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Effect of ractopamine hydrochloride on growth promotion in guinea pigs (*Cavia porcellus*)

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Abstract: Ractopamine hydrochloride (R) is commonly used in the livestock industry, mainly in pork and beef, to improve feed efficiency and productivity. In the present study, the use of R at different doses (20 ppm, 30 ppm, or 40 ppm) on growth performance and carcass characteristics of guinea pigs (male and female) was explored with a view to achieving greater use of this species for human consumption. Thirty-two clinically healthy guinea pigs at an average age of 5 weeks were used. The daily gain, feed over gain ratio, Kleiber ratio, carcass yield, and muscle thickness of the thigh in millimeters were measured. The results obtained were compared to a control group treated with only purified water, without R. Arithmetically, the measured variables showed benefits in the groups treated with R, with the results of Kleiber ratio and muscle thickness of the right thigh demonstrating statistical significance ($P < 0.05$). In the present work, the noncastrated males treated with R exhibited a more positive effect than the females ($P = 0.006$). The results suggest that the use of R as a growth promoter could benefit the productivity of guinea pigs for human consumption.

Key words: Beta-adrenergic agonist, Kleiber ratio, meat production

1. Introduction

Ractopamine hydrochloride (R) is a β-adrenergic agonist that belongs to the phenethanolamines group. It is commonly used in the livestock industry (primarily pork and beef) to improve feed efficiency and productivity (1,2).

R is an organic molecule that binds to β-adrenergic receptors at the skeletal muscle and adipocyte cell membrane levels, and it activates the Gs1 protein. The α-subunit of the Gs protein activates adenylate cyclase, an enzyme that produces cyclic adenosine monophosphate (cAMP). This molecule produces its effect by binding to the regulatory subunit of protein kinase A to release a catalytic subunit that phosphorylates a large number of intracellular proteins. Intracellular proteins have vital functional roles for a range of functions, ranging from allowing Ca^{2+} to enter the cell, key to cellular functionality, to mediating protein synthesis in myocytes and energy consumption in adipocytes (3-6).

In pigs, the use of R has been reported with different performance results for females, males, and castrated males (7). R acts directly on the utilization of nutrients for fat accumulation in protein synthesis, which leads to an increase in the lean meat content in the carcasses of animals fed diets to which the product was added (8,9).

The use of R as a growth promoter is approved in many countries, including the United States, Canada, Mexico, Peru, Ecuador, and Brazil (10).

Some investigations have used R on guinea pigs (cavies) and other rodents. In the specific case of the guinea pig, which is a laboratory animal model, metabolites of R were traced and measured in different concentrations and in different tissues, such as skeletal muscle (11–14). The production of this species for human consumption has historically occurred in countries on the American continent, including Andean countries such as Peru and Ecuador (15,16); currently, it is also found in other parts of the world, such as some regions of Cameroon (17–19). Despite the regional importance and the traceability of R reported in tissues from *Cavia porcellus*, no previous reference was found about the possible productive effect

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for the species. Therefore, the present work focuses on the possible productive effect of R on guinea pigs by measures of the animal body (weight, carcass, muscular thickness).

2. Materials and methods

2.1. Animals

This study was approved by the Institutional Committee of Research, Care, and Use of Experimental Animals (CICUAL) of the Faculty of Veterinary Medicine and Zootechnics at the National Autonomous University of Mexico (UNAM), according to Mexican Official Regulation NOM-062-ZOO-1999.

Thirty-two clinically healthy guinea pigs of the Dunkin Hartley line (16 females and 16 males) at an average age of 5 weeks were used. The cavies were obtained and maintained throughout the experiment in the Animal House of the Unit of Chemical and Biological Inspection of the Faculty of Veterinary Medicine and Zootechnics at UNAM.

The cavies used for the study were nonmedicated for at least 30 days prior to the trial. Once in the experiment, the animals (304.6 ± 4.8 g body weight [BW]) were fed with a commercial brand diet specific to the species to cover their nutritional needs (Guinea Pig ®, Cargill-Purina-Mexico). Potable water was made available ad libitum throughout the experiment. The animals were reared and then euthanized at the age of 2.5 months in compliance with the regulations for the care and use of animals in research. Animals were reared in acrylic cages (one per individual) with the recommended vital space for the species, 1504 cm^2 of floor and 17 cm of height per animal. The environment was enriched by adding a hard PVC tube that allowed hiding and housing, and all procedures were conducted with respect to the Mexican normativity on animal welfare (current Animal Protection Law of Mexico City). There was no withdrawal time for R, and animals were not deprived of food or water prior to euthanasia.

2.2. Study design

The number of animals used for the study (n = 32) was determined using the method described by Montgomery (20) for a two-factor experimental design (A: sex; B: dose), based on a significant minimum difference (D = 100 g) between the average levels of the ractopamine factor, and with an expected standard deviation of BW of σ = 50 g, a minimum power of 90%, and a significance level of α = 0.05 in the contrasts.

The experiment was carried out in accordance with a factorial arrangement design of two factors (A: sex: male, female; B: ractopamine dose: 0, 20, 30, 40 ppm, respectively). The sample (n = 32) was randomly assigned via computer-generated lists into 4 repetitions for every combination of these factors (male-female × R dose).

In accordance with the research proposal, R doses were administered daily. A proportional dose was calculated for each animal in volumes up to 1 mL, with the dilution in accordance with the study group. The specific dose was calculated based on the feed consumption per animal (6 g/100 g BW daily) (21) throughout the experiment. The control group received only purified water without R (0 ppm). In all cases, administration was performed orally with the individual use of one sterile needleless syringe per animal.

The groups were constructed as follows:

R 40 (n = 4 females and 4 males, received a daily dose of R 40 ppm),

R 30 (n = 4 females and 4 males, received a daily dose of R 30 ppm),

R 20 (n = 4 females and 4 males, received a daily dose of R 20 ppm),

C (control group, n = 4 females and 4 males, received a daily dose of purified water, without R).

2.3. Drug preparation

The R source (PAFMINE®, ractopamine hydrochloride, 2%) used for the study was provided by PAFFA S.A. de C.V, Mexico; all preparations utilized in this trial were prepared in a sterile environment with laminar flux air turnover and the resulting preparation was bottled prior to filtration with Durapore filters (0.45 μm, Millipore, Mexico). The aqueous formulation of R was prepared from powder and dissolved in sterile purified water each week. For preparation, 50-mL vials were bottled, sealed, and stored at room temperature, avoiding direct light exposure. This preparation was stable as assessed by stability testing performed at 2 weeks, as quantified and corroborated by HPLC.

2.4. Sample collection and analysis

The daily intake of food and water and the BW were recorded per animal per day. The animals were euthanized at the end of the experiment (week 4) by exposure to carbon dioxide. The individual BW was registered prior to euthanasia. Immediately after euthanasia, the viscera, fur, and front and back feet were severed from the body and the carcass weight was recorded. The legs and hips were separated at the third lumbar vertebra level (L3) and weighed separately, after which the right thigh was measured at the halfway point (between the patella and the head of the femur at the intersection with the hip; to register the muscular thickness, measured caudocranially) using a Vernier tool and measured in millimeters. Then the carcasses and samples were packaged, frozen, and stored at –20 °C for a later analysis not shown in this work.

The variables considered included the following:

1. Common farm and productive measures: the individual BWs of each group; weight gain (G = initial BW – final BW); and feed conversion rate (=feed consumed over weight gain) (Table 1) (21).

Table 1. Means ± SEM for the productive data of guinea pigs treated with different oral doses of ractopamine hydrochloride (n = 32).

Group	Sex[a]	Initial BW[b] (g)	Final BW[b] (g)	Total feed intake (g)	Daily feed intake (g)	Weight gain (g)	Daily gain (g)	KR[c] (%)	G/F[d] (g)
R 0	M	298.1 ± 2.7	545.8 ± 23.9	1011.7 ± 28.5	34.9 ± 0.98	248.7 ± 23.1	8.58 ± 0.79	38.9 ± 2.5	0.25 ± 0.02
R 0	F	308.4 ± 5.76	496.2 ± 17.94	908.8 ± 58.4	31.3 ± 2.01	187.8 ± 18.07	6.48 ± 0.62	31.6 ± 2.34	0.21 ± 0.01
R 20	M	299.3 ± 0.25	535.0 ± 25.05	959.0 ± 114.9	33.1 ± 2.61	235.7 ± 25.3	8.13 ± 0.87	37.5 ± 2.49	0.23 ± 0.002
R 20	F	306.9 ± 7.07	521.1 ± 11.74	998.3 ± 54.6	34.4 ± 1.88	214.2 ± 5.16	7.39 ± 0.17	34.9 ± 0.35	0.22 ± 0.01
R 30	M	307.6 ± 6.26	570.8 ± 9.57	941.5 ± 73.1	32.4 ± 2.52	263.2 ± 10.13	8.49 ± 0.33	40.1 ± 1.19	0.29 ± 0.03
R 30	F	302.8 ± 11.9	518.7 ± 32.9	977.1 ± 40.3	33.7 ± 1.39	215.9 ± 22.6	6.97 ± 0.73	35.1 ± 2.16	0.22 ± 0.02
R 40	M	302.5 ± 60.9	584.8 ± 26.8	952.7 ± 42.3	32.9 ± 1.45	282.3 ± 25.9	9.1 ± 0.84	41.9 ± 2.5	0.30 ± 0.03
R 40	F	309.4 ± 7.5	508.8 ± 26.2	948.3 ± 39.2	32.7 ± 1.35	199.4 ± 20.8	6.43 ± 0.67	32.9 ± 2.3	0.21 ± 0.02

[a] M, male; F, female; [b] BW, body weight; [c] KR, Kleiber ratio; G/F, [d] gain over feed.

2. Kleiber ratio (growth rate / $BW^{0.75}$) or KR (22).

3. The hot carcass weight, with head and kidneys included (21), and its yield in percentage ([hot carcass weight / body weight prior to euthanasia] × 100) (Table 2).

4. Right thigh muscular thickness in millimeters.

2.5. Statistical analysis

The results are described by graphs and summaries of estimates. To compare the means between groups for each variable, analysis of variance for a two-factor experimental design with interaction was carried out, with a previous verification of the normality of each of the variables and the homogeneity of the variances between the groups. The analysis of results was performed using the program JMP 10.0.0 (SAS Institute, Inc., 2012).

The linear model corresponding to the proposed design was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \begin{cases} i = 1, \ldots, a: sex \\ j = 1, \ldots, b: RAC\ dose \\ k = 1, \ldots, r: replication \end{cases}$$

Y_{ijk} = Response variable
μ = General average
α_i = Sex effect
β_j = R dose effect
$(\alpha\beta)_{ij}$ = Sex × R dose interaction
ε_{ijk} = Error associated with each result, ~ N_{ID} (0, σ2)

Table 2. Means ± SEM for the carcass data of guinea pigs treated with different doses of oral ractopamine hydrochloride.

Group	Sex[c]	Carcass (g)	Yield (%)	Viscera (g)	Thigh and hip (g)	Thigh Ø (mm)
R 0	M	271.4 ± 7.4	49.8 ± 1.3	133.5 ± 11	71.4 ± 3	[x]33.4 ± 1
R 0	F	246.4 ± 4.1	49.8 ± 1.9	134.95 ± 9	68.2 ± 2	[a]32.23 ± 0.5
R 20	M	271 ± 9.4	50.7 ± 0.6	128.6 ± 5	73.8 ± 1	[x, y]34.69 ± 2
R 20	F	259.9 ± 9.7	49.8 ± 1.1	140.5 ± 8	70.0 ± 3	[a, b]33.29 ± 2
R 30	M	299.5 ± 8.2	52.4 ± 1	140.6 ± 4	77.3 ± 3	[y]38.6 ± 1
R 30	F	255.4 ± 16	49.8 ± 4	132.3 ± 10	72.3 ± 3	[a, b]36.65 ± 1
R 40	M	293.8 ± 10	50.4 ± 1.5	136.8 ± 7	79.5 ± 3	[y]39.85 ± 1
R 40	F	266.4 ± 10	52.5 ± 1	113.9 5 ± 7	71.7 ± 3	[b]37.75 ± 1

[x, y] Levels not represented by the same letter are significantly different (P < 0.005) among males.
[a, b] Levels not represented by the same letter are significantly different (P < 0.02) among females.
[c] M, male; F, female.

3. Results

Performances and carcass data are presented in Tables 1 and 2, respectively. The difference between the means of the right thigh thickness in R (37.4 mm) vs. C (32.8 mm) groups was statistically significant (P < 0.0001) (Figure 1). A difference in the thickness of the right thigh was seen in favor of males compared with females, as shown in Figure 2.

In Figure 3, a type of relation is shown: when the carcass is bigger, then the right thigh muscular thickness is larger, which is dependent on greater R use.

There were no significant differences among KR means at different R doses or between groups fed R vs. fed C. When the KR means were compared by sex (male vs. female) in the C group, they were not significantly different (P = 0.07). When we did the same for the R groups (male 39.6% vs. female 34.3%), we found a significant difference (t ratio = 3.04, P = 0.0064), as shown in Figure 4.

4. Discussion

The R groups in general showed better results, but they did so only numerically (see Table 1). In males, the best observed score was seen in the average daily gain and the rate of gain over feed consumed (R 40, 9.1, and 0.30, respectively) but without significance compared to the C group. These values, which are economically important

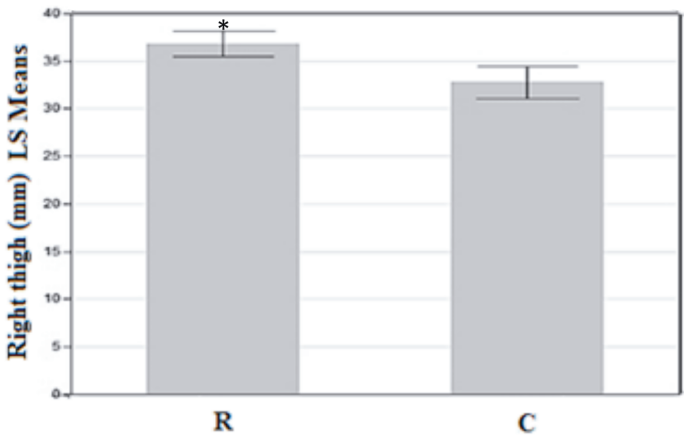

Figure 1. Least square means, right thigh thickness (mm), and a comparison between the animals treated with ractopamine hydrochloride (R) in doses of 20, 30, or 40 ppm and the control group (C), which received only purified water without ractopamine: 0 ppm. *: P < 0.0001.

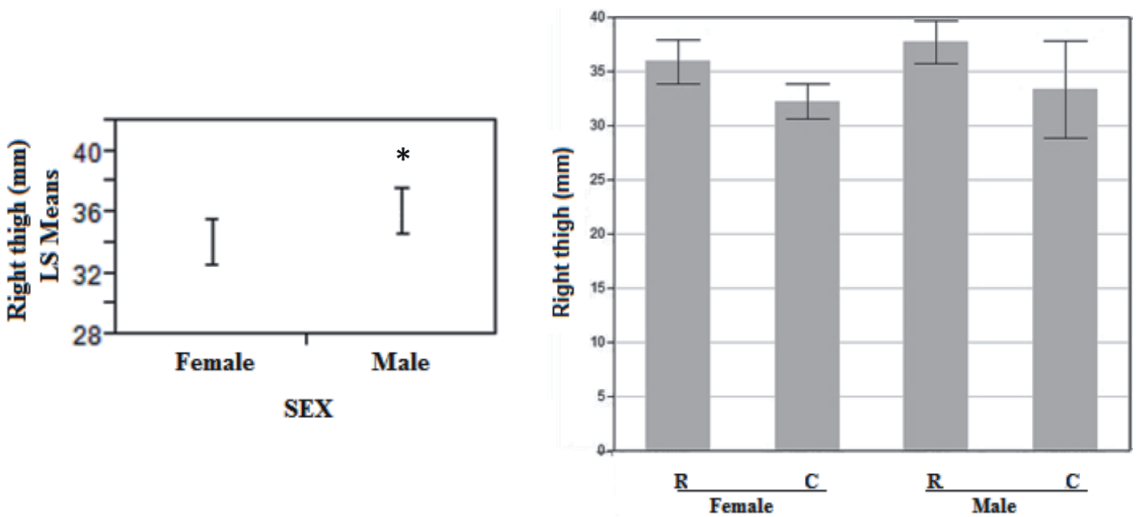

Figure 2. The least square means and right thigh thickness in mm, with a difference between males (36.1 mm) and females (34.1 mm). *: P = 0.058.

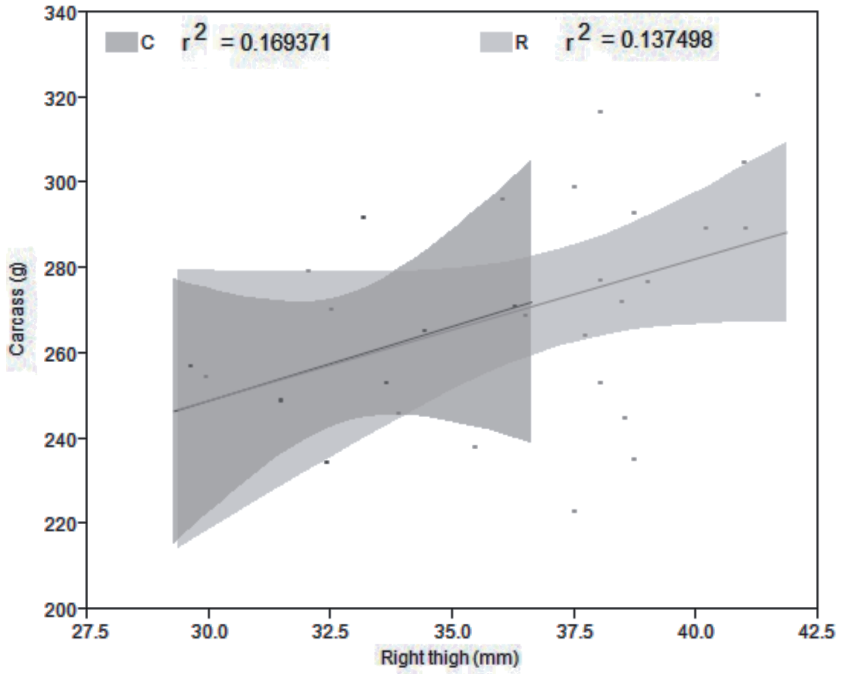

Figure 3. The relation between carcass weight in grams and the right thigh muscular thickness (mm), and a comparison between animals treated with ractopamine hydrochloride (R; P = 0.0893) and the control group (C; P = 0.3111).

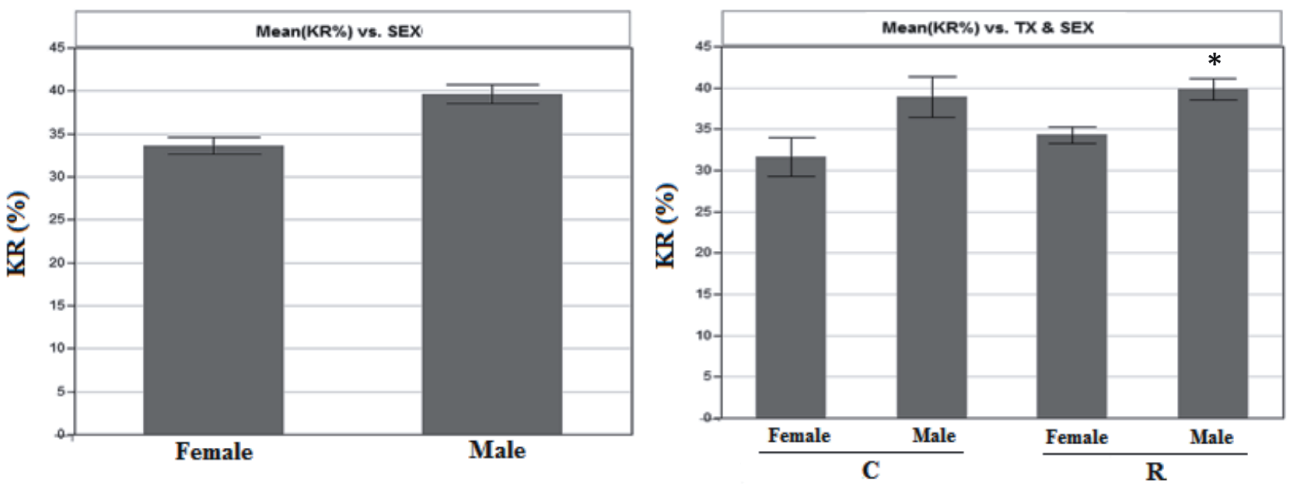

Figure 4. Comparison of the Kleiber ratio (KR) by sex, the same as for the control group (C) and the ractopamine hydrochloride group (R; *: P = 0.0064).

in the animal farming industry, made us consider the genetic line chosen for this work, the Dunking Hartley. This guinea pig was selected for laboratory purposes (23) instead of a line that is usually applied for farm objectives, such as the Andina, Peruvian, and Inti, among others that are specialized in growth and meat production (15,16,24).

From this perspective, we found that other details must be considered, including those neglected in other experimental works that used cavies or rats in the laboratory to examine R (11–13), such as the following: in farm research (15,16,19) cavies are separated into groups, which also stimulates feed consumption due to competition and better socialization, because these animals are gregarious. In our study, we chose cages for individual growth and never considered that this could be a challenge for the animal. Additionally, farmed cavies are

generally fed green fiber (e.g., green lucerne) as a part of the daily diet. Because it is not common in routine laboratory practice to provide green fiber, we did not do so, which could explain why the cavies were seen eating wood starch sporadically. However, we did not pay too much attention to this practice because the omission did not cause any clinical problem in terms of the animals' health.

The KR (growth rate/metabolic mass) is a more typical measurement, a more significant variable, and a better productive indicator than others, such as the average daily gain and feed conversion ratio (22,25–28). Higher values, up to 100%, are more desirable. Considering the data in this experiment, despite the positive result for groups with R use (the whole mean for the C [KR = 35%] and R [KR = 37%] groups), our study only showed an absence of a significant difference.

However, when the KR was applied to compare the treatments or groups by sex, the analysis did show significant differences ($P < 0.05$), as follows: the male values were overall higher than the female values ($t = 2.22$, $P = 0.034$, difference between least squares means = 2.8516 at week 4); this effect is expected for the species, where the male is bigger than the female (7,27), and was observed in R 40 males ($t = 3.1177$, difference between least squares means = 4.297, $P = 0.0103$ at week 2; $t = 3.301$, difference between least squares means = 3.335, $P = 0.0082$ at week 4) and R 30 males ($t = 1.968$, difference between least squares means = 1.99, $P = 0.047$ at week 4). In contrast, it was obvious that the R 40 female group had a lower KR (30%) compared with the other female R groups (Table 1). These data may indicate that a higher R dose could be detrimental for females; i.e. it seems that R at a lower dose such as R 20 could be sufficient for females.

Our results (or data) were insufficient to be determined as significant for all of the variables. In fact, we had expected a different result, e.g., something similar to that observed in species such as pigs or steers, because the observations of those species presented a statistical significance when R was used and the same sex was compared in terms of performance or carcass values (1,7). What we found differed from the results reported for pigs, where castrated males (surgically or chemically) showed the best result with R use (7). In the present work, noncastrated males with R use showed a positive effect that was better than the demonstrated effect in females ($P = 0.006$, interaction of sex by treatment $P = 0.13$).

The genetic line chosen for this work, Dunking Hartley, is a laboratory animal that is not specialized in farm objectives (meat production); moreover, we think that gregarious behavior and feeding habits should be considered important elements in future experimental works when this animal is the model of study or the main objective. However, the present work sets a precedent for the use of R in cavies for farm purposes due to an observed effect (KR and muscular thigh thickness) at different doses for males and females. Thus, further experiments with a proper genetic line for meat production, such as the Peruvian or Inti line, are needed to corroborate the benefits of R in the species.

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