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Effects of Dietary Beta-Adrenergic Agonist, Terbutaline, on Carcass Characteristics and Blood Attributes in Japanese Quails (*Coturnix coturnix japonica*)

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Abstract

The effect of dietary Terbutaline, a beta adrenergic agonist, on carcass characteristics and blood attributes in 288 Japanese quails was studied from 21 through 49 days of age. Dietary treatments included four levels of Terbutaline [0 (T_0), 1 (T_1), 3 (T_3), and 5 (T_5) mg/Kg of the diet]. Quails were bled at the end of the trial for biochemical assays and the carcass characteristics were then measured. The relative weights of breast and drumstick muscles were increased in birds treated with 3 and 5 mg/Kg diet of Terbutaline ($P<0.05$). Regardless of Terbutaline dietary levels included, the weight of subcutaneous and abdominal fat pad was significantly decreased in treated birds ($P<0.05$). The relative weight of heart was increased in T_3 treatment group and that of liver was increased in all birds receiving Terbutaline as compared with the control group ($P<0.05$). Irrespective of the dosing level, the percentage of protein in breast muscle was higher in Terbutaline-treated birds ($P<0.05$). However, the fat percentage in drumstick muscle was reduced in birds treated with 3 and 5 mg/Kg diet of Terbutaline, but its protein percentage was increased in T_1, T_3, and T_5 ($P<0.05$). A significant increase in plasma levels of free fatty acids was found in all birds that received Terbutaline ($P<0.01$) as compared with the control. Overally, the data suggested that dietary Terbutaline had a profound positive effect on carcass composition, but a minimal one on the plasma metabolites (glucose, cholesterol, and triglyceride) of Japanese quails.

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Introduction

A major concern in meat animal production is to efficiently produce an acceptable meat as a source of protein for human (Abolghasemi *et al*., 2007). Beta-adrenergic agonists have considerable effects on carcass characteristics (Mersmann, 1989a,b). The effects of beta agonists in improved feed utilization, increased leanness, increased dressing percentage, increased weight gain, increased protein deposition, and decreased carcass fat have been documented (Beermann, 1987). These effects have been demonstrated in several species, including chicken (Abolghasemi *et al*., 2007), lamb (Beermann *et al*., 1987), pork (Weber *et al*., 2006), and guinea pig (Zamiri and Ehsani, 1995).

Dalrymple *et al*. (1984) reported that feeding Clenbuterol, a beta agonist, to broilers improved the growth rate, feed efficiency, carcass yield, and protein accretion; whereas, the fat deposition was decreased. If beta adrenergic agonists are to be useful in elucidating the mechanisms of fat and protein metabolism, then the broad spectrum of effects of beta agonists necessitates further studies. Terbutaline is a β2-adrenergic receptor agonist which is used as a fast-acting bronchodilator.

To our knowledge, the effects of the oral administration of Terbutaline on carcass traits and blood attributes of quails have not been addressed previously. Therefore, this trial aims to study the effects of Terbutaline on carcass characteristics and blood attributes at the second half of meat quail production period.

Materials and Methods

Animals and treatments

A total of 288 male Japanese quails (*Coturnix coturnix japonica*) (weighing 97.77 ± 2.09 g) were separated from their female counterparts using the feather sexing, randomly selected at 16 d of age and fed on a conventional diet through 21 d of age. The quails were then randomly assigned to 8 treatment groups with 3 replicates of 12 birds each. Four levels of dietary Terbutaline (Iran Hormone Co., Tehran, Iran) and two times of slaughter were considered. The birds received either of 0 (T_0), 1 (T_1), 3 (T_3), or 5 (T_5) mg Terbutaline per Kg of diet from 21 through 42 d of age. Half of the birds in each dietary level of Terbutaline were immediately slaughtered in week 6 (42 d; W_6). The remaining birds were kept one more week to pass a withdrawal period prior to slaughter in wk 7 (49 d; W_7) of age. The treatments were then as follows: T_0W_6, T_0W_7, T_1W_6, T_1W_7, T_3W_6, T_3W_7, T_5W_6, and T_5W_7. The basal diet was formulated to meet NRC (1994) requirements and was provided *ad libitum*. The birds had a free access to fresh water under a 16L:8D photoschedule. Weight gain and feed consumption were recorded on a weekly basis to calculate feed conversion ratio. After a one-hour feed deprivation, the birds were humanely killed by decapitation. Different dissections of carcass, liver, heart, abdominal fat pad, and subcutaneous fat were weighed.

Table 1. Ingredients and chemical composition of basal diet

Ingredients	Percentage
Corn	48.40
Soybean meal	39.80
Corn gluten meal	8.70
Oil	0.10
Dicalcium phosphate	0.90
Oyster shell	1.16
Vitamin premix[1]	0.24
Mineral premix[2]	0.24
Salt	0.32
DL-Methionine	0.07
L-Lysine	0.07
Chemical Composition	
ME (Kcal/Kg)	2900
Crude Protein (%)	26
Calcium (%)	0.80
Available Phosporous (%)	0.30

[1]Vitamin premix provided per Kg of diet: Vitamin A, 1650 IU; Vitamin D3, 750 IU; Vitamin E, 12 IU; Vitamin K3, 2 mg; Vitamin B12, 0.003 mg; Thiamin, 2 mg; Riboflavin, 4 mg; Biotin, 0.3 mg; Niacin, 40 mg; Pyridoxin, 3 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Choline chloride, 200 mg.
[2]Mineral premix provided per Kg of diet: Manganese, 60 mg; Copper, 5 mg; Iodine, 0.3 mg; Cobalt, 0.20 mg; Selenium, 0.2 mg; Zinc, 25 mg; Iron, 120 mg.

At 49 d, the birds (n = 12 birds per treatment) were bled from the jugular vein and blood samples were collected in EDTA-coated tubes. Plasma samples were prepared following the centrifugation at 3000 RPM for 5 mins and stored at -20°C for the future assay of glucose, cholesterol, and triglyceride, using commercially available kits (Zist Chem. Co., Tehran, Iran). The plasma samples were also tested to measure the level of total free fatty acids (FFA) by the procedure described by Soloni and Sardina (1973). Furthermore, specimens from drumstick and breast muscle were frozen until analyzed for moisture, crude protein, and ether extract (AOAC, 2000). A sample of liver was also frozen for the determination of total lipid content according to Folch *et al*. (1957).

Statistical analysis

Statistical treatment of data involved an analysis of variance using a model with Terbutaline and time as two factors in a factorial arrangement. Data were analyzed using the GLM procedure of SAS (2002). When a significant difference was found, means were compared using the Duncan's multiple range tests at $P \leq 0.05$. The statistical model was as follows: $Y_{ijk} = \mu + T_i + P_j + TP_{ij} + b(BW_{ijk} - BW) + E_{ijk}$;

Where, Y_{ijk} = K^{th} observation in J^{th} period and I^{th} treatment, μ = overall mean, T_i = effect of I^{th} treatment, P_j = effect of J^{th} feeding period, BW = average body weight, b = regression coefficient of examined traits on body weight at 16 d of age, BW_{ijk} = the body weight as a covariate, E_{ijk} = residual effect.

Results

Feeding period (time), expect for the FFA, had no effect on the traits studied. The interaction of time and level of Terbutaline was not significant on the traits (data not tabulated). Further, Terbutaline had no effect on feed intake, body weight, and feed conversion ratio (Table 2).

Table 2. Effects of different dosing levels of Terbutaline on body weight gain, feed intake and feed conversion ratio in Japanese quails (Mean ± SEM)[1]

Trait	T_0	T_1	T_3	T_5	*P-value*
Body weight gain (g)	237.75±3.12	240.12±4.41	241.66 ± .68	247.95±2.55	0.7
Feed intake (g)	702.86±6.30	705.06±3.00	704.50±2.98	703.50±4.69	0.9
Feed conversion ratio	5.11±0.01	5.09±0.01	5.10±0.01	5.07±0.01	0.7

[1]The birds received either of 0 (T_0), 1 (T_1), 3 (T_3), or 5 (T_5) mg Terbutaline/Kg of diet from 21 through 42 d of age.
No significant difference was observed between treatments in each trait (*P>0.05*).

Table 3 shows the effects of different dosing levels of Terbutaline on carcass characteristics. Birds in T_3 and T_5 group recorded a higher drumstick weight as compared with T_0 and T_1 counterparts. The breast weight in T_3 and T_5 was higher in comparison with the control group. The highest (0.86±0.02) and lowest (0.72±0.03) records of heart relative weight were recorded for T_3 and T_0 treatment groups, respectively. The weight of the liver was increased in the all Terbutaline-dosed birds. The same birds showed a decrease in the weight of subcutaneous fat and abdominal fat pad as compared with the control group.

Table 3. Effects of different dosing levels of Terbutaline on carcass characteristics in Japanese quails as percent of live body weight (Mean ± SEM)[1]

Trait	T_0	T_1	T_3	T_5	*P-value*
Drumstick	14.33±0.01 ^{b}	14.33±0.02 ^{b}	15.50±0.02 ^{a}	15.50±0.01^{a}	0.0003
Breast	22.90±0.05 ^{b}	23.50±0.03 ^{ab}	24.70±0.03^{a}	24.20±0.04^{a}	0.01
Heart	0.72±0.03^{c}	0.78±0.02^{bc}	0.86±0.02^{a}	0.82±0.02^{ab}	0.002
Liver	1.40±0.06^{b}	1.80±0.04^{a}	1.90±0.06^{a}	1.70±0.05^{a}	0.004
Subcutaneous and abdominal fat	3.00±0.19^{a}	2.20± 0.19^{b}	2.10±0.16^{b}	2.00±0.13^{b}	0.003

[1]The birds received either of 0 (T_0), 1 (T_1), 3 (T_3), or 5 (T_5) mg Terbutaline/Kg of diet from 21 through 42 d of age.
[a-c]Means within a row having different superscripts are significantly different (*P<0.05*).

The chemical composition of meat in Terbutaline-treated birds is summarized in Table 4. The percentage of breast fat and breast moisture showed no differences among the treatment groups. Drumstick fat percentage was decreased in T_3 and T_5, although that of T_1 group did not differ from the control birds. Dietary Terbutaline, regardless of the dosing level, was associated with a higher content of breast protein. However, the medium (T_3) and high (T_5) dosing levels of Terbutaline resulted in an increase in the drumstick protein and moisture contents.

Table 4. Effects of different levels of Terbutaline on meat chemical composition of Japanese quails in dry matter (Mean ± SEM)[1]

Trait	T_0	T_1	T_3	T_5	*P-value*
Breast fat (%)	0.11±0.012	0.11±0.027	0.10±0.012	0.10±0.009	0.666
Drumstick fat (%)	0.23±0.028^a	0.19±0.010^a	0.13±0.006^b	0.14 ±0.009^b	0.0002
Breast protein (%)	80.46±1.45^b	83.74±0.99^a	84.17±0.91^a	85.67±0.72^a	0.001
Drumstick protein(%)	74.89±2.39^c	78.63±1.51^b	84.63±0.61^a	83.58±1.03^a	<0.0001
Breast moisture (%)	71.24±0.30	71.49±0.29	71.68±0.22	71.39±0.17	0.248
Drumstick moisture(%)	72.47±0.42^b	72.24±0.44^b	74.94±0.14^a	74.52±0.24^a	<0.0001

[1]The birds received either of 0 (T_0), 1 (T_1), 3 (T_3), or 5 (T_5) mg Terbutaline/Kg of diet from 21 through 42 d of age.

[a-c]Means within a row having different superscripts are significantly different ($P<0.05$).

Plasma levels of triglyceride, glucose, and cholesterol as well as liver contents of triglyceride and total lipid were not influenced by the dietary treatment (Table 5). Dietary Terbutaline resulted in an increase in the plasma level of free fatty acids in the birds receiving Terbutaline.

Table 5. Effects of different dosing levels of Terbutaline on plasma metabolites, liver triglyceride, and total liver lipid in Japanese quails (Mean ± SEM)[1]

Trait	T_0	T_1	T_3	T_5	*P-value*
Triglyceride (mg/dL)	78.51±3.80	74.98±3.70	71.82±1.85	73.68±3.16	0.497
Glucose (mg/dL)	325.60±6.01	331.39±5.41	336.56±5.55	329.53±5.79	0.546
Cholesterol (mg/dL)	60.60 ±4.68	156.23±4.68	149.72±3.94	155.46±3.86	0.341
Free fatty acids (mg/dL)	8.14±0.36^b	9.89±0.33^a	9.90 ±0.35^a	9.57 ±0.24^a	<0.0001
Liver triglyceride (mg/g)	8.80±0.68	8.82±0.54	10.47±0.56	9.95 ±0.49	0.332
Total liver lipid (mg/g)	30.39±2.21	29.36±1.30	38.12±2.13	36.29±1.54	0.325

[1]The birds received either of 0 (T_0), 1 (T_1), 3 (T_3), or 5 (T_5) mg Terbutaline/Kg of diet from 21 through 42 d of age.

[a-b]Means within a row having different superscripts are significantly different ($P<0.05$).

Discussion

In the present experiment, feed intake, body weight gain, and feed conversion ratio did not differ among the treatment groups. However, Shahneh *et al*. (2011) reported an improvement in the body weight gain and feed conversion ratio in quails treated with Salbutamol. Merkley and Garwood (1989) showed no difference in feed conversion ratio in quail lines fed Cimatrol. The effects of beta agonists on the rate of gain and feed consumption are less pronounced; however, a modest increase in the rate of gain and/or a decrease in feed consumption to yield have been suggested in a number of studies (Buyse *et al*., 1991; Hamano *et al*., 1998; Ansari *et al*., 2002). If the efficiency is to be evaluated on the basis of the product obtained (i.e. lean meat or carcass muscle mass), then the efficiency of production would be markedly improved even if no changes are observed in weight gain or feed consumption (Mersmann., 1989b). Morgan *et al*. (1989) reported that chickens fed Cimatrol had heavier leg muscles compared with the control group, but no differences were observed in their breast muscle. Using Salbutamol has been suggested to be associated with an increase in the drumstick weight in broilers (Ansari *et al*., 2002). Gwartney *et al*. (1991) showed heavier leg muscles in 42-d-old Cimatrol-fed birds without a withdrawal period than either that from age-matched Cimatrol-fed birds with a 7-day withdrawal period or from control group. Abolghasemi *et al*. (2007) as well as Hamano *et al*. (1998) also observed an increase in the weights leg and pectoral muscles in broilers. Beermann *et al*. (1987) indicated that beta agonists increase the muscle size via hypertrophy, possibly though decreasing the rate of muscle protein degradation. Young *et al*. (1990) reported that using Cimatrol in the culture of leg muscles from 12-day old chick embryos, resulted in an increase in the quantity of the myofibrillar fraction and of myosin heavy chain and in a slight increase in quantity of total cellular protein. Increased muscle cell protein has been attributed to a combination of enhanced synthesis rate via increased mRNA and decreased degradation rate (Gwartney *et al*., 1992). It has also been shown that the beta adrenergic agonists reduced cathepsins activity or increased concentration of protease inhibitors (Gwartney *et al*., 1991). Reduction in fat weight in the current treated quails is in general agreement with the previous reports (Dalrymple *et al*., 1984; Zamiri and Ehsani., 1995). Merkley and Garwood (1989) observed no effect of Cimatrol on fat and protein contents. An increase in blood flow to the skeletal muscle enhances the process of hypertrophy by delivery of increased amounts of substrates and energy sources for protein synthesis (Mersmann, 1989b). Likewise, increased blood flow to adipose tissue might be envisioned to carry non-etherified fatty acids away from the tissue to enhance the lipid degradation process (Beermann *et al*., 1987; Eisemann *et al*., 1988). Buyse *et al*. (1991) reported that the relative liver weight was not affected by Clenbuterol in broilers. Muramatsu *et al*. (1991) as well as Merkley and Garwood (1989), however, observed a decrease in liver weight. Protein and lipid content, as well as enzyme activity may influence the liver weight (O'hea and leveille, 1969). In the current study, the total liver triglyceride and lipid were not affected by the dietary treatments, but they tended to be higher at 3 and 5 ppm dosing levels as compared with the 0 and 1 ppm ones. The effects of beta agonists on heart weight have been inconsistent. Salbutamol increased the relative heart weight in guinea pigs (Zamiri and Ehsani, 1995), but it had no effect on this trait in Japanese quails (Shahneh *et al*., 2011). In our study, the addition of Terbutaline had no effect on heart weight.

Beta agonists increased moisture content of the carcass in some studies (Mersmann, 1989b). Carcass moisture reported to be significantly lower in quails feed Cimatrol (Merkley and Garwood, 1989). Weber *et al*. (2006) reported that Ractopamine decreased the total lipid content of the loin muscle, with no effect on its moisture. In the present work, the moisture of drumstick muscle in T_3 and T_5 groups were significantly higher as compared with T_0 and T_1 treatment groups. The different responses might be due to the difference in the level of beta agonists included in the diet, or the duration of time it was fed prior to slaughter. Plasma levels of cholesterol, triglyceride, and glucose were not affected in this trial. Buyse *et al*. (1991) reported that the chronic administration of Clenbuterol did not affect plasma glycerol, glucose or triglyceride, although plasma level of VLDL was decreased in Clenbuterol-fed chickens. However, Abolghasemi *et al*. (2007) reported an increase in the plasma level of cholesterol, triglyceride, and glucose in chicks receiving Terbutaline. These researchers also reported that the level of cholesterol was increased, but that of triglyceride was not affected in chickens fed with Salbutamol (Ansari *et al*., 2002). The pharmacodynamic properties of a particular beta agonist administrated to a particular species are expected to be influenced by genetic, sex, and age-borne variations in drug metabolism and delivery systems. Although in poultry, it seems that liver is the target organ for beta agonists through decreasing the fat content (O'hea and leveille, 1969), the elevation of plasma FFA concentration following the administration of a beta agonist is an indication of activated adipocyte lipolytic system which may be originated from a hormone-sensitive lipase activity (Eisemann *et al*., 1988). On the other hand, prolonged feeding of beta agonists had no effect on lipogenic activity (Coleman *et al*., 1988). Acute and chronic increases in plasma FFA were found in steers Eisemann *et al*. (1988) and lambs Beermann *et al*. (1987) fed with Cimatrol. Many factors such as animal, beta agonists, feeding pattern, temperature, humidity, season, breed/line source, cage design, and animal density in cage or pens may influence the final outputs.

Overally, the data demonstrated that Terbutaline could affect the carcass composition and some blood metabolites of quails in a similar manner to the activity of a number of beta agonists in several mammalian and avian species. Studies on the carcass residues of such compounds are needed prior to making any practical recommendations.

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