, respectively, while plasma T3 concentration was not increased by dietary T4. Both T4 and T3 retarded growth and increased feed conversion at the higher dose levels while food intakes were not changed significantly. Relative skeletal muscle weights tended to be increased by T4 and T3 probably because body fat contents were decreased intensely by the treatments as indicated by the decrement of abdominal fat. Both heat production and muscle protein breakdown were increased in a dose-dependent manner by either T4 or T3, and the potency of T4 was about one fourth of that of T3. In conclusion, thyroxine is active and has roles in heat production and skeletal muscle protein breakdown.

Key words: chicken, heat production, muscle protein breakdown, thyroxine, triiodothyronine

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Introduction

Triiodothyronine (T3) is 3–4 times as potent metabolically as thyroxine (T4), and T4 can be converted to T3 in the peripheral tissues catalyzed by 5′-deiodinase (Larsen *et al.,* 1981). About 80% and 60% of the circulating T3 has been reported to be derived from the peripheral tissues in man (Larsen *et al.,* 1981) and rat (Naguyen *et al.,* 1993), respectively. Thus, it is generally thought that T3 is an active hormone.

On the other hand, it has been shown that activities of circulating and intracellular thyroid hormones vary from one tissue to another tissue. Plati *et al.* (1992) have reported that the concentration of T4 increases in liver following pattern similarly to that of plasma T4, but in eye, heart, and, especially, brain, T4 increases to a greater degree than expected from the circulating T3 levels, indicating some organ requires T4 and other requires T3. It has also been reported that while intracellular conversion of T4 to T3 is essential for the optimal thermogenic capacity of brown adipose tissue, T3 is not required for the cold-induced activation of lipoprotein lipase activity (Reiter *et al.,* 1990). From these observations, we thought that T4 is also an active hormone. Indeed, our *in vitro* experiment showed that T4 stimulates cell differentiation but not protein degradation and apoptosis in primary chick muscle cells, while all events are stimulated by T3 (Nakashima *et al.,* 1998). However, there is no *in vivo* study, to our knowledge, showing the difference of the effects of T4 and T3 in chicken.

The present experiment was conducted to compare the effects of T4 and T3 on growth performance and organs weights in addition to heat production and muscle protein metabolism in broiler chicken *in vivo* in order to confirm our previous results showing that T4 is active and has roles especially in the regulation of skeletal muscle protein metabolism (Nakashima *et al.,* 1998).

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Materials and Methods

The animal experiment was conducted in accordance with the guidelines of Kagoshima University. Male broiler chicks (Chanky), kindly supplied by a commercial hatchery (Kagoshima Chicken Foods Co. Ltd., Kagoshima, Japan) at one day of age, were placed in an electrically-heated brooder until 12 days of age, and they were provided *ad libitum* with water and a commercial starter diet for the first 12 days. On Day 12, 84 birds were selected to have similar body weights for the experiment. There were 2 birds in each replicate, housed in wire-bottomed aluminum cages (0.49×0.40×0.67 m), and 6 replicates in each treatment; replicates were assigned to one of 7 treatments. The basal diet (3,200 kcal ME and 200 g crude protein per kg) was made mainly from maize and purified soya-bean protein as reported by Hayashi *et al.* (1993). The basal

diet was fed *ad libitum* for the preconditioning periods (12–15 days of age), then the control diet (basal diet) or the 6 experimental diets containing 1.2, 3.6 or 10.8 mg/kg T4 and 0.3, 0.9 or 2.7 mg/kg T3 were fed *ad libitum* throughout the experimental period from 15 to 27 days of age. The levels of T4 were set according to the results of our previous report (Suthama *et al.,* 1989). The T3 levels were set as one forth of respective T4 level because it is generally thought that T3 is 3–4 times as potent as T4. The experiment was conducted in a temperature controlled environment (25±1℃) with a 14 h light: 10 h dark cycle. Food consumption was recorded daily and body weights were determined every 3 days.

Heat productions were measured using open chamber systems. The chamber (0.68×0.30×0.30 m) was partitioned to have 2 rooms, and the space where the birds were placed was restricted to 0.30 m wide and 0.34 m long. At Day 26, food and water were withdrawn 1 hour before the pairs of the birds were transferred to the chambers. Concentrations of oxygen and carbondioxide in the respiratory gasses were monitored during the 2 hours experimental period by an oxygen meter (Model OX 61, Yokogawa Co. Ltd., Tokyo, Japan) and a carbondioxide indicator (Model RI-411A, Riken Keiki Co. Ltd., Tokyo, Japan) respectively. Heat productions were then calculated according to the report of Romijn and Lokhorst (1961).

At the end of the experimental period, the birds were killed by decapitation and blood was collected to measure plasma concentrations of T4 and T3. The birds were dissected to remove heart, liver, pairs of pectoral profundus muscles and abdominal fat. Since pectoral muscles are easily dissected and their lipid contents are lower than those of the thigh muscles, pectoral muscle weights were used as a measure of skeletal muscle growth. However, it has been reported that the rate of growth of breast muscle is similar to that of thigh muscle between 2 and 4 weeks of age (Kang *et al.,* 1985).

Plasma T4 was measured by enzyme-immuno assay using a commercial kit (Cobas core T4, Roche Diagnostics, Indianapolis, IN, USA) as reported previously (Hayashi *et al.,* 1994). However, we prepared our own T 4 standard solutions because plasma T4 concentration of the chicken is not in the range of the standard in the kit. Plasma T3 was measured also by a commercial enzyme-immunoassay kit (Elisa-T3, International Reagents Corp., Kobe, Japan). Excreta samples were collected daily for 3 days from 23 days of age and pooled to measure rates of muscle protein breakdown (Kd) by N^{τ}-methylhisitidine (MH) excretion as described and discussed by Hayashi *et al.* (1985). MH was determined by the method of Hayashi *et al.* (1987). Briefly, whole excreta sample was homogenized with 100 m*l* water and 2 g of the homogenate was hydrolyzed with 6 M HCl at 110℃ for 24 h, and MH in the hydrolyzed sample was separated from acid and neutral amino acids by an ion-exchange column (7×69 mm, Dowex 50×8, 200–400 mesh, pyridine form). The MH fraction was then evaporated, and the residue dissolved in the mobile phase (15 mM sodium octane sulphonate in 20 mM potassium phosphate) was subjected to high-performance liquid chromatography that utilized a reversed-phase separation with ion-pairing using Zorbax ODS column (4.6×150 mm) and post-column fluorescence derivatization using orthophthalaldehyde. Dietary and muscle MH contents were similarly analyzed.

The fractional rate of muscle protein breakdown was then calculated as follows. Skeletal muscle comprises 29.3% of the live weight in the 4-week-old male broiler chicken and MH concentration of the muscle is 0.606 μmol/g (Maeda *et al.,* 1984). Thus, the pool size of MH in the skeletal muscle was estimated to be 178 μmol/ kg body weight. Since the diet contained MH (0.04 μmol/g), it was subtracted from the total excreted MH derived from body tissues and this was multiplied by 0.8 to give the excreted MH derived from the skeletal muscle as reported by Hayashi *et al.* (1985). The fractional breakdown rate was calculated by dividing the amount of excreted MH, derived from skeletal muscle, by the amount of MH in the skeletal muscle.

Statistical analysis was conducted using Statistical Analysis System (1998). A one way ANOVA model was used, and mean values were compared using Turkey's multiple-range test. Results were considered significant at $P<0.05$.

Results

Plasma T4 and T3 concentrations are shown in Table 1. Plasma T4 concentration was about 6 fold of that of T3 in the normal chickens, and the hormones concentrations in the treatment groups were significantly reflected by their dietary levels. But plasma T4 concentrations were not influenced by the T3 treatment. The plasma T3 concentration was not changed also by dietary T4, indicating that there was little peripheral conversion of T4 to T3. Thus, the effects of dietary T4 observed in the present experiment are due mainly to its own actions. Although the dietary T4 levels were 4 times of respective T3 levels, plasma concentrations of T4 were 10.0, 9.7 and 22.9 times of respective T3 levels.

Table 2 shows the relative effects of T4 and T3 on growth, feed intake and feed conversion. Growth was inhibited in the groups fed diets containing 10.8 mg/kg T 4 and 2.7 mg/kg T3. However, in other treatment groups, there were no significant differences. Feed conversion ratios were increased significantly in 10.8 mg T4 group, but in other treatment groups, there were no significant differences. As the plasma T4 level of 10.8 mg T4 group was extraordinary high (74 times of the control), growth might be inhibited significantly.

Table 3 shows weights of a pair of pectoral profundus muscle, heart and abdominal fat. The relative weights of the pectoral muscles were increased significantly by both treatments probably because body fat contents were decreased as was indicated by changes in abdominal fat

Table 1. **Effects of dietary triiodothyronine (T3) and thyroxine (T4) on their plasma concentrations**

	Control	Dietary T3 (mg/kg)			Dietary T4 (mg/kg)		
		0.3	0.9	2.7	1.2	3.6	10.8
T3 (ng/m*l*)	2.1±0.5^{c}	7.5±7.1^{bc}	18.0±12.6^{b}	38.3±21.5^{a}	2.0±0.9^{c}	2.6±0.6^{c}	3.3±1.0^{c}
T4 (ng/m*l*)	11.8±3.4^{c}	7.8±1.7^{c}	8.0±1.5^{c}	8.3±2.1^{c}	75.3±17.2^{c}	174±40^{b}	877±201^{a}

Values are means±SD, and means in the same row without common superscript are significantly different ($P<0.05$).

Table 2. **Effects of dietary triiodothyronine (T3) and thyroxine (T4) on body weight gain, feed intake and feed conversion**

	Control	Dietary T3 (mg/kg)			Dietary T4 (mg/kg)		
		0.3	0.9	2.7	1.2	3.6	10.8
Initial body weight (g)	316±5.1	316±6.1	320±9.8	320±9.5	318±8.2	316±8.4	318±7.3
Body weight gain (g/12 days)	403±31^{ab}	439±39^{a}	373±57^{b}	326±27^{c}	413±33^{ab}	395±28^{ab}	309±45^{c}
Feed intake (g/12 days)	690±38	775±56	685±135	607±47	743±112	727±110	612±87
Feed conversion (g consumed/g gain)	1.71±0.06^{b}	1.77±0.10^{b}	1.84±0.22^{ab}	1.87±0.13^{ab}	1.79±0.15^{b}	1.83±0.16^{ab}	1.98±0.12^{a}

Values are means±SD, and means in the same row without common superscript are significantly different ($P<0.05$).

Table 3. **Effects of dietary triiodothyronine (T3) and thyroxine (T4) on weights of pectoral muscles, heart and abdominal fat**

	Control	Dietary T3 (mg/kg)			Dietary T4 (mg/kg)		
		0.3	0.9	2.7	1.2	3.6	10.8
Pectoral profundus muscle weight (g/kg body weight)	26.7±1.7^{b}	27.8±1.1^{a}	28.4±0.1^{ab}	28.3±1.8^{b}	26.7±1.3^{ab}	27.6±1.0^{ab}	26.2±0.9^{b}
Heart weight (g/kg body weight)	4.7±0.8^{d}	6.0±0.4^{bc}	6.9±0.6^{b}	8.8±1.2^{a}	5.5±0.6^{cd}	6.4±0.3^{bc}	6.7±1.0^{b}
Abdominal fat weight (g/kg body weight)	7.2±2.1^{a}	6.0±2.6^{a}	1.9±0.5^{b}	0.7±0.5^{b}	6.3±2.5^{a}	2.4±1.1^{b}	0.5±0.4^{b}

Values are means±SD, and means in the same row without common superscript are significantly different ($P<0.05$).

Table 4. **Effects of dietary triiodothyronine (T3) and thyroxine (T4) on heat production and rate of muscle protein breakdown**

	Control	Dietary T3 (mg/kg)			Dietary T4 (mg/kg)		
		0.3	0.9	2.7	1.2	3.6	10.8
Heat production (kcal/kg body weight)	76.7±7.8^{b}	90.1±10.5^{a}	101.8±8.3^{a}	110.7±15.1^{a}	94.5±19.2^{ab}	91.0±10.0^{ab}	105.4±9.8^{a}
Rate of muscle protein breakdown (%/day)	3.8±0.4^{b}	5.4±1.7^{ab}	5.3±0.6^{ab}	6.5±2.7^{a}	4.1±7.0^{b}	5.6±1.3^{ab}	7.0±2.1^{a}

Values are means±SD, and means in the same row without common superscript are significantly different ($P<0.05$).

weights. The abdominal fat weights were decreased dose-dependently by both hormones. The heart weights were increased dose dependently by the hormones and it was more than twice of the control when 2.7 mg T3/kg diet was given. It seems that T3 specifically influences on heart.

Table 4 summarizes the effects of T3 and T4 on heat production and skeletal muscle protein breakdown rate estimated from MH excretion. Heat productions were significantly increased by both hormones and the lowest dose levels of both hormones were sufficient to stimulate heat production. Heat production was expressed as kcal/

kg BW because a pair of birds was used for the measurement. The rates of skeletal muscle protein breakdown were also significantly increased by both T4 and T3 dose-dependently.

Discussion

The aim of the present study was to support our hypothesis that T4 is active and has roles in chicken skeletal muscle protein metabolism although it is usually believed that T3 is an active hormone. The present experiment was designed based on the assumption that the potency of T3 is about 4 times that of T4, and thus dietary levels of T3 (0.3, 0.9, 2.7 mg/kg diet) were set as one forth of those of T4 (1.2, 3.6, 10.8 mg/kg diet). However, plasma concentrations of the hormones were not parallel to the dose levels. Plasma T4 concentrations were 6, 15 and 74 times of the normal level in the groups of 1.2, 3.6 and 10.8 mg T 4 / kg diet, respectively, and plasma T3 concentrations were 4, 9 and 18 times of the normal levels in the groups of 0.3, 0.9 and 2.7 mg T3/kg diet, respectively. These results show that T3 turns over much faster than T4 although the two plasma thyroid hormones have been reported to have identical t 1/2 ranged between 2 and 8 hours (Epple and Stetson, 1980).

Plasma T3 was not increased by dietary T4. This indicates that peripheral 5′-deiodinase activity is low in chickens. The present results are consistent with the observation of Chanoine *et al.* (1993) showing that thyroid gland is a major source of circulating T3. It is also probable that 5′-deiodinase is under the feed-back regulation as has been reported by Halperin *et al.* (1994).

Our previous study has shown that body weight gain and skeletal muscle growth are stimulated and muscle protein turnover rate is increased by dietary T4 (Suthama *et al.,* 1989). However, in the present study, significant increase in muscle weight due to T4 treatment could not be observed while body weight gain and pectoral muscle weights were increased significantly in the group of 0.3 mg T3/kg diet. At the highest levels, both T4 and T3 significantly decreased body weight gain while the muscle weights were not changed. The high levels of thyroid hormones decreased body fat content but not skeletal muscle content. Both thyroid hormones similarly reduced abdominal fat weight in a dose dependent manner. Abdominal fat weights were less than 1/10 of the control group in both groups of 2.7 mg T3/kg diet and 10.8 mg T 4/kg diet. This is consistent with the increase in heat production. Heat production was also significantly increased dose-dependently by both hormones.

The potency of these two hormones may vary with tissues. Although the present experiment was not designed to clarify the difference between tissues of the responsibility to the hormones, the heart weight, for instance, responded more to T3 than T4 especially when the dose levels were high. Both hormones significantly increased heart weight, but heart weight of 2.7 mg T3/kg diet group was about 1.4 times heavier than that of 10.8 mg T4/kg diet group. The potency of dietary T3 to increase heart weight is much stronger than that of T4. It is well known that important actions of thyroid hormone are to increase heart rate and cardiac output. Thyroid hormone disturbance has been observed to exert profound effects on cardiac function which result from the modification of the myofibrillar remodeling (Machackova *et al.,* 2005). It has also been reported that cardiac hypertrophy induced by thyroid hormones may involve reduced proteolysis (Hjalmarson *et al.,* 1975), indicating that thyroid hormones differentially affect skeletal and cardiac muscles protein metabolism.

Skeletal muscle protein breakdown was accelerated dose-dependently by the hormones, and the potency of T3 was about 4 times higher than that of T4. These effects of thyroid hormones on skeletal muscle protein metabolism might contribute to the changes in heat production because muscles comprise a major part of the body and both protein synthesis and breakdown are energy consuming processes. Skeletal muscle protein breakdown seemed to be affected more intensely than heat production by thyroid hormones. However, further study is needed to clarify the relationship between heat production and skeletal muscle protein breakdown affected by T4 and T3. The thermogenic effects of thyroid hormones are well investigated, but there have been no report, to our knowledge, comparing the proteolytic activities of T3 and T4. The present result is consistent with our previous results (Nakashima *et al.,* 1998) showing that T4 is active and plays important metabolic roles especially in protein metabolism in chicken skeletal muscle cells although T3 is generally thought to be the active form of thyroid hormone. It is plausible that T 4 plays major roles in the regulations of heat production and skeletal muscle protein metabolism in animals, because normal plasma level of T4 is about 6 times of that of T3, and T4 is more stable in the plasma than T3.

In conclusion, the present study strongly supports the idea that T4 is active and the potencies to stimulate heat production, and skeletal muscle protein breakdown are about one forth of those of T3 in chickens.

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