Effects of a β -Adrenergic Agonist on Growth Performance and Protein Metabolism in Broilers Treated with or without an Antithyroid Substance

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ABSTRACT : To determine the interrelationship between thyroid status and the reparitioning action of clenbuterol (CLE) in broilers, two-week-old female chickens were fed diets containing an antithyroid substance, propylthiouracil (PTU, 0 or 0.3%), CLE (0 or 1 mg/kg), or both for 18 days in a 2×2 factorial design experiment. Muscle weights (breast muscle, *gastrocnemius* and *peroneus longus*) increased only in the normal chickens fed CLE. As absolute mass, protein of leg muscle quantitatively increased in the CLE-fed normal birds. In contrast, inhibition of the CLE-induced protein accretion, especially of *peroneus longus*, occured in the PTU group. A quantitative increase in DNA was observed in leg muscles of the normal chickens, but no DNA response to CLE was shown in the PTU-treated chickens. The decreased RNA in leg muscles of the PTU group was more reduced by CLE feeding. Although not statistically significant, the reduced degradation rate of whole muscle protein in normal chickens fed CLE was not confirmed in the PTU-fed group. The present study, therefore, concluded that metabolic action of thyroid hormones was a prerequisite for the hypertrophic effect of β-agonist in broilers. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 788-793*)

Key Words : Clenbuterol, Propylthiouracil, Growth Performance, Broiler

INTRODUCTION

Dietary administration of β-adrenergic agonist (β-agonist), a potent growth promoter, has been shown to increase protein accretion and decrease fat deposition in meat-producing animals (Reeds and Mersmann, 1991; Wellnereiter, 1991). Most observations indicate that the increase in protein accretion in the β-agonist-fed animals is attributable to a lowered rate of fractional muscle protein degradation (Hamano et al., 1994; Morgan et al., 1989; Reeds et al., 1986; Yang and McElligott, 1989). However, chickens are thought to be generally insensitive to β-agonist stimulation (Wellenreiter, 1991; Yang and McElligott, 1989). The authors have focused on interrelationships with nutritional conditions that maintain and improve the repartitioning action of β-agonist (Hamano et al., 1994, 1998).

Furthermore, the dramatic effects of β-agonist depend on dose levels or length of treatment (Buyse et al., 1991; Dalrymple et al., 1984; Gwartney et al., 1992). The lack of sensitivity of tissues to this agent seems to be associated with β-receptor density on the cells (Kim et al., 1992; Yang and McElligott, 1989). Thyroid hormones have been shown not only to stimulate protein turnover rates of skeletal muscle directly (Evers, 1989; Suthama et al., 1991), but also to regulate β-adrenergic receptor density in several tissues as same as glucocorticoids do (Stiles, 1991). In addition, a previous study found a positive correlation in that the hypertrophic effect of a β-agonist, clenbuterol (CLE), was stimulated when broilers were treated with a thyroid hormone, thyroxine (Hamano et al., 1995). However, the involvement of thyroid status in the growth response of chickens to β-agonist has not been fully understood.

The present study was conducted to determine the effects of CLE on the performance and muscle protein metabolism of chickens when thyroid function was lowered by administration of an antithyroid substance.

MATERIALS AND METHODS

One-hundred day-old female broiler chicks (*Ross*) were housed in battery brooders and fed a pretreatment diet (23% crude protein, 3,200 kcal of metabolizable energy/kg) until 2 weeks of age. The chicks were weighed at 2 weeks of age. Twenty-four chicks that had a similar body weight were divided into 4 groups for a 2×2 factorial arrangement (n=6); then the birds were housed in individual cages and fed experimental diets and water *ad libitum* throughout the experiment period of 18 days. An antithyroid substance, 0.3% propylthiouracil (PTU, Sigma, St. Louis, MO, USA) was added to a basal diet (21% crude protein, 3,100 kcal of metabolizable energy/kg; see Hamano et al., 1995). For a normal thyroid condition, the basal diet alone was given. Furthermore, 1 mg/kg of CLE (Sigma, St Louis, MO., USA) was added to half of basal or PTU diet.

At 4 weeks of age, the birds in each group were weighed and killed using anesthesia injection (pentobarbital sodium); abdominal fat, breast muscle, and leg muscles (*gastrocnemius* and *peroneus longus*) were removed and weighed. The skeletal muscles were stored at -85℃ and used for chemical analysis for

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protein, DNA, and RNA.

Protein concentration in the muscle was determined by a modified Lowry procedure (Markwell et al., 1978). DNA was analyzed using fluorometric procedure with Hoechist 33258 reagent (Labarca and Paigen, 1980). Determination of RNA was conducted using a previously reported procedure (Hamano et al., 1998). Finally, excreta from individual chickens (n=5) were collected for the last 3 days of the experimental period. A portion of the excreta sample was used for analysis of 3-methylhistidine. The fractional rates of myofibrillar protein degradation were estimated by the 3-methylhistidine excretion using the hight performance liquid chromatographic method of Hayashi et al. (1987). The fractional rate of protein synthesis was calculated in accordance with the mathematical model of Funabiki et al. (1976).

All data were statistically analyzed using a two-way analysis of variance. When a significant interaction between thyroid state and CLE treatment was detected in each parameter within each thyroid condition, a significant difference of the means between control and CLE-treated chickens was tested by Student's t test.

RESULTS

Table 1 shows the effects of CLE on growth performance of chickens with or without PTU treatment. The adiministration of PTU decreased ($p<0.01$) final body weight of chickens by 18%. However, CLE feeding did not affect whole body growth, regardless of the PTU administration. In absolute mass of tissue weight, no effects of PTU and CLE on abdominal fat weight were observed, independent of the decreased body weight in the PTU group. Leg muscle weight showed significant interactions between PTU and CLE ($p<0.05$), but breast muscle did not. The PTU treatment resulted in a 16% decrease in weight of mixed leg ($p<0.01$), consisting of *gastrocnemius* and *peroneus longus*. This mixed leg muscle mass in the CLE-fed chickens increased by 20% only when the birds were maintained under normal conditions. In the PTU-treated group, CLE did not affect leg muscle weight. In addition, each tissue weight of *gastrocnemius* and

Table 1. Effects of clenbuterol (CLE) on tissue weights in broilers treated with 0 or 0.3% propylthiouracil (PTU)

Thyroid state	Normal		PTU		Pooled SEM[1]	Analysis of variance[2]		
CLE treatment	Control	CLE	Control	CLE		Thyroid state	CLE	Interaction
Final body weight (g)	1,235	1,294	1,054	1,032	32	**	NS	NS
Tissue weight (g)								
Abdominal fat	13.36	13.96	14.36	18.39	1.23	NS	NS	NS
Breast muscle	73.01	74.70	57.20	58.51	2.59	**	NS	NS
Leg muscle[3]	19.42[a]	23.29[b]	18.41	17.54	0.62	**	NS	*
Gastrocnemius	10.31[a]	12.37[b]	10.02	9.44	0.36	*	NS	*
Peroneus longus	9.11[a]	10.92[b]	8.39	8.10	0.30	**	NS	*

[1] Values represent pooled standard error of the means (n=6). [2] ** $p<0.01$, * $p<0.05$, NS: no significance.
[3] Values consist of the weights of *gastrocnemius* and *peroneus longus*
[a,b] Significant difference between control and CLE treatment in each thyroid state ($p<0.05$).

Table 2. Effects of clenbuterol (CLE) on tissue weight per body weight (BW) in broilers treated with 0 or 0.3% propylthiouracil (PTU)

Thyroid state	Normal		PTU		Pooled SEM[1]	Analysis of variance[2]		
CLE treatment	Control	CLE	Control	CLE		Thyroid state	CLE	Interaction
Tissue weight (g/kg BW)								
Abdominal fat	10.48	10.76	13.70	17.64	1.09	*	NS	NS
Breast muscle	58.98	57.54	54.05	56.35	1.06	NS	NS	NS
Leg muscle[3]	15.68[a]	18.04[b]	17.61	17.00	0.34	NS	NS	*
Gastrocnemius	8.30[a]	9.59[b]	9.57	9.11	0.21	NS	NS	*
Peroneus longus	7.38	8.45	8.04	7.90	0.18	NS	NS	NS

[1] Values represent pooled standard error of the means (n=6). [2] ** $p<0.01$, * $p<0.05$, NS: no significance.
[3] Values consist of the weights of *gastrocnemius* and *peroneus longus*
[a,b] Significant difference between control and CLE treatment in each thyroid state ($p<0.05$).

Table 3. Effects of clenbuterol (CLE) on muscle composition in broilers treated with 0 or 0.3% propylthiouracil (PTU)

Thyroid state / CLE treatment	Normal		PTU		Pooled SEM[1]	Analysis of variance[2]		
	Control	CLE	Control	CLE		Thyroid state	CLE	Interaction
Protein (mg/g)								
Breast muscle	219.1	215.9	208.6	202.7	3.6	NS	NS	NS
Gastrocnemius	205.5	209.6	184.9	181.2	3.7	**	NS	NS
Peroneus longus	218.3	218.2	186.6	163.5	5.9	**	NS	NS
DNA (mg/g)								
Breast muscle	0.81[a]	0.69[b]	0.64[a]	0.83[b]	0.03	NS	NS	**
Gastrocnemius	0.70[a]	1.01[b]	0.67	0.65	0.03	**	**	**
Peroneus longus	0.87	0.90	0.71	0.73	0.02	**	NS	NS
RNA (mg/g)								
Breast muscle	1.38	1.22	1.31	1.42	0.04	NS	NS	NS
Gastrocnemius	1.33	1.19	0.93	0.54	0.07	**	**	NS
Peroneus longus	1.44	1.28	0.95	0.56	0.08	**	**	NS

[1] Values represent pooled standard error of the means (n=6). [2] ** p<0.01, NS: no significance.
[a,b] Significant difference between control and CLE treatment in each thyroid state (p<0.05).

peroneus longus was proportional to the mixed muscle weight.

In proportion to body weight (table 2), abdominal fat was significantly (p<0.05) increased by the PTU treatment, but no effect of CLE on PTU-induced adipose tissue accretion was observed. Percentage of breast muscle was also unchanged by the CLE feeding. However, the combined leg muscle and *gastrocnemius* in the composition were about 15% heavier in the CLE-fed chickens than in controls only when birds were fed the normal diet. *Peroneus longus* weight did not respond to PTU or CLE treatment.

Table 3 shows the effects of CLE on muscle composition in chickens with or without PTU treatment. Although PTU decreased (p<0.01) protein concentration of leg muscles, no significant effect of CLE was obserbed. As a significant interaction (p<0.01) was found, DNA concentration of breast muscle was lowered (p<0.05) in the CLE-fed chickens, but increased (p<0.05) in the treated birds when fed the PTU diet. In contrast, an increase in DNA concentration due to CLE was detected in *gastrocnemius* of chickens fed the normal diet (p<0.05). When birds were fed PTU, the DNA concentration in *gastrocnemius* or *peroneus longus* was not influenced by the CLE administration. A similar depression was observed in the DNA concentration of breast muscle in normal chickens, but change in the DNA was not associated merely with the protein concentration (per wet weight). An decrease in RNA

Table 4. Effects of clenbuterol (CLE) on absolute mass of protein, DNA and RNA in muscle of broilers treated with 0 or 0.3% propylthiouracil (PTU)

Thyroid state / CLE treatment	Normal		PTU		Pooled SEM[1]	Analysis of variance[2]		
	Control	CLE	Control	CLE		Thyroid state	CLE	Interaction
Protein (g)								
Breast muscle	15.95	16.15	12.07	11.83	0.63	NS	NS	NS
Gastrocnemius	2.12	2.60	1.85	1.74	0.09	**	NS	NS
Peroneus longus	1.97[a]	2.37[b]	1.57[a]	1.33[b]	0.09	**	NS	**
DNA (mg)								
Breast muscle	59.22	51.77	36.76[a]	48.81[b]	2.48	**	NS	*
Gastrocnemius	7.22[a]	12.46[b]	6.72	6.22	0.57	**	**	**
Peroneus longus	8.01[a]	9.83[b]	5.93	5.92	0.40	**	NS	*
RNA (mg)								
Breast muscle	98.7	89.7	74.6	81.3	3.2	**	NS	NS
Gastrocnemius	13.4	14.8	9.2[a]	5.0[b]	0.8	**	NS	**
Peroneus longus	13.0	14.0	7.9[a]	4.5[b]	0.8	**	NS	*

[1] Values represent pooled standard error of the means (n=6). [2] ** p<0.01, * p<0.05, NS: no significance.
[a,b] Significant difference between control and CLE treatment in each thyroid state (p<0.05).

concentration occurred in the CLE-fed chickens regardless of PTU treatment ($p<0.01$).

In the present study, thus, a distinct interrelation between thyroid state and CLE was not found in muscle composition as concentration per wet weight. Table 4 shows the results of absolute mass of protein, DNA and RNA in muscles. In normal chickens, CLE increased ($p<0.05$) protein content in *peroneus longus* by 20%, but decreased ($p<0.05$) it by 15% when birds were fed PTU. In other muscle, the effect of CLE on protein content tended to be similar to that on *peroneus longus* (not significant). A significant interaction between thyroid status and CLE was detected in muscle DNA content. A decrease in DNA ($p<0.01$) occurred in the muscles in PTU-fed chickens. Although DNA in breast muscle of normal chickens tended to be lowered by CLE feeding, CLE increased ($p<0.05$) it in the PTU group. When animals were given the normal diet, an increase in DNA was detected in *gastrocnemius* and *peroneus longus* treated with CLE ($p<0.05$), but not in the muscle of the PTU-fed chickens. The PTU treatment decreased the absolute mass of RNA in the muscles ($p<0.01$). Furthermore, RNA content in leg muscles was markedly reduced ($p<0.05$) by CLE feeding only when chickens received PTU.

Table 5 shows the effects of CLE on protein/DNA and RNA/DNA ratios in chickens treated with or without PTU. Significant interactions between PTU andCLE were observed in protein/DNA ratios of breast muscle and *peroneus longus* ($p<0.05$). In the normal chickens, the ratio of protein/DNA was higher in the CLE-treated group than in controls. In contrast, the PTU treatment lowered the CLE-induced ratio of protein/DNA in breast muscle ($p<0.05$). This ratio in *gastrocnemius* decreased in CLE-fed chickens of the normal group. When animals were fed PTU, the ratio was unchanged due to CLE. In addition, no effect on the protein/DNA ratio was detected in *peroneus longus*. The RNA/DNA ratio in muscles showed no significant interaction between PTU and CLE. However, CLE feeding lowered this ratio of leg muscles, regardless of the PTU administration.

Table 6 shows the effects of CLE on muscle protein turnover rate in chickens with or without PTU treatment. When chickens were fed the normal diet, CLE tended to reduce the fractional degradation rate of protein by 18%, although not significantly. In the PTU group, the magnitude of the difference of this rate between control and CLE treatment was smaller

Table 5. Effects of clenbuterol (CLE) on protein/DNA and RNA/DNA in muscle of broilers treated with 0 or 0.3% propylthiouracil (PTU)

Thyroid state	Normal		PTU		Pooled SEM[1]	Analysis of variance[2]		
CLE treatment	Control	CLE	Control	CLE		Thyroid state	CLE	Interaction
Protein/DNA (mg/mg)								
Breast muscle	272	313	332[a]	250[b]	12	NS	NS	*
Gastrocnemius	294[a]	208[b]	283	277	10	NS	**	*
Peroneus longus	249	242	271	228	9	NS	NS	NS
RNA/DNA (mg/mg)								
Breast muscle	1.70	1.73	2.05	1.74	0.06	NS	NS	NS
Gastrocnemius	1.90	1.18	1.37	0.82	0.09	**	**	NS
Peroneus longus	1.64	1.42	1.35	0.80	0.08	**	**	NS

[1] Values represent pooled standard error of the means (n=6). [2] ** $p<0.01$, * $p<0.05$, NS: no significance.
[a,b] Significant difference between control and CLE treatment in each thyroid state ($p<0.05$).

Table 6. Effects of clenbuterol (CLE) on fractional rates of protein degradation and protein synthesis in skeletal muscle of broilers treated with 0 or 0.3% propylthiouracil (PTU)

Thyroid state	Normal		PTU		Pooled SEM[1]	Analysis of variance[2]		
CLE treatment	Control	CLE	Control	CLE		Thyroid state	CLE	Interaction
Fractional rate[3] (%/d)								
Protein degradation	3.15	2.59	2.44	2.04	0.17	NS	NS	NS
Protein synthesis	8.97	8.65	6.19	6.77	0.42	**	NS	NS

[1] Values represent pooled standard error of the means (n=5). [2] ** $p<0.01$, NS: no significance.
[3] Protein turnover rate was estimated from 3-methylhistidine excretion.

than that of the normal group. The fractional synthesis rate of protein was not influenced by CLE feeding, while PTU administration reduced this rate.

DISCUSSION

In our laboratory, Kobayashi et al. (1994) confirmed that PTU administration resulted in hypothyroidism of chickens at a minimum of 0.0075 - 0.0150% in the diet. In the present study, the 0.3% PTU administration results in the markedly decreased growth rate and the increased abdominal fat accretion as body composition attributable to inhibition of thyroid hormone synthesis or release. CLE was reported to improve the muscle growth rate in diabetic rats (Yang and McElligott, 1989). Takahashi et al. (1993) found that CLE prevented the increase in abdominal fat weigh induced by corticosterone administration. However, CLE did not improve the PTU-reduced performance of chickens. Thus, the findings of this study support the idea that sufficient thyroid function is at least prerequisite for CLE-induced growth reponse, and that β-adrenergic responsiveness of tissues possesses an indirect relationship with thyroid hormones (Hamano et al., 1995). With regard to breast muscle growth, this correlation was unclear in this study. This different response between breast and leg muscles could be associated with the proportion of red fiber, namely the difference in β-receptor density on the cell (Morgan et al., 1989; Watson-Wright and Wilkinson, 1986).

Most studies exhibited reduced DNA concentrations with protein accretion in muscle of animals that received β-agonist (Gwartney et al., 1992; Hamano et al., 1998; Reeds and Mersmann, 1991; Yang and McElligott, 1989). These findings suggested that the increased muscle mass with protein accretion resulted over cellular hypertrophy, increased muscle cell size. Generally, DNA concentration in used to estimate cell number (Simon, 1989). Morgan et al. (1989) reported that increased DNA is not prerequisite for diagnosing muscle hypertrophy due to β-agonist. In contrast, the present results showed that CLE increased absolute mass and protein accretion with enhanced DNA content in *gastrocnemius* when chickens were fed the normal diet (table 4). The hypertrophic effect of CLE may depend on muscle type or might result from an increased number of non-muscle cells such as dipocytes, satellite cells and fibroblasts. Grants et al. (1990) and Roberts and McGeachie (1992) reported that β-agonist stimulated proliferation of satellite cells. In fact, the dietary CLE enhanced the protein/DNA ratio, which is an index of cell size (Simon, 1989), in breast muscle, but reduced it in *gastrocnemius*.

Thyroid hormones have also been reported to affect DNA synthesis (Evers, 1989). The decreased DNA in the muscles of the PTU-fed chickens would result from reduced DNA syhthesis, as compared with that in normal birds. In this condition, no effet of CLE on DNA content was observed, but the reason for this effect on breast muscle DNA was obscure in this study. Thus, although the present DNA result might be inadequate to assess the muscle cell number alone, the attenuation of thyroid function led, at least, to lack of or reduced the β-adrenergic response of muscle cells.

The PTU-induced protein accumulation was closely related to RNA content or the RNA/DNA ratio, namely protein synthesis capacity, in muscles. Thyroid hormone supplementation stimulates fractional rates of protein synthesis and degradation in skeletal muscle (Evers, 1989; Suthama et al., 1991). In the present study, the fractional synthesis rate of whole skeletal muscle protein, estimated from 3-methylhistidine excretion, was also markedly decreased by PTU administration. However, CLE supplementation did not prevent this inhibition of protein synthesis (Table 6). Generally, the fractional synthesis rate of muscle does not respond to β-agonist in chickens (Hamano et al., 1994; Morgan et al., 1989; Muramatsu et al., 1990). Conversely, in the PTU-fed chickens, the more diminished RNA content and RNA/DNA ratio occurred in the CLE-fed chickens (Tables 4 and 5), whereas no change in fractional synthesis rate due to CLE was detected in the PTU group. Previous studies also exhibited a decrease in RNA concentration in breast muscle of broilers fed CLE (Hamano et al., 1995; 1998). In normal conditions, CLE would depress the proteolysis responsible for protein accretion. Relative to normal conditions, when a lowered fractional rate of protein degradation was induced by PTU, no response in this fractional rate to CLE appeared. These findings suggested that thyroid hormone-induced protein turnover was necessary to maintain the dramatic effect of CLE in protein accretion.

Over all, thyroid status apparently affected the dramatic effect of CLE on performance and protein accretion in broilers. In responsiveness of tissue to β-agonists, thyroid hormones and glucocorticoids have been shown to affect β-receptor density on the cells and post-receptor events (Stiles, 1991). We found previously that thyroxine administered to broilers stimulated the hypertrophic effect of CLE (Hamano et al., 1995). The cause may not necessarily be the increased β-receptor number, but rather interaction with a rising protein turnover rate. Moreover, increased uric acid excretion in broilers treated with corticosterone was reduced by CLE (Takahashi et al., 1993). The impact of CLE on proteolysis may have been favorable when protein turnover rates rose. In the case of lowered thyroid function, metabolic response of chickens to CLE was markedly reduced or negated. The present study revealed that the metabolic action of

thyroid hormone is a prerequisite for the muscle response to elicit the repartitioning action of β-agonist.

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