EFFECTS OF CIMATEROL ON THE GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND TISSUE METABOLISM IN BROILER CHICKS FED DIFFERENT DIETARY ENERGY

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Summary

This study was a sequential experiment consisting of feeding trial and *in vitro* culture studies. Feeding trial was conducted by 2 × 2 × 2 factorial design with two cimaterol levels (0, 0.25 mg/kg), two energy levels (3,200, 2,900 ME kcal/kg) and two sexes. In starting period (0-21 days) broilers were fed diets containing two energy level without dietary supplementation of cimaterol. During finishing period (21-42 days) cimaterol groups were fed cimaterol supplemented diets. *In vitro* cultures were carried out to study the cellular metabolism of protein and fat in liver and adipose tissues prepared from chicks used in feeding trials.

Body weight gain was significantly improved by the administration of cimaterol to experimental diets by 2.4% ($p < 0.05$). Feed intake was reduced by cimaterol administration at the high energy level, but this trend was reversed at low energy level. Feed efficiency was improved by cimaterol administration and at high energy level the difference (5.7%) was significant ($p < 0.05$). The administration of cimaterol had no effects on percentage of abdominal fat content, giblet and neck. There was little difference in carcass yield between control and cimaterol treated group. The administration of cimaterol had no effect on nutrient metabolizability or carcass composition. The results of *in vitro* studies with liver tissues showed that cimaterol increased the lipolytic activities ($p < 0.05$) and decreased lipogenic activities ($p < 0.05$). In *in vitro* studies with acinar cell of liver tissues, cimaterol increased the amount of retained protein and decreased secreted protein at high energy level, but the trend was opposite at low energy level.

(**Key Words**: Cimaterol, Broiler, Lipolysis, Lipogenesis, Retained Protein, Secreted Protein, Acinar Cell Culture)

Introduction

Major objectives of animal and avian growth research are to improve the rate and efficiency of gain, and meat quality. Since feed accounts for major cost (approximately 70% of the cost of broiler production) in animal production, even small improvement in feed efficiency is economically important. Carcass fat in broilers has continually increased since producers began using high energy broiler rations (Donaldson, 1985). Not only is carcass fat viewed by healthy conscious consumers as undesirable, but it has been demonstrated that the lack of improvement in feed efficiency is associated with increased carcass fat in broilers (Chery et al., 1978). It is apparent that a product which improves growth, increases efficiency of feed utilization, increases carcass protein and decreases carcass fat would be beneficial to the broiler industry.

At this background, beta-adrenergic agonists (β-agonists) are particularly attractive as growth promoters. Recently, it has been shown that the dietary administration of beta-adrenergic agonist such as cimaterol or clenbuterol improve animal growth performance and carcass composition in various animals (Baker et al., 1984; Dalrymple et al., 1984a,b; Emery et al., 1984; Beerman et al., 1985b; Jones et al., 1985). Several reports (Baker et al., 1984; Dalrymple et al., 1984a,b) have identified that the β-adrenergic agonists (β-agonists) such as clenbuterol [benzyl alcohol, 4 - amino - R - (t - butyl - amino) methyl 3,5 - dichloro] or cimaterol (CL263, 780), when added to the diets of steers, lambs, poultry and pigs, increase the muscle mass and decrease fat accretion which have been attributed to a shift in nutrient partitioning. In addition, many β-

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agonists are potent and orally active so that parenteral and "in feed" formulation are possible.

The objectives of this study were as follows; 1) to determine the effects of the level of dietary cimaterol and energy on growth performance, carcass quality and nutrient utilization in broilers, 2) to investigate lipogenic and lipolytic activity in liver and adipose tissue, and 3) to compare protein synthetic activity in liver acinar cell culture.

Materials and Methods

To investigate the effects of cimaterol on the growth performance and carcass quality of broilers at two energy level and sex, 1,200 Hanil Poultry Farm's Arbor Acres broiler chicks, male 600 (average weight 43.25 g), female 600 (average weight 43.80 g) were used as shown in table 1. All treatments in this experiment had 5 replicates with 60 birds in each replicate.

Birds were fed two basal diets (no cimaterol) for 0-3 weeks; which were formulated to contain the same crude protein level (23%) and two metabolizable energy levels (3,200, 2,900 kcal/kg) as shown in table 2. During 4-6 weeks the birds were fed 4 different diets (table 2); two of which were added with cimaterol (0.25 mg/kg) at each dietary energy level.

The birds were grown in floor-pens, each pen holding 30 males and 30 females. Birds were housed in a room with 24 hours illumination and forced fan air ventilation. One day old chicks were allocated to the experimental groups to have uniform mean body weight. Diets and water were fed *ad libitum* throughout the experimental periods. Body weight and feed intake were recorded at 21 and 42 days.

TABLE 1. EXPERIMENTAL DESIGN

Cimaterol (mg/kg)	0		0.25	
Metabolizable energy (mg/kg)	3,200	2,900	3,200	2,900
Replication	5	5	5	5
Bird/replication[1]	60	60	60	60
Total	300	300	300	300

[1] Each pen contained 30 males and 30 females.

To determine the nutrient metabolizability of the experimental diets, a trial was carried out by a total collection method for 8 days (5 preliminary days, 3 collection days) upon the termination of feeding trial. At 42 day three male and female chicks per treatment were selected for metabolizability trial. All chicks were caged in metabolic cages individually and the experimental diets and water were fed *ad libitum*. Total excreta collected from same birds were dried in an air-forced drying oven at 60℃ for 72 hours and analyzed for proximate composition.

To evaluate the carcass composition four male and female chicks per treatment were randomly selected at 42 day. These chicks were fed cimaterol-free finisher diet for 3 days. These selected birds were slaughtered. Slaughter procedure consisted of stunning, decapitation, bleeding, scalding and defeathering. Weight of whole carcass, fat pad, neck and giblet were determined. The carcass sample that was removed of bone, giblet, neck, abdominal fat was individually collected to determine carcass composition. These carcass sample was freeze-dried and ground with 1 mm mesh Willy mill and analyzed for composition by AOAC (1990) methods.

To investigate protein metabolism in liver acinar cell, and lipolytic and lipogenic activity in liver tissue, liver tissues were collected from each male and female bird per treatment. To study protein metabolism, liver tissues were trimmed free of large pieces of connective and adipose tissue. Isolation and culturing of liver acinar cells were performed (Park et al., 1979). The cell were plated on plastic tissue culture dishes (approximately 10^6 cell/dish). The basic culture medium was Eagle's 1 × MEM (Eagle, 1959) with 0.2% (wt/vol) glucose, 5% (vol/vol) fetal bovine serum

TABLE 2. FORMULAR AND CHEMICAL COMPOSITION OF EXPERIMENTAL DIETS

ME (kcal/kg)	Starter (0-3 wks)		Grower (4-6 wks)				Finisher (7 wk)
	3,200	2,900	3,200	2,900	3,200	2,900	3,200
Ingredients (%)							
Corn	61.4	57.9	67.2	61.9	67.2	61.9	70.1
Soybean meal (40%)	15.8	28.9	16.5	28.0	16.5	28.0	17.7
Corn gluten	5.7	3.1	3.9	0.4	3.9	0.4	3.0
Fish meal (60%)	11.9	5.0	7.3	4.1	7.3	4.1	3.9
Wheat bran	0	1.8	0	2.5	0	2.5	0
Tallow	2.4	0	2.2	0	2.2	0	2.25
Limestone	0.7	1.2	0.7	0.7	0.7	0.7	1.1
Dicalcium phosphate	0.7	0.7	0.7	1.1	0.7	1.1	0.7
Lysine	0.1	0.1	0.3	0.1	0.3	0.1	0.1
Methionine	0.3	0.3	0.2	0.2	0.2	0.2	0.15
Salt	0.2	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral[2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Anticoccidant[3]	0.1	0.1	0.1	0.1	0.1	0.1	
Cimaterol (mg/kg)	0	0	0	0	0.25	0.25	0
Total	100	100	100	100	100	100	100
Chemical composition :							
Metabolic energy (kcal/kg)[4]	3,200	2,900	3,200	2,901	3,200	2,901	3,201
Crude protein (%)	21.73	21.18	20.31	18.96	20.31	18.96	18.0
Ca (%)	1.03	1.03	0.93	0.92	0.93	0.92	0.90
P (%)	0.64	0.66	0.66	0.71	0.66	0.71	0.57

[1] Vitamin mixture contains the followings in 1 kg: Vitamin A, 10,000 IU; Vitamin D, 1,500 IU; Vitamin E, 15 mg; Vitamin K, 5 mg; Vitamin B_2, 8 mg; Vitamin B_{12}, 0.008 mg; Ca-d-pantothenate, 8 mg; Niacin, 25 mg; Folic acid, 0.4 mg; Biotin, 0.2 mg; Choline, 500 mg; pyridoxine, 1 mg; B. H. T. 125 mg.

[2] Mineral mixture contains the followings in 1 kg: Co, 0.85 mg; I, 1.29 mg; Zn, 100 mg; Mg, 110 mg; Cu, 8.75 mg; Se, 0.15 mg; Fe, 35 mg.

[3] Monensin was used.

[4] Calculated value.

and antibiotics. Dishes were incubated for 18 hours at 37℃ under a gaseous atmosphere of 5% CO_2 in O_2. Four dishes were set up for each observation with contents pooled after incubation for subsequent analysis. The [^{3}H]-lysine (0.5 μ Ci/ml) was added to culture medium to determine the amount of *in vitro* secreted protein and retained protein. At the termination of the 18 hours incubation, cells were collected, pooled, and centrifuged at 1,000×g at 4℃ for 10 minutes. Supernatant and pellet were separated and used to determine the amount of secreted protein and retained protein, respectively.

To determine lipogenic activity, liver tissues of each bird were divided into five 10-20 mg tissue slices. These liver tissue slices (10-20 mg) were incubated for 120 minutes at 37℃ in 3 ml of medium under a gaseous atmosphere of 5% CO_2 in O_2. The incubation medium (Krebs-Ringer bicarbonate buffer) also contained 25 mM HEPES, 0.5 mM glucose, 3% bovine serum albumin and 0.5 μCi [^{14}C]-glucose. Incubation was terminated by placing vials on ice. After taking out tissue slices from medium, total lipids in the tissue were extracted by the method of Dole and Meinertz (1960). After the extracts were dried, radioactivity incorporated into total lipid slices was determined by a liquid scintillation counter

(LC 100C). Lipolytic activity was measured in Krebs-Ringer bicarbonate buffer (KRB) with one-half of the indicated Ca^{2+}, 4% fatty-acid-poor Fraction V bovine serum albumin and 5.56 mM glucose. Tissue slices (approximately 100 mg) were incubated for 120 minutes at 37℃ in 3 ml of medium under a gaseous atmosphere of 5% CO_2. Incubation was terminated by placing vials on ice. The medium was filtered through cheese cloth to remove the tissue and stored at −20℃ until analysis. Non-esterified fatty acids in the medium were extracted and titrated according to method of Kelly (1965).

All proximate analyses were conducted by the AOAC (1990) method. Analysis of variance was carried out and means were compared by Duncan's multiple range test using Proc Anova Procedure of SAS (1986) package program with IBM-PC compatible computer.

Results and Discussion

Performance of broilers

The results in bird performance as affected by dietary treatments are presented in table 3. Both weight gain during the 0-42 days and final

TABLE 3. EFFECTS OF CIMATEROL ON BODY WEIGHT GAIN, FEED INTAKE AND EFFICIENCY IN BROILER FED TWO ENERGY LEVELS (0-42 DAYS)

Variable	Treatment	3,200	3,200-C[1]	2,900	2,900-C[1]
Init. weight (g)	Male	43.25	43.60	43.30	43.94
	Female	44.91	44.10	43.27	42.90
	Mean	44.08	43.85	43.29	43.42
42 day	Male	2,180.00	2,179.29	2,088.67	2,176.57
weight (g)	Female	1,860.00	1,894.05	1,773.33	1,838.00
	Mean	2,019.21	2,036.67	1,931.00	2,006.73
Body weight	Male	2,136.75	2,135.69	2,045.37	2,132.63
gain (g)	Female	1,815.09	1,849.95	1,730.06	1,795.10
	Mean	1,975.13	1,992.82	1,887.71	1,963.31
Feed intake (g)		4,139.00	3,984.42	3,776.25	3,971.94
Feed efficiency		2.10	1.98	2.00	2.02

Probability;

	42-day weight	Body weight gain	Feed intake	Feed efficiency
Energy	0.0048	0.0107	0.0195	0.2290
Sex	0.0001	0.0001	–	–
Cimaterol	0.0487	0.0301	0.5524	0.1090
Energy × sex	0.2853	0.3988	–	–
Energy × cimaterol	0.4529	0.2308	0.0315	0.1048
Sex × cimaterol	0.7151	0.4732	–	–
Energy × sex × cimaterol	0.3364	0.6817	–	–

[1] C: cimaterol added.

body weight were significantly affected by dietary energy level, cimaterol and sex and there was no evidence for any interaction between these main effects. The inclusion of cimaterol resulted in an increase in weight gain and 42 day body weight of 2.4% ($p < 0.05$). Several studies have shown that the β adrenergic agonist such as cimaterol and clenbuterol improved growth performance and carcass composition of broilers and of other various animals (Dalrymple et al., 1984a,b; Ricks et al., 1984; Jones et al., 1985; Beerman et al., 1986; Bohorov et al., 1987; Williams et al., 1988). These reports are consistant with present results. The response in terms of final body weight to increasing the dietary energy concentration was 3.0% ($p < 0.05$). There was a significant energy × cimaterol interaction in feed intake. Cimaterol enhanced feed intake at the low energy level and reduced it at the high energy level. This was also reflected in the results in relation to feed efficiency by 5.7% reduction ($p < 0.05$) at the high energy level but had no effect at the low energy level.

Carcass characteristics

The effects of cimaterol on carcass characteristics are summarized in table 4. There were no significant differences among the experimental

TABLE 4. EFFECTS OF DIETARY CIMATEROL ON THE GIBLET, NECK, ABDOMINAL FAT AND CARCASS YIELD OF THE BROILERS (% OF LIVE WEIGHT)

Variable	Treatment	3,200	3,200-C[1]	2,900	2,900-C[1]
	Male	5.14	4.79	4.86	5.31
Giblet	Female	5.22	5.05	5.09	4.61
	Mean	5.18	4.92	4.98	4.96
	Male	3.97	4.53	3.72	3.56
Neck	Female	3.37	3.79	3.71	4.00
	Mean	3.67	4.16	3.72	3.78
Abdominal	Male	1.52	2.16	1.79	1.42
fat	Female	1.72	2.61	1.34	2.01
	Mean	1.62	2.39	1.57	1.72
Carcass	Male	66.16	66.84	66.00	67.16
yield	Female	65.95	66.85	68.15	67.79
	Mean	66.06	66.85	67.08	67.48

Probability	Giblet	Neck	Abdominal fat	Carcass yield
Energy	0.6242	0.3959	0.0574	0.1355
Sex	0.7999	0.2155	0.1921	0.2184
Cimaterol	0.5415	0.1104	0.0202	0.3124
Energy × sex	0.3421	0.0087	0.7408	0.1892
Energy × cimaterol	0.5485	0.1649	0.2322	0.7285
Sex × cimaterol	0.3297	0.5991	0.2167	0.5332
Energy × sex × cimaterol	0.1736	0.3369	0.5228	0.4331

[1] C: cimaterol added.

groups in weight of giblet, neck, abdominal fat or carcass yield by the administration of cimaterol. Giblet was the lowest in the 3,200-cimaterol group and the highest in the 3,200-no cimaterol group. Abdominal fat was significantly increased with cimaterol administration. These results are contrary to other reports (Dalrymple et al., 1984a,b, 1985) which indicated that abdominal fat was reduced with cimaterol administration. There was little difference in carcass yield between control and cimaterol treated group.

Nutrient metabolizability

The results of nutrient metabolizability affected by dietary treatment are presented in table 5. The results of the analysis of variance provided no evidence for any significant effect of cimaterol on the metabolizability of dry matter, crude protein, ether extract or total carbohydrate. All of these parameters, however, were significantly

TABLE 5. EFFECTS OF CIMATEROL ON THE NUTRIENTS METABOLIZABILITY OF BROILERS (%)

Variable	Treatment	3,200	3,200-C[1]	2,900	2,900-C[1]
Dry matter	Male	74.62	75.15	71.15	75.06
	Female	75.95	74.48	71.04	70.12
	Mean	75.29	74.82	71.28	72.59
Crude protein	Male	47.96	59.32	39.70	28.21
	Female	53.44	58.32	45.09	45.01
	Mean	50.70	58.82	42.25	36.61
Ether extract	Male	82.39	90.58	76.17	77.70
	Female	91.79	89.96	74.31	85.47
	Mean	87.09	90.27	75.24	81.59
Ash	Male	32.90	56.98	39.52	38.28
	Female	32.59	55.31	34.98	55.58
	Mean	32.93	56.15	37.25	46.93
Total carbohydrate	Male	86.60	85.17	84.25	76.61
	Female	85.73	86.49	79.71	84.28
	Mean	86.17	85.83	81.98	80.45

Probability ;

	Dry matter	Crude protein	Ether extract	Ash	Total carbohydrate
Energy	0.0048	0.0070	0.0132	0.7191	0.05511
Sex	0.4472	0.0938	0.0832	0.2204	0.3607
Cimaterol	0.8283	0.8944	0.3340	0.0071	0.3053
Energy × sex	0.2356	0.1954	0.6569	0.2035	0.3392
Energy × cimaterol	0.3850	0.0445	0.9819	0.0410	0.3079
Sex × cimaterol	0.1222	0.7732	0.4010	0.8049	0.1605
Energy × sex × cimaterol	0.4675	0.5903	0.2612	0.6630	0.3888

[1] C: cimaterol added.

increased by raising the energy concentration of the diet. The metabolizability of the ash component of the diets was highly variable but was nevertheless significantly increased by cimaterol.

Carcass composition

Carcass compositions as influenced by dietary cimaterol are summarized in table 6. In this study neither cimaterol administration nor energy level affected carcass composition. Crude protein content was significantly higher in males than in females ($p < 0.05$) but other carcass composition was similar for the sexes. The effects of cimaterol on carcass composition were not found, which is contrary to results of others who reported that β-agonist such as clenbuterol and cimaterol reduced the fat content and increased the protein content in various animals (Dalrymple et al., 1984a,b; Baker et al., 1984; Emery et al., 1984; Jones et al., 1985; Hanrahan et al., 1986; Kim et al., 1987; Han et al., 1990). No responce by cimaterol in body composition in present studies may be the results of withdrawal period imposed to the experimental bird for the last three days of the feeding trial.

Lipolytic and lipogenic activity

The effects of cimaterol on lipolytic and lipogenic activity in the liver are presented in table 7. Lipolytic activity in liver tissue was

TABLE 6. EFFECTS OF FEEDING CIMATEROL AT TWO ENERGY LEVELS ON THE CARCASS COMPOSITION OF BROILERS (%)

Variable	Treatment	3,200	3,200-C[1]	2,900	2,900-C[1]
Moisture	Male	65.44	65.78	66.18	69.16
	Female	69.96	67.29	70.07	67.45
	Mean	67.20	66.54	68.13	68.31
Crude protein	Male	19.60	18.99	19.89	20.14
	Female	18.52	19.18	19.06	17.71
	Mean	19.06	19.09	19.48	18.93
Ether extract	Male	14.39	14.40	12.85	9.85
	Female	11.47	12.79	10.01	14.12
	Mean	12.93	13.60	11.43	11.99
Ash	Male	0.92	0.92	1.06	1.02
	Female	0.93	0.95	0.97	0.91
	Mean	0.93	0.94	1.02	0.97

Probability ;

	Moisture	Crude protein	Ether extract	Ash
Energy	0.3095	0.7047	0.2312	0.0645
Sex	0.1753	0.0242	0.6511	0.1618
Cimaterol	0.7144	0.5522	0.4729	0.4462
Energy × sex	0.4473	0.1303	0.4124	0.0441
Energy × cimaterol	0.6471	0.4279	0.9138	0.2955
Sex × cimaterol	0.1038	0.8120	0.0707	0.9571
Energy × sex × cimaterol	0.4445	0.1176	0.2133	0.7622

[1] C: cimaterol added.

TABLE 7. EFFECTS OF DIETARY CIMATEROL AT DIFFERENT ENERGY LEVELS ON LIPOLYTIC AND LIPOGENIC ACTIVITY IN LIVER INCUBATED *IN VITRO*[2]

Variable	Treatment	3,200	3,200-C[1]	2,900	2,900-C[1]
Lipolytic[3]	Male	0.081	0.068	0.041	0.078
activity	Female	0.047	0.075	0.072	0.078
	Mean	0.064	0.072	0.056	0.078
Lipogenic[4]	Male	20.86	11.94	18.40	16.18
activity	Female	34.77	9.09	20.44	8.73
	Mean	27.82	10.52	19.42	12.46

Probability :	Lipolytic activity	Lipogenic activity
Energy	0.9937	0.6152
Sex	0.8801	0.825
Cimaterol	0.0293	0.0653
Energy × sex	0.0326	0.5220
Energy × cimaterol	0.2417	0.4222
Sex × cimaterol	0.6512	0.3098
Energy × sex × cimaterol	0.0066	0.7769

[1] C: *cimaterol added.*

[2] Each value represents the mean of five determinations (n = 5).

[3] μeq nonesterified fatty acid (NEFA) released mg/cell in 120 minutes

[4] n moles glucose incorporated into total lipid mg/cell in 120 minutes.

TABLE 8. EFFECTS OF DIETARY CIMATEROL AT DIFFERENT ENERGY LEVELS ON RETAINED PROTEIN AND SECRETED PROTEIN IN ACINAR CULTURE OF BROILER LIVER[1]

Variable	Treatment	3,200	3,200-C[4]	2,900	2,900-C[4]
Retained	Male	81.04	94.58	105.40	87.60
protein[2]	Female	75.53	76.75	84.27	95.66
(dpm/mg × 10^{-2} protein)	Mean	78.29	85.67	94.84	91.63
Secreted	Male	5.40	5.30	11.34	11.02
protein[3]	Female	6.78	5.74	6.11	8.18
(dpm/mg × 10^{-2} protein)	Mean	6.09	5.52	8.73	9.6

Probability ;	Retained protein (dpm/mg × 10^{-2} protein)	Secreted protein (dpm/mg × 10^{-2} protein)
Energy	0.0196	0.2751
Sex	0.2459	0.3732
Cimaterol	0.9060	0.8553
Energy × sex	0.0744	0.8111
Energy × cimaterol	0.5843	0.6132
Sex × cimaterol	0.7842	0.6849
Energy × sex × cimaterol	0.5301	0.2911

[1] Acinar cells from liver were plated and incubated for 18 hours in the medium; Each value represents the mean of four determinations (4 culture dishes/determination).

[2] The amount of retained protein was determined by the incorporation of [^{3}H]-lysine (0.5 μCi/ml) into acini

[3] The amount of secreted protein was determined by the incorporation of [^{3}H]-lysine (0.5 μCi/ml) into TCA insoluble material.

[4] C: cimaterol added.

decreased in female birds with cimaterol administration. *In vitro* lipogenic activity in liver was decreased with cimaterol administration ($p < 0.05$). This results is consistant with that of Fain and Garcia-Sainz (1983), who reported that β-agonist increased lipolysis as a result of stimulation of β-receptors. Even though the difference was not significant, *in vitro* lipogenic activity in liver tissue was decreased with cimaterol administration ($p = 0.065$). Beta-agonists are substituted catecholamines which act on the β adrenergic receptors to stimulate cyclic AMP production (Fain and Garcia-Sainz, 1983), resulting in reduced lipogenesis and enhanced lipolysis (Thornton et al., 1984; Duquette and Muir, 1985). A direct effect, at least on lipolysis, is supported by long-term elevation in circulating plasma free fatty acids in cimaterol-treated lambs (Beermann et al., 1985a).

Protein synthesis

Effects of cimaterol on retained and secreted protein in liver acinar culture are summarized in table 8. Even though difference was not significant, at 3,200 kcal/kg energy level synthesis of retained proteins was increased by cimaterol administration. The increase was higher in males than in females. At 2,900 kcal/kg energy level the synthesis of retained protein was decreased with cimaterol administration. With regard to secreted protein, it was not significantly affected by cimaterol administration. At 3,200 kcal/kg energy level secreted protein was decreased with cimaterol administration, but at 2,900 kcal/kg energy level this trend was reversed.

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