Excessive Levels of Dietary Protein and Energy Induce Lack of Growth Promoting Effects of Clenbuterol in Broilers

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ABSTRACT : The present study examined the effects of excessive dietary protein and energy on growth response to clenbuterol in broilers. The chicks were allocated into 6 groups at 14d old, and used for a 3 × 2 factorial experiment. Birds were fed six diets, the control diet containing 21% crude protein (CP) and 3,100 kcal of metabolizable energy ME/kg, a high protein (30% CP) or a high energy (3,500 kcal/ ME/kg) diet, with or without 1 ppm clenbuterol, for 18 d. Clenbuterol feeding markedly decreased ($p < 0.05$) body weight gain by 23% in the high energy group. Feed intake was also decreased ($p < 0.05$) by clenbuterol administration across diet treatments. Abdominal fat weight was reduced ($p < 0.05$) by clenbuterol only when chickens were fed the high energy diet. Clenbuterol increased ($p < 0.05$) leg muscle weight in the control diet group, but decreased ($p < 0.05$) it in the high energy group. Muscle protein concentration was increased by 11% in leg muscle only of the birds at the high energy level. In leg muscle, clenbuterol enhanced the protein/DNA ratio by 18%, except for the high protein group. These results indicate that feeding a diet containing excessive amounts of protein and more energy than normal did not necessarily improve growth response to clenbuterol.
(**Key Words:** β-Adrenergic Agonist, Clenbuterol, Dietary Protein, Dietary Energy, Growth Performance)

INTRODUCTION

Dietary supplementation with β-agonists has been shown to increase skeletal muscle protein accretion and to decrease adipose tissue mass (Wellenreiter, 1991; Weppelman, 1984; Yang and McElligott, 1989). However, the mechanisms leading to muscle hypertrophy and reduction in adipose tissue accretion have not been well known, since there are conflicting findings, depending on animal species, sex, types of agents, dose levels or duration of treatment (Dalrymple et al., 1984; Gwartney et al., 1992; Morgan et al., 1989; Reeds et al., 1986; Yang and McElligott, 1989).

Moreover, the mode of action of β-agonists depends on dietary levels of nutrients. A previous study (Hamano et al., 1994) reported that feeding a diet containing 11% crude protein (CP) brought about a negative growth performance for clenbuterol-treated broilers, as compared with birds fed a normal diet (21% CP). In pigs, dietary supplementation with β-agonists enhanced protein and energy requirements to maintain maximal growth performance (Dunshea et al., 1993; Mitchell et al., 1991; Oksbjerg et al., 1994). Thus, variation in dietary status affects the protein-sparing effect of β-agonists in growing animals.

In addition, whether β-agonist-fed broilers result in increased requirements of dietary protein or energy is unclear (Wellenreiter, 1991). Kim et al. (1991) reported that the greatest growth response to a β-agonist cimaterol was observed when the broiler chickens were fed a diet containing high-protein (19% CP) with high-metabolizable energy (ME) of 3,200 kcal/kg, during the period of 4 to 7 wk of age, although not significantly. The growth-promoting impact of β-agonists, however, may not necessarily occur under any dietary conditions that are higher in levels of protein and energy than normal, even if administration of the agents enhances nutrient use efficiency for the growth. The present study was focused on whether feeding a high-protein or-energy diet, especially excessive levels, maintains the β-agonist-induced growth performance and results in the lack of growth-promoting action of the agents in broilers.

This study was, therefore, conducted in order to examine the effects of excessive levels of dietary protein and energy on growth response to clenbuterol in broiler chickens.

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MATERIALS AND METHODS

Day-old female broiler chicks (*Ross breed*) were housed in battery brooders until 14d old. Thirty chicks with similar initial body weight were selected and allocated into 6 groups of 5 birds each at 14d old. The chicks were then housed in individual wire cages in room maintained at 25℃, and used for a 3 × 2 factorial experiment design. Experimental diets containing different levels of either protein or energy, with or without 1 ppm clenbuterol (Sigma, St. Louis, MO., USA) were provided to each group. Composition of diets is shown in table 1. The control diet (CD), containing 21% CP and 3,100 kcal ME/kg, was based on requirements of the Japanese Feeding Standard (National Research Council of Agriculture, 1992). The level of CP in the CD was lower than that of the NRC requirement for the starter period. Relative to the CD, 30% CP (HP) and 3,500 kcal ME/kg (HE) in diets were used as excessive levels of protein and energy, respectively.

Table 1. Composition of experimental diets (%)

Diet[1]	CD	HP	HE
Ingredients			
Isolated soybean protein[2]	17.4	27.9	17.6
Ground yellow corn	77.6	65.1	71.4
Corn oil	–	–	6.0
Vitamin and mineral mixture[3]	3.4	3.4	3.4
DL-methionine	0.26	0.37	0.26
L-lysine HCl	0.16	0.23	0.13
Glycine	0.20	0.27	0.20
L-threonine	0.05	0.07	0.04
Cellulose[4]	0.77	2.48	0.85
Analytical composition			
CP (%)	21.5	30.0	21.3
ME[5] (kcal/kg)	3,154.0	3,160.0	3,500.0

[1] CD, control diet; HP, high protein diet; HE, high energy diet.
[2] Fujipro-R®, Fujiseiyu Co., Ltd., Osaka, Japan.
[3] Vitamin and mineral mixture per kg diet: 10,000 IU vitamin A; 2,000 ICU vitamin D_3; 5.0 mg α-tocopherol acetate; 2.5 mg vitamin K_3; 1.51 mg cyanocobalamin; 7.7 mg riboflavin; 1.5 mg thiamin nitrate; 5.28 mg calcium panthothenate; 10.4 mg folic acid; 18.2 mg nicotinic acid; 1.5 mg pyridoxine hydrochloride; 0.11 mg biotin; 880 mg choline chloride; 7.2 g $CaCO_3$; 17.8 g $CaHPO_4 \cdot 2H_2O$; 2.9 g NaCl; 5.0 mg $CuSO_4 \cdot 5H_2O$; 0.13 g $MnSO_4 \cdot 6H_2O$; 0.13 g $ZnSO_4 \cdot 7H_2O$; 50 mg $FeSO_4 \cdot 7H_2O$ and 0.6 mg KI.
[4] Cellulose Powder D (40-100 mesh), Toyo Roshi Co., Ltd., Tokyo, Japan.
[5] Calculated value.

These experimental diets were given *ad libitum* for 18 d. At 18 d, chickens were killed using an anesthesia (Nembutal® Injection, Abbott Laboratories, North Chicago, IL., USA) injected intravenously; then abdominal fat, breast muscle and leg muscle, consisting of the *gastrocnemius* and *peroneus longus*, were removed and weighed. The muscles were immediately stored at −85℃, and used for chemical analysis of protein, DNA and RNA.

Protein concentration was determined by the biuret method of Gornall et al. (1949) using BSA as a standard. DNA and RNA, isolated from muscles (Shibko et al., 1967), were colorimetrically quantitated by using diphenylamine reaction (Richards, 1974) and orcinol reaction (Lin and Schjeide, 1969), respectively. Two-way ANOVA as a 3 × 2 factorial arrangement was used in analysis of data. When a significant interaction between dietary nutrient level and clenbuterol treatment was detected, the means of clenbuterol-treated group within each dietary level were compared with their controls (t - test).

RESULTS AND DISCUSSION

There were significant interactions ($p < 0.05$) between dietary state and clenbuterol treatment in final body weight and body weight gain (table 2). In chickens given HP or HE diet, clenbuterol decreased ($p < 0.05$) final body weight, but did not in those fed CD. The influence of clenbuterol on body weight gain was consistent with final body weight, although negative response to β-agonist was not statistically confirmed in chickens fed HP diet (table 2). Dietary clenbuterol decreased ($p < 0.05$) feed intake, but the extent of reduced feed intake was greater in HP (5%) and HE (15%) groups than in CD group (2%). However, neither dietary condition nor clenbuterol statistically affected average feed intake (% body weight) or feed efficiency, while feeding the HP diet tended to reduce this feed intake as compared with other dietary groups.

The negative correlations between β-agonist and nutritional state have been shown in pigs (Bracher-Jakob and Blum, 1990) and rats (Perez-Llamas et al., 1991), given a low protein diet. A previous study also indicated that clenbuterol markedly reduced body weight gain and feed intake when broilers were fed a 11% CP diet (Hamano et al., 1994). Thus, growth response to β-agonist is variable under conditions of insufficient dietary protein supply. The present results furthermore noted that ability of clenbuterol to use protein and energy for whole body growth was limited, even though over large amounts of nutrients required for growth were supplied to the

Table 2. Effects of clenbuterol on body weight gain, feed intake and feed efficiency in broiler chickens fed either an excessive protein or energy diet

Diet[1] Treatment	CD		HP		HE		Pooled SEM	Analysis of variance[3]		
	Control	Clen-buterol	Control	Clen-buterol	Control	Clen-buterol		Diet	Clen-buterol	Interac-tion
Final body weight (g)	1,176	1,187	1,160[a]	1,100[b]	1,314[a]	1,071[b]	22	NS	*	*
Body weight gain (g/18 d)	925	934	909	848	1,062[a]	817[b]	22	NS	*	*
Feed intake (g/18 d)	1,531	1,508	1,420	1,350	1,646	1,402	29	NS	*	NS
Average feed intake (% body weight)	7.24	7.06	6.79	6.85	6.97	7.31	0.09	NS	NS	NS
Feed efficiency[2]	0.60	0.62	0.64	0.63	0.65	0.59	0.01	NS	NS	NS

[1] CD, control diet; HP, high protein diet; HE, high energy diet.
[2] Body weight gain/feed intake.
[3] * $p < 0.05$; NS, no significance.
[a,b] Means within a row with no common superscript in each dietary group differ significantly ($p < 0.05$).

treated chickens.

The effects of clenbuterol on tissue weight are shown in table 3. A significant interaction between dietary condition and clenbuterol supplementation was detected in abdominal fat weight ($p < 0.05$). Clenbuterol did not affect abdominal fat weight in chickens fed CD. In the HP feeding group, this tissue weight was markedly lighter than those groups of CD and HE, but no significant effects of clenbuterol was observed. However, the remarkable accretion of abdominal fat induced by feeding the HE diet was profoundly inhibited by clenbuterol treatment. The percentage of abdominal fat weight to the live weight in HE group was also decreased by clenbuterol. The distinct response to clenbuterol for reduction in adipose tissue accretion could be elicited in a state consuming high energy diet. Many studies noted that dietary supplementation with a β-agonist brought about decreased fat accretion in broiler chickens (Buyse et al., 1991; Dalrymple et al., 1984; Wellenreiter, 1991). Morgan et al. (1989) confirmed that the typical response to cimaterol, reduced fat deposition, was significantly different between 38- and 56-d-old broilers. No effect of

Table 3. Effects of clenbuterol on tissue weight and percentage in broiler chickens fed either an excessive protein or energy diet

Diet[1] Treatment	CD		HP		HE		Pooled SEM	Analysis of variance[3]		
	Control	Clen-buterol	Control	Clen-buterol	Control	Clen-buterol		Diet	Clen-buterol	Interac-tion
Tissue weight (g)										
Abdominal fat	13.1	17.3	6.9	6.4	24.6[a]	8.6[b]	1.7	*	NS	*
Breast muscle	65.5	64.0	68.0	56.5	66.2	59.3	1.8	NS	NS	NS
Leg muscle	33.2[a]	37.7[b]	37.3	37.8	37.0[a]	32.0[b]	0.7	NS	NS	*
Tissue percentage[2] (g)										
Abdominal fat	1.09	1.41	0.57	0.56	1.86[a]	0.78[b]	0.12	**	NS	*
Breast muscle	5.53	5.40	5.85	5.12	5.24	5.53	0.11	NS	NS	NS
Leg muscle	2.82	3.18	3.22	3.44	2.83	3.00	0.05	**	**	NS

[1] CD, control diet; HP, high protein diet; HE, high energy diet.
[2] Tissue weight × 100/live weight.
[3] ** $p < 0.01$; * $p < 0.05$; NS, no significance.
[a,b] Means within a row with no common superscript in each dietary group differ significantly ($p < 0.05$).

clenbuterol on fat deposition is likely to occur until 35 d old (Hamano et al., 1994, 1995). In addition, the β-adrenergic responsiveness in adipose tissue of chickens, inducing lipolysis, is insensitive to stimulation of β-agonist as compared with mammals (Wellenreiter, 1991; Weppelman, 1984). Thus, subsequent response to clenbuterol, leading to the marked reduction in adipose tissue accretion, would be associated with capacity for fat deposition in adipocytes.

Neither dietary condition nor clenbuterol supplementation affected breast muscle weight or its percentage. In leg muscle weight, there was a significant interaction between dietary nutrient level and clenbuterol treatment ($p < 0.05$). The administration of clenbuterol significantly increased ($p < 0.05$) leg muscle weight as absolute mass in ckickens given CD, but did not affect the weight at HP level. On the other hand, the significant depression in this muscle mass due to clenbuterol was exhibited in broilers fed HE diet ($p < 0.05$), and resulted from the retarded body weight gain. The muscle weight, as a percentage of live weight, significantly increased in chickens treated with clenbuterol, independent of the nutritional states. Different responses to clenbuterol between these two muscle types are possibly explained by functional characteristics of muscle that proportion of red fiber is greater in leg muscle than in breast muscle (Morgan et al., 1989). Furthermore, the β-receptor density on red fiber is higher than that on white fiber (Watson-Wright and Wilkinson, 1986). In regard to nutritional status, higher levels of protein and energy than control in the present study resulted in no improvement of muscle weight or the lack of β-adrenergic response.

Table 4 shows the effects of clenbuterol on muscle composition in broilers. Although a significant decrease in protein concentration was observed in breast muscle of chickens treated with clenbuterol, clenbuterol increased the concentration in leg muscle, independent of dietary levels of protein or energy. The most impressive increase in protein deposition for leg muscle was indicated only in HE group supplemented with clenbuterol. Recent studies noted that, with muscle hypertrophy caused by β-agonist, DNA dilution appeared (Gwartney et al., 1992; Morgan et al., 1989; Hamano et al., 1995; Yang and McElligott, 1989). The present study observed no influence of clenbuterol on muscle DNA concentration, even though the excessively high protein and energy supplies enhanced DNA concentration in both types of muscle ($p < 0.01$).

Table 4. Effects of clenbuterol on protein, DNA and RNA concentration in breast and leg muscles of broiler chickens fed either an excessive protein or energy diet

Diet[1] / Treatment	CD		HP		HE		Pooled SEM	Analysis of variance[2]		
	Control	Clen-buterol	Control	Clen-buterol	Control	Clen-buterol		Diet	Clen-buterol	Interac-tion
Protein (mg/g)										
Breast muscle	215.0	207.6	203.2	197.5	209.7	208.7	1.4	**	*	NS
Leg muscle	175.6	177.7	170.5	171.5	171.1	189.4	1.9	NS	*	NS
DNA (mg/g)										
Breast muscle	0.36	0.38	0.44	0.47	0.46	0.48	0.01	**	NS	NS
Leg muscle	0.44	0.37	0.51	0.52	0.59	0.56	0.06	**	NS	NS
RNA (mg/g)										
Breast muscle	1.85	1.41	1.92	1.75	2.51	2.24	0.09	**	**	NS
Leg muscle	1.52	1.42	0.93	0.76	2.47	2.45	0.12	**	NS	NS
Protein/DNA (mg/mg)										
Breast muscle	602	549	462	418	465	435	14	**	*	NS
Leg muscle	411	486	339	335	293	344	14	**	*	NS
RNA/DNA (mg/mg)										
Breast muscle	5.18	3.74	4.36	3.71	6.01	4.65	0.20	**	**	NS
Leg muscle	3.47	3.80	1.88	1.48	4.15	4.42	0.23	**	NS	NS

[1] CD, control diet; HP, high protein diet; HE, high energy diet.
[2] ** $p < 0.01$; * $p < 0.05$; NS, no significance.

RNA concentration in breast muscle rose ($p < 0.01$) in HP and HE feeding groups as compared with CD group, and decreased ($p < 0.01$) in clenbuterol-fed chickens. A marked reduction in leg muscle RNA was confirmed when chickens fed HP rather than CD and HE diets. In this case, clenbuterol administration did not affect the RNA concentration. Protein/DNA ratio in breast and leg muscles decreased ($p < 0.01$) in HP and HE groups, as compared with chickens fed CD. A reduction in the protein/DNA occurred in breast muscle of chickens treated with clenbuterol ($p < 0.05$). In contrast, this ratio in leg muscle increased ($p < 0.05$) due to clenbuterol, but no impact was quantitatively detected in the HP group. The increased muscle protein accretion with clenbuterol feeding resulted in enhanced muscle cell size. The high levels of dietary protein and energy were responsible for an increase in DNA concentration. This parameter, however, would assess a potential impact not only on a population of muscle cells, but on other of non-muscle cells. Thus, a general ability of β-agonists to promote muscle growth was possibly diminished or remained unchanged, even if nutritional status stimulated metabolic activity of protein turnover and DNA synthesis remarkably.

Many investigations have demonstrated a lack of change in fractional synthesis rates due to treatment with β-agonists (Hamano et al., 1994; Morgan et al., 1989; Muramatsu et al., 1990; Reeds et al., 1986; Reeds and Mersmann, 1991). The present study also agreed that clenbuterol did not elevate protein synthesis in muscle. Rather, reduced protein degradation would lead to muscle hypertrophy in clenbuterol-fed chickens. Conversely, results of this study found a depressed RNA/DNA ratio with RNA dilution in breast muscle ($p < 0.01$).

Dietary supplementation with β-agonist has been advocated to increase dietary protein and energy requirements in pigs (Dunshea et al., 1993; Mitchell et al, 1991; Oksbjerg et al., 1994; Reeds and Mersmann, 1991). Kim et al. (1991) also noted in broilers that cimaterol caused the highest increase in growth performance in broilers fed a high protein (19% CP) with high energy (3,200 kcal ME/kg) diet during the period of 4 to 7 wk of age, although not significantly. Dietary requirements to maintain the maximal perfomance may rise in β-agonist-fed chickens. In accordance with this possibility, elevated dietary levels of protein or energy are probably necessary to elicit maximal protein deposition from β-agonist stimulation. Table 5 and 6 show the ratios of muscle composition to dietary protein or energy consumed, respectively. The ratio of muscle protein/protein consumption and protein/ME consumption increased ($p < 0.01$) in leg muscle of the clenbuterol-treated chickens, independent of the nutritional states, but not in breast muscle. In breast muscle of the HP group, both ratios were slightly lowered by the clenbuterol feeding. Thus, in the leg muscle, clenbuterol stimulates utilization of protein and

Table 5. Effects of clenbuterol on ratios of muscle composition to dietary protein consumed in broiler chickens fed either an excessive protein or energy diet

Diet[1] / Treatment	CD		HP		HE		Pooled SEM	Analysis of variance[2]		
	Control	Clen-buterol	Control	Clen-buterol	Control	Clen-buterol		Diet	Clen-buterol	Interac-tion
Protein/protein consumed (mg/g)										
Breast muscle	42.4	41.1	32.7	27.6	39.6	41.8	1.3	**	NS	NS
Leg muscle	17.6	20.7	15.0	16.0	18.1	20.4	0.5	**	**	NS
DNA/protein consumed (μg/g)										
Breast muscle	71.6	75.1	70.7	65.8	86.1	96.0	2.8	NS	**	NS
Leg muscle	120.0	132.5	44.8	48.0	135.6	130.5	7.8	**	NS	NS
RNA/protein consumed (μg/g)										
Breast muscle	364	279	312	245	507	445	21	*	**	NS
Leg muscle	153	166	82	71	256	266	15	**	NS	NS

[1] CD, control diet; HP, high protein diet; HE, high energy diet.
[2] ** $p < 0.01$; * $p < 0.05$; NS, no significance.

Table 6. Effects of clenbuterol on ratios of muscle composition to dietary ME consumed in broiler chickens fed either an excessive protein or energy diet

Diet[1] Treatment	CD		HP		HE		Pooled SEM	Analysis of variance[2]		
	Control	Clen-buterol	Control	Clen-buterol	Control	Clen-buterol		Diet	Clen-buterol	Interac-tion
Protein/ME consumed (mg/kcal)										
Breast muscle	2.89	2.80	3.10	2.62	2.41	2.55	0.08	NS	NS	NS
Leg muscle	1.20	1.41	1.43	1.52	1.10	1.24	0.04	**	**	NS
DNA/ME consumed (μg/kcal)										
Breast muscle	4.88	5.12	6.71	6.25	5.24	5.84	0.19	NS	**	NS
Leg muscle	8.18	9.03	4.25	4.56	8.25	7.94	0.39	**	NS	NS
RNA/ME consumed (μg/kcal)										
Breast muscle	24.8	19.0	29.6	23.3	30.9	27.1	1.2	*	*	NS
Leg muscle	10.4	11.3	7.7	6.7	15.6	16.2	0.75	**	NS	NS

[1] CD, control diet; HP, high protein diet; HE, high energy diet.
[2] ** $p < 0.01$; * $p < 0.05$; NS, no significance.

energy for protein accretion, although no significant interaction between dietary states and clenbuterol was observed. This ability of clenbuterol was inapplicable to breast muscle. The different responses of muscles would also be attributable to an aforementioned involvement of muscle characteristics. Moreover, the results of muscle protein accretion rate to the constant consumption of nutrients indicate that availability of the dietary nutrients was affected especially when the dietary ratio of protein-to-energy changed.

No significant interaction between dietary states and clenbuterol were also detected in the ratio of DNA to protein or ME consumption. With regard to RNA/protein consumption and RNA/ME consumption, these ratios in breast muscle decreased in the clenbuterol-treated chickens as compared with controls, although this influence possessed no relationship with dietary condition. This findng indicates that clenbuterol probably reduced protein turnover rate, especially protein synthetic capacity, even if the high protein or energy level resulted in stimulating the protein turnover of muscles.

The present results showed depression in, or no improvement of, the clenbuterol-induced growth performance or muscle protein deposition, associated with dietary nutrient levels. This would have relation to protein-to-energy ratio in diet (Reeds and Mersmann, 1991). More investigation of the suitable protein-to-energy ratio is needed. Therefore, the present study concluded that the excessive supply of protein and energy is responsible for the lack of the growth-promoting action of β-agonist in broilers.

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