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SALBUTAMOL AFFECTS BODY COMPOSITION OF THE GUINEA PIG

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ABSTRACT

Effects of salbutamol, a beta-adrenergic agonist, on body composition of guinea pigs were investigated. Thirty individually caged male guinea pigs were weighed and allotted to two groups. One group (n=20) was administered with salbutamol by tube, at 4 mg per kg BW per day (treatment group) once daily for 30 consecutive days, and the other group (n=10) served as control. Mean initial body weights of treatment and control groups were 403.5 and 430.3 g, respectively.

A pelleted diet was fed *ad libitum* and daily orts were weighed for determination of daily feed consumption. The experiment was continued for 30 days. The guinea pigs were reweighed, anesthetized with ether, and blood samples were collected via heart puncture before slaughter (10 guinea pigs per group).

Salbutamol treatment did not affect final body weight, mean daily gain and feed-to-gain ratio; however, the treatment group consumed less feed. Weights of carcass, skin, kidneys, lungs, testes and "longissimus muscle" were not different between the two groups. Heart weight was increased but liver weight was decreased with salbutamol treatment. Physically separated fat from several

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depots was significantly reduced in the salbutamol group. Carcass fat content was decreased but carcass protein content was increased in salbutamol fed guinea pigs. Carcass moisture content was not affected by treatment. Serum levels of lipase, glucose, cholesterol and triacylglycerols were increased by salbutamol.

Results of this experiment demonstrated that salbutamol affected carcass composition of guinea pigs in a manner similar to the activity of a number of beta-adrenergic agonists in several mammalian and avian species. The results also support the view that effects of beta-adrenergic agonists on carcass composition is a general property of these agents, and are applicable to a wider range of animal species.

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تأثیر سالبیوتامول بر ترکیب بدن خوکچه هندی

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چکیده

اثرسالبیوتامول بر ترکیب بدن خوکچه هندی بر رسی شد. تعداد ۳۰ خوکچه هندی پس از وزن شدن به طور تصادفی به دو گروه تقسیم و در قفس های انفرادی نگهداری شدند. به یک گروه (۲۰ خوکچه هندی) روزانه چهار میلی‌گرم سالبیوتامول به ازای هر کیلوگرم وزن بدن خورانده شد (گروه تیمار) وگروه دیگر(۱۰ خوکچه هندی) به عنوان شاهد در نظرگرفته شد. میانگین وزن این دو گروه به ترتیب ۴۰۳/۵ و ۴۳۰/۳ گرم بود.

هر روز در ساعت هشت بامداد مقداری غذای پلت شده، به میزانی بیش از نیاز روزانه، به قفس افزوده شد. پیش از افزودن غذا به قفس، غذای زیادی روز پیش وزن شد تا مقدار غذای مصرفی برآورد گردد. آزمایش برای ۳۰ روز ادامه یافت و در پایان خوکچه‌ها دوباره وزن و نمونه خون (ده خوکچه در هر گروه) از طریق قلب گرفته شد.

سالبیوتامول تأثیر معنی داری بر میانگین وزن نهائی (کشتار)، افزایش وزن روزانه و بازدهی مصرف غذا نداشت اما مصرف غذا را در مقایسه با گروه شاهد کاهش داد. وزن لاشه، پوست، کلیه‌ها، شش‌ها، بیضه‌ها و ماهیچه *Longissimum dorsi* بین دو گروه تفاوتی نشان نداد. سالبیوتامول چربی جدا شده از بدن و چربی لاشه را کاهش داد اما میزان پروتئین لاشه را تغییر نداد. میزان لیپاز، گلوکز، کلسترول و تری اسیل گلیسرول‌ها ی سرم خون تحت تأثیر سالبیوتامول افزایش یافت.

یافته‌های این آزمایش نشان داد که تأثیر سالبیوتامول بر ترکیب لاشه خوکچه هندی همانند اثر بتاآدرینرژیک آگونیست‌ها در دیگر گونه‌های پستانداران و طیور است. این نتایج همچنین نشان می دهند که تأثیر بتاآدرینرژیک آگونیست‌ها بر ترکیب لاشه، از ویژگی های عمومی این ترکیبات بوده و برای گروه گسترده‌تری از حیوانات قابل تعمیم است.

INTRODUCTION

Beta-adrenergic agonists cause profound effects on carcass characteristics in mice (4, 11), rats (4, 12), sheep (3, 5, 13, 16, 19, 32, 38), cattle (24, 25, 26, 27, 37), poultry (9, 17, 36), pigs (1, 7, 18, 21, 33, 34, 35), rabbits (14) and mink (29). A summary of the responses of the mammalian (sheep, cattle and swine) and avian (chicken, duck, quail, and turkey) species to various beta-adrenergic agonists has recently been published (36).

It is generally believed, mainly on the basis of their effects in food animals, that beta-adrenergic agonists offer interesting tools for elucidation of the mechanisms of fat and protein metabolism. The most extensively studied species, i.e., cattle, sheep, swine and poultry, have been selected for increased growth rate and tend to store large amounts of fat. If beta-adrenergic agonists are to be useful in elucidating the mechanisms of fat and

protein metabolism, a wider range of species should be studied. However, few studies have been reported in other species.

To the best of the authors' knowledge, the effect of beta-adrenergic agonists on body composition of guinea pigs has not been reported. This experiment was, therefore, carried out to investigate the effect of the beta-adrenergic agonist salbutamol, on feed consumption, dissectable fat, carcass composition and weights of several organs in this species. Several blood constituents, indicative of fat metabolism, were also measured.

MATERIALS AND METHODS

Thirty adult male guinea pigs, obtained from the Medical School Laboratory Animal Center, were weighed, allotted to two groups and kept in individual cages. Average body weight (SE) at the start of the experiment was 430.3 g (64.7) for the control (n=10) and 403.5 g (44.2) for the treatment group (n=20). A single dose of salbutamol, obtained from Darou Pakhsh - Iran, was orally administered at 4 mg per kg BW per day, once daily for 30 days by using a tube. Beta-adrenergic agonists are generally administered at low doses in the feed which maintains blood levels over the entire 24 hr. In this experiment, it was not feasible to mix salbutamol with the pelleted feed; therefore, salbutamol was administered by drenching and at a dose larger than those reported in the literature because of the very short half-life of beta-adrenergic agonists in blood. A pelleted guinea pig diet (20% crude protein, 2 Mcal metabolizable energy per kg), purchased from Pars Animal Feed Co.-Tehran, was fed *ad libitum* and daily orts were weighed for determination of feed consumption. No adjustments were made for the amount of feed lost in the bedding. Water was always freely available in bottles.

Guinea pigs were weighed at the end of the experiment (slaughter weight), anesthetized with ether, and blood samples were taken via heart puncture for determination of serum lipase, glucose, cholesterol and triacylglycerols; serum samples were kept at -25 °C until analysis. Ten of the salbutamol treated guinea pigs were matched by slaughter weight (mean, 533.0 g; SE, 67.4) with the control (mean, 546.7 g; SE, 68.1) guinea pigs; the slaughter weights of the two groups were not statistically different. The guinea pigs were then decapitated and the carcass, skin, liver, heart, lungs, kidneys, testes and "longissimus muscle" were weighed, using a Mettler digital balance. Weights of physically separated adipose tissue depots including, cardiac, renal, gastro-intestinal, pelvic, axillary and dorsal fats were also determined. Axillary and dorsal fats are isolated subcutaneous fat depots seen in this species. We also noticed an isolated fat depot associated with hind legs.

Due to difficulty in deboning, whole carcasses were minced thoroughly and kept at -25 °C until used for chemical analysis. Crude protein was determined by Kjeldahl (N × 6.25), fat by ether extract and water content by vacuum oven drying (2). Serum glucose (30), cholesterol (20) and triacylglycerols (15) were determined by an autoanalyzer, and lipase was determined by titration (31) in the Medical School Laboratory. Lipase activity is expressed in terms of units, and one unit is the amount of lipase which splits one micromole of substrate per minute. The data were subjected to analysis of covariance with the appropriate body weight as covariate (28). The level of significance was set at $P>0.10$.

RESULTS

The effects of salbutamol on body weight change, feed consumption, daily weight gain and feed to gain ratio are shown in Table 1. The values reported are the means for 10 control and 20 salbutamol fed guinea pigs.

reported are the means for 10 control and 20 salbutamol fed guinea pigs. Salbutamol did not affect mean daily gain ratio; however, it decreased total feed consumption by 12.2% and feed intake per kg initial body weight by 6.8% as compared with the control guinea pigs (P=0.001).

For carcass studies, ten of the salbutamol treated guinea pigs were matched by slaughter weight (mean, 533.0 g; SE, 67.4) with the control (mean, 546.7 g; SE, 68.1) ones; the slaughter weights of the two groups were not statistically different. The value of 511.5 g for the mean slaughter weight of salbutamol group in Table 1 belongs to 20 guinea pigs initially allotted to this treatment. Hot and cold carcass weights, and cold carcass weight as a percentage of slaughter weight (dressing percentage) are shown in Table 1. Salbutamol did not affect the hot and cold carcass weights and the dressing percentage.

Table 1. Effects of salbutamol on mean (SE) body weight, feed consumption, and carcass weight in guinea pigs.

Parameter	Control	Salbutamol
Initial body weight (g)	430.3 (64.7)	403.5 (44.2)
Slaughter weight (g)	546.7 (68.1)	511.5 (42.3)
Feed consumption (g)	1231 (204)	1081 (121)*
Feed per kg BW (kg)	2.863 (0.003)	2.680 (0.02)*
Hot carcass weight (g)†	373.3 (47.3)	369.4 (48.7)
Empty cold carcass (g)	235.6 (32.1)	239.3 (34.9)
Dressing% (cold)	42.2 (1.4)	43.8 (1.1)
Daily gain (g)	3.88 (0.36)	3.64 (0.23)
Feed to gain ratio	12.14 (2.78)	11.18 (1.86)

* Significantly different from control (P=0.001).

† Including the internal organs.

Mean liver weight per animal (P=0.014) and per kg body weight (P=0.001) was decreased in the guinea pigs receiving salbutamol as compared with the control (Table 2). Salbutamol decreased the heart weight per animal (P=0.025) but did not affect heart weight per kg body weight (P=0.14; Table 2). Mean weights of skin, kidneys, testes, lungs and "longissimus muscle" either absolute (Table 2) or per kg body weight (data not shown) were not different between the two groups.

Table 2. Effects of salbutamol on mean (SE) organ weights in guinea pigsn.

Organ weight	Control	Salbutamol	P
Liver- g/animal	23.30 (2.87)	18.80 (2.56)	0.014
Liver- g/kg BW	100.50 (2.87)	80.80 (3.85)	0.001
Heart- g/animal	1.93 (0.84)	2.15 (0.26)	0.025
Heart- g/kg BW	8.40 (0.44)	9.40 (0.47)	NS
Skin- g/animal	74.10 (8.53)	70.5 (10.37)	NS
Kidneys- g/animal	4.40 (0.34)	4.30 (0.28)	NS
Testes- g/animal	3.10 (0.53)	3.10 (0.60)	NS
Lungs- g/animal	4.50 (0.53)	4.50 (0.50)	NS
Longissimus muscle - g/animal	12.40 (1.64)	12.00 (1.36)	NS

NS Not significant (P > 0.10).

Table 3 shows effects of salbutamol on physically separated adipose depots in guinea pigs. Mean weights of physically separated fat were generally lower for the salbutamol fed guinea pigs. The effect was more pronounced when weights were expressed per kg body weight (Table 3).

Effects of salbutamol on carcass composition are shown in Table 4. Moisture content of the carcass between control and salbutamol fed groups did not differ. The carcass fat content was decreased but protein content was increased in guinea pigs receiving salbutamol (Table 4).

Mean levels of serum lipase, glucose, cholesterol and triacylglycerols were all higher in salbutamol fed guinea pigs as compared with the control ones (Table 5).

Table 3. Effects of salbutamol on physically separated fat in guinea pigs [mean,(SE)].

Dissected fat	Control	Salbutamol	P
Cardiac- g/animal	0.43 (0.09)	0.32 (0.80)	0.09
Cardiac- g/kg BW	1.60 (0.18)	1.20 (0.15)	0.01
Renal- g/animal	5.08 (1.75)	2.90 (0.98)	0.05
Renal- g/kg BW	17.00 (4.36)	9.50 (2.18)	0.013
Pelvic- g/animal	4.74 (1.69)	2.75 (0.05)	NS
Pelvic- g/kg BW	15.80 ((4.23)	9.50 (1.73)	0.04
GIT-g/animal †	11.40 (4.50)	6.90 (2.75)	NS
GIT-g/kg BW	37.00 (11.57)	21.10 (6.16)	0.05
Forelegs- g/animal	4.80 (1.23)	3.40 (1.07)	0.053
Forelegs- g/kg BW	17.60 (2.75)	11.50 (2.27)	0.003
Hind legs- g/animal	5.00 (1.45)	3.00 (0.94)	0.023
Hind legs- g/kg BW	17.40 (3.54)	10.10 (2.02)	0.004
Dorsal- g/ animal	4.20 (0.94)	2.90 (0.69)	0.008
Dorsal- g/kg BW	15.50 (1.83)	10.90 (1.35)	0.001

NS Not significant ($P > 0.10$).

† Gastrointestinal tract.

Table 4. Effect of salbutamol on carcass composition of guinea pigs [mean, (SE)].

Composition	Control	Salbutamol	p
Moisture content (%)	66.40 (1.33)	66.50 (1.61)	NS
Fat (%) in carcass			
-DM basis	26.10 (3.41)	19.50 (2.71)	0.001
Protein (%) in carcass			
-DM basis	57.20 (3.22)	64.60 (2.52)	0.003
Protein (%) in carcass	18.80 (0.44)	21.30 (0.37)	0.001
Protein			
(g in cold carcass)	44.30 (5.53)	50.90 (7.74)	0.006

NS Not significant (P > 0.10).

Table 5. Effects of salbutamol on serum composition of guinea pigs [mean, (SE)].

Composition	Control	Salbutamol	P
Lipase (UNIT)	0.46 (0.03)	0.52 (0.05)	0.012
Glucose (MG/DL)	91.40 (1.43)	99.30 (2.10)	0.002
Cholesterol (MG/DL)	27.50 (1.06)	36.00 (1.29)	0.001
Triacylglycerols (MG/DL)	51.00 (2.32)	59.40 (2.52)	0.025

DISCUSSION

Results of this experiment demonstrated that salbutamol altered carcass composition of guinea pigs in a manner similar to the activity of several other beta-adrenergic agonists in other mammalian species (14, 17, 29).

Daily gain and final body weight (BW) of guinea pigs fed salbutamol did not differ from controls (Table 1). Similarly, salbutamol feeding did not change the growth rate and slaughter weight in pigs (8, 35). Salbutamol fed guinea pigs consumed about 12% less feed relative to the control animals ($P=0.001$), and although there was about 8% improvement in feed -to- gain ratio over the control guinea pigs, the difference was not significant (Table 1). Variable responses have been observed for effects of beta-adrenergic agonists on feed intake, growth rate and feed efficiency in different species (1, 3, 5, 14, 18, 19, 24, 27, 36, 37).

There were no differences in weight and percentage of carcasses between salbutamol treated and control guinea pigs (Table 1). Heavier hot and cold carcass weights and greater dressing percentage have been reported with feeding salbutamol to pigs (8, 35). Jones *et al.* (18) did not find any differences due to cimaterol in slaughter and hot carcass weights of pigs; however, salbutamol resulted in a higher dressing percentage. Cimaterol implants increased carcass weight gain and dressing percentage in lambs (13). Carcass weight and dressing percentage of lambs fed cimaterol were also improved (5, 19). Baker *et al.* (3) found increased dressing percentage in lambs fed clenbuterol, but Hamby *et al.* (16) reported no changes in dressing percentage with this compound. Carcass weight of steers increased with $L_{644,969}$ (37). Cimaterol increased dressing weight and percentage in rabbits (14). Effects of salbutamol on weights of several internal organs of guinea pigs were also studied in the present experiment (Table 2). Liver weight in salbutamol fed guinea pigs decreased by 19% relative to the control. Similar findings have been reported for salbutamol in pigs (35) and

for $L_{644,969}$ in Friesian steers (25). However, cimaterol did not affect liver weight in rabbits (14), pigs (18) and lambs (13, 19).

Heart weights of salbutamol fed guinea pigs were about 10% heavier compared with the control animals (Table 2); however, heart weight per kg BW was not different between the two groups. Effects of cimaterol on heart weight have been quite variable; it was increased by cimaterol in rabbits (14) but was decreased in pigs (18). Cimaterol decreased lamb heart weight in one study (13) but had no effects in another (19). The beta-adrenergic agonist $L_{644,969}$ decreased the heart weight in steers (25).

Salbutamol did not affect weights of skin, kidneys, testes and lungs in guinea pigs (Table 2). Decreased pelt weight in lambs due to cimaterol (13) and decreased hide weight in steers due to $L_{644,969}$ (25) have been reported. Cimaterol increased kidney weights in swine (18), but no effects were noted in rabbits (14) and lambs (13). The beta- adrenergic agonist $L_{644,969}$ decreased kidney and lung weights in steers (25). Beta-adrenergic agonists have generally resulted in a greater cross sectional area of the "longissimus muscle" (LD area) in farm animals (3, 13, 19, 25, 27, 35, 37).

There was a 36% reduction in amount of physically separated fat in salbutamol fed guinea pigs (Table 3). This included the dissected cardiac, renal, pelvic and gastrointestinal fats, as well as special fat depots in the guinea pig associated with the dorsum (dorsal fat), forelegs (axillary fat) and hind legs. Reductions between 25 to 43% in weights of individual fat depot (Table 3) were noted in the treated guinea pigs which is in general agreement with the published data (3, 5, 13, 25, 27); however, Wheeler and Koohmaraie (37) did not find any effect of $L_{644,969}$ on cardiac, renal and pelvic fat percentages in steers.

Due to difficulty in deboning guinea pig carcasses, whole carcasses were minced for chemical analysis. Salbutamol affected carcass composition of guinea pigs in a manner similar to the activity of several beta-adrenergic agonists in other species (see refs in the introduction). Fat content of the

carcass decreased by 25%, but protein content increased by 13% compared with the control. Beta-adrenergic agonists increased moisture content of the carcass in some studies (3, 13, 27) but not in others (18, 19, 25, 35). There is an inverse relationship between the fat and moisture content of the carcass soft tissues, but in the present study, moisture content of the carcass (soft tissues and bones) was not different between treated and control guinea pigs. Carcass bone and compositions could not be determined in this experiment; therefore, grinding of the bones with soft tissues might have influenced the fat, protein and moisture values of the carcass.

Serum levels of lipase, triacylglycerols, cholesterol and glucose increased 13, 16, 31 and 8%, respectively, in the treated guinea pigs relative to the control (Table 5). Systemic effects of beta-adrenergic agonists have been reported in some species. Acute and chronic increases in plasma nonesterified fatty acids were found in steers (10) and pigs (22) with clenbuterol and in lambs with cimaterol (6, 19). Blood glucose decreased in pigs treated with salbutamol (35), and chronically increased in steers with clenbuterol (10). However, Ricks *et al.* (27) did not find any changes in glucose concentration of steers fed clenbuterol. Plasma triacylglycerol concentration was two times higher and fatty acid 24% higher in cimaterol fed lambs (19).

It has been reported that cimaterol decreased plasma insulin levels in lambs (6) which could explain the increased levels of serum glucose due to salbutamol treatment of the guinea pigs in the present experiment. This could also be due to increased level of naturally occurring catecholamines and their effects on gluconeogenesis in the liver. Clenbuterol infusion increased plasma norepinephrine levels and blood flow to the adipose tissues in pigs (23). Decreased food consumption of the salbutamol treated guinea pigs was probably due to increased concentration of blood glucose.

Changes in the blood concentration of hormone sensitive lipase, cholesterol and triacylglycerols are consistent with the effects of the beta-adrenergic agonists on body metabolism.

The observed differences between the effects of salbutamol in the present experiment and those reported for various beta-adrenergic agonists in other species might be due to species variation, dose, duration of treatment, route of administration, receptor down-regulation and different mechanisms of action of various beta-adrenergic agonists in different species (36).

Results of this experiment demonstrated that salbutamol affected carcass composition of guinea pigs in a manner similar to the activity of a number of beta-adrenergic agonists in several mammalian and avian species. The results support the view that effects of beta-adrenergic agonists on carcass composition is a general property of these agents, and are applicable to a wider range of animal species.

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