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Ractopamine effect on lipid metabolism and GLUT4 amount of finishing pigs

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Abstract: Ractopamine (RAC) causes fat deposition and/or fatty acid synthesis reduction and increases the rate of protein synthesis and muscle growth. However, there are few scientific studies detailing the mechanism of action of RAC and its possible metabolic pathways in swine. The objective of this study was to evaluate the effect of RAC on lipid metabolism of finishing pigs. Subcutaneous and retroperitoneal fat, muscle, and blood samples were collected at slaughter. Forty pigs were fed different RAC levels (ppm): 0, 5, 10, 15, and 20. RAC did not affect lipoprotein lipase activity in any of the tissues. There were no changes in insulin levels, but a linear increase in serum triacylglycerol, total cholesterol, and HDL-cholesterol (HDL-c) was seen. The insulin-dependent glucose transport (GLUT4) and fatty acid synthase amounts present in animals' adipose tissue did not differ, but the muscle GLUT4 presented a negative quadratic effect. The smallest GLUT4 amount (0.959) was estimated for 15.5 ppm of RAC. Serum glucose increased linearly, while a linear decrease in glycogen content was detected. The results indicate that RAC acts upon lipid metabolism in order to stimulate lipolysis, while there are changes in carbohydrate metabolism that might support lean growth in these animals.

Key words: β-Adrenergic agonist, lipoprotein lipase, fatty acid synthase, GLUT4

1. Introduction

Fat deposition in animals can be beneficial or undesirable, depending on the quantity and location. In pork production, the understanding of lipid metabolism and its regulatory mechanisms contributes to the development of strategies capable of altering the fat deposition in different animal tissues, thereby improving the meat quality; supplementation with ractopamine (RAC) is one such example (1).

It is believed that RAC, a β-adrenergic receptor agonist, can control the fat in pigs through important metabolic pathways related to fat tissue deposition and fat release by activating specific β-adrenergic receptors as a result of its chemical structure and pharmacological properties that are similar to those of catecholamines. This helps to produce carcasses with lower fat levels, resulting from the better nutrient partitioning that supports muscle growth rather than fat deposition (2).

Some of the effects of RAC supplementation on lipid metabolism in pigs include the regulation of gene expression related to the breakdown of body fat and the genes that regulate enzymes involved in lipogenesis (3,4). As other studies are still necessary to help to understand the possible pathways of this drug in pigs, metabolically important enzymes, such as lipoprotein lipase, also require further investigations in this animal model. Therefore, the aim of this study was to evaluate enzymes from fat and muscle cells related to lipid synthesis and glucose metabolism as well as serum biochemical parameters of finishing pigs fed different levels of RAC.

2. Materials and methods

2.1. Experimental procedure

All animals were cared for in accordance with the principles promulgated by ethical guidelines and the experimental protocol was approved by the Ethics Committee in Animal Experimentation of the Federal University of Lavras.

Forty commercial crossbred pigs (TOPIGS) from the Swine Experimental Centre of the Animal Science Department, Federal University of Lavras, barrows and

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gilts, were used to collect samples of subcutaneous (SAT) and retroperitoneal (RPAT) adipose tissues, the *longissimus dorsi* (MT), and blood. Throughout the experimental period, the animals were housed in individual pens with a concrete floor (2.3 × 1.5 m) equipped with semiautomatic feeders and access to water ad libitum. Animals were fed twice daily at 0700 and 1600 hours. The treatments were: control diet formulated with corn and soybean meal, without RAC; diet + 5 ppm RAC; diet + 10 ppm RAC; diet + 15 ppm RAC, and diet + 20 ppm RAC. Table 1 presents the composition and the percentage values of the experimental diets.

Table 1. Composition and percentage values of experimental diets for finishing pigs fed with different levels of ractopamine.

	Experimental diets				
	0 ppm	5 ppm	10 ppm	15 ppm	20 ppm
Ingredient composition (%)					
Corn	71.43	71.43	71.43	71.43	71.43
Soybean meal	22.73	22.73	22.73	22.73	22.73
Soy oil	2.50	2.50	2.50	2.50	2.50
Dicalcium Phosphate	0.92	0.92	0.92	0.92	0.92
Calcium Carbonate	0.67	0.67	0.67	0.67	0.67
Salt	0.36	0.36	0.36	0.36	0.36
Mineral premix[1]	0.10	0.10	0.10	0.10	0.10
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10
DL-Methionine 99%	0.16	0.16	0.16	0.16	0.16
L-Lysine HCL 78%	0.35	0.35	0.35	0.35	0.35
L-Threonine 98%	0.17	0.17	0.17	0.17	0.17
Clay	0.490	0.465	0.440	0.415	0.390
Tylan[3]	0.02	0.02	0.02	0.02	0.02
Ractosuin[4]	0.00	0.025	0.05	0.075	0.10
Nutritional composition					
Crude protein (%)	16.20	16.20	16.20	16.20	16.20
ME (kcal/kg)	3310	3310	3310	3310	3310
Digestible Lysine (%)	1.003	1.003	1.003	1.003	1.003
Digestible methionine (%)	0.400	0.400	0.400	0.400	0.400
Digestible threonine (%)	0.717	0.717	0.717	0.717	0.717
Available phosphorus (%)	0.263	0.263	0.263	0.263	0.263
Calcium (%)	0.559	0.559	0.559	0.559	0.559

[1]Composition, per kg: calcium: 98.800 mg; cobalt: 185 mg; iron: 26.650 mg; copper: 15.750 mg; iodine: 1.470 mg; manganese: 41.850 mg; zinc: 77.999 mg.
[2]Composition, per kg: folic acid: 116.55 mg; pantothenic acid: 2333.5 mg; biotin: 5.28 mg; niacin: 5.600 mg; pyridoxine: 175 mg; riboflavin: 933.3 mg; thiamine, 175 mg; vitamin A: 1,225,000 UI; vitamin D_3: 315,000 UI; vitamin E: 1.400 mg; vitamin K_3: 700 mg; vitamin B_{12}: 6.825 mg; selenium: 105 mg; antioxidants: 1.500 mg.
[3]Antibiotic made from granulate tylosin.
[4]Ractopamine hydrochloride 2.05%.

2.2. Sample collection

The samples of SAT, RPAT, MT, and blood were collected at the moment of slaughter, which occurred at the end of the 28-day experimental period. Slaughtering was performed after electrical stunning in a commercial facility, according to the rules of the Brazilian Federal Inspection Service. All slaughtered animals were fasted for 12 h prior to slaughter.

Fragments of SAT and RPAT with a thickness of at least 1 cm and a depth of 1 cm were collected in duplicate during evisceration immediately after slaughter. Fragments of MT were collected in similar sizes as adipose tissue, also in duplicate, from the left side of the carcasses of the pigs. Both procedures were carried out carefully using sterile instruments. Each fragment collected was stored in resistant plastic bags, individually identified with labels and immediately frozen in liquid nitrogen. The collected tissue was divided and transported in liquid nitrogen, and then stored in a freezer (–80 °C) until analysis.

The blood was collected in duplicate during bleeding, which was performed by cutting the great vessels of the neck, with the animals positioned vertically and suspended by one of their hind limbs. Blood sample storage was done in test tubes with no anticoagulant, and samples were individually identified. The material was kept under refrigeration (2 °C to 8 °C) during transport, which lasted 30 min. In the Physiology and Pharmacology Sector, samples were centrifuged at 1000 × *g* for 15 min to separate serum, which was stored at –20 °C in polyethylene centrifuge tubes for later analysis of the following metabolic parameters: glucose (GLU), total cholesterol (TC), HDL-cholesterol (HDL-c), and triacylglycerol (TAG). Serum used for insulin (IN) measurement assay remained stored in a freezer at –80 °C.

2.3. Total lipoprotein lipase activity analysis

In order to perform this analysis, fragments weighing 150 mg of SAT, RPAT, and MT were homogenized in buffer containing detergents (0.2 M Tris/HCl, pH 8.3, 5000 UI/mL heparin, 1 mg/mL BSA, and 5 mg/mL sodium deoxycholate) (5) and total lipoprotein lipase (LPL) activity was measured using a lecithin emulsified substrate (6) containing [9,10-3H]-triolein and plasma from rats fasted 24 h as a source of apoCII. The reaction was quenched with extraction mixture (7) and [3H] fatty acids released were quantified by liquid scintillation spectroscopy (Beckman LS 7600). The enzyme activity was expressed as moles of [3H] fatty acid released per milligram of evaluated tissue per minute.

2.4. Glycogen content determination

To measure the glycogen content, glycogen extraction from samples of 200 mg of MT was performed in boiling water for 1 h using a KOH solution (30%), followed by addition of saturated Na_2SO_4 and ethanol (100%) to precipitate the glycogen from alkaline digestion. The material was centrifuged at 978 × *g* for 10 min, and then the glycogen was rinsed with distilled water and precipitated again using 100% ethanol followed by centrifugation. The glycogen precipitate was dissolved in 10 mL of distilled water and the final solution was stored in 15-mL tubes. A mixture of anthrone was carefully added to a 1-mL aliquot of the final dilution with subsequent heating of the samples in boiling water for 10 min. The absorbance was read in a spectrophotometer at 620 nm. Different glucose concentrations were used to plot a calibration curve.

2.5. Western blotting analysis

Semiquantitative analyses were performed only on barrow samples, since these animals showed a better response pattern to RAC. Proteins were obtained from SAT and MT samples using a lysis buffer (1% Triton X-100, 100 mM Tris/HCl, pH 8.0, 10% glycerol, 5 mM EDTA, 200 mM NaCl, 1 mM DTT, 1 mM PMSF, 25 mM NaF, 2.5 mg/mL leupeptin, 5 mg/mL aprotinin, and 1 mM sodium orthovanadate). Lysates were centrifuged at 10,000 × *g* for 10 min at 4 °C and quantified using the Bradford assay reagent from Bio-Rad (Hercules, CA, USA).

Twenty micrograms of protein from the SAT and MT was separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Bio-Rad). A molecular weight standard (Bio-Rad) was run in parallel to estimate molecular weight. Membranes were blocked overnight at 4 °C in Tris-buffered saline-Tween (TBST; 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.1% Tween 20) containing 5% dried milk. After blocking, membranes were incubated at 4 °C overnight with anti-ICAM-1 (1:1000), anti-RANKL (1:1000), anti-OPG (1:2000), or α-tubulin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), used as an internal control (1:1000), diluted in TBST containing 5% dried milk for the analyses of gingival proteins. Membranes were then incubated at room temperature for 60 min with secondary antibody conjugated with peroxidase (1:5000) diluted in TBST containing 5% dried milk.

Finally, the bands recognized by the specific antibody were visualized using a chemiluminescence-based ECL system (Amersham Biosciences, Piscataway, NJ, USA) and exposed to an X-ray film for 30 min (Eastman Kodak, Rochester, NY, USA). A computer-based imaging system (Image J, Bethesda, MD, USA) was used to measure the intensity of optical density of bands.

2.6. Biochemical parameters

GLU, TC, and HDL-c concentrations from serum were respectively quantified by the glucose oxidase, cholesterol oxidase, and modified PEG 6000 methodologies using Doles (Goiânia, GO, Brazil) enzymatic assays according to the protocol of the manufacturer. TAG was quantified by the Enzyme-Trinder method using the LABTEST

(Lagoa Santa, MG, Brazil) enzymatic test, according to the manufacturer's protocol. The IN concentration was quantified by ELISA Millipore insulin kit (Millipore, St Charles, MO, USA) according to manufacturer's methods.

2.7. Statistical analysis

The data were analyzed in a randomized block design with 5 treatments and 8 replications. After testing for normality and homogeneity of variances (Shapiro–Wilk and Levene test, respectively), data were subjected to analysis of variance using SPSS 17.0 for Windows. Multiple regression analyses through the third degree were performed. The results were considered significant when $P < 0.05$.

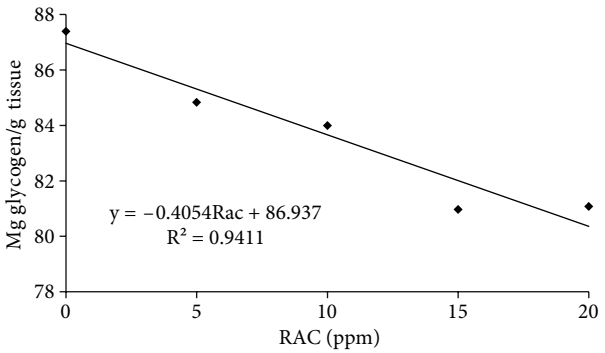

Figure 1. Muscle glycogen versus ractopamine (RAC) levels on diets for commercial hybrid finishing pigs, barrows and gilts.

3. Results

There was no significant difference among the different levels of RAC on LPL total activity on blood vessels around SAT, RPAT, or MT (Table 2) of pigs.

A linear decrease ($P < 0.01$) in the amount of glycogen stored in skeletal muscle tissue of the pigs was observed with increasing doses of dietary RAC after 28 days of treatment (Figure 1).

Figure 2 shows the bands of insulin-dependent glucose transporter (GLUT4) (54 kDa) as well as the enzyme fatty acid synthase (FAS) (250 kDa) in SAT of barrows fed different levels of RAC. Figure 3 shows the bands of 54 kDa GLUT4 in the muscle tissue of barrows fed with different levels of RAC.

As demonstrated in Table 3, GLUT4 and FAS in SAT were not affected by different levels of RAC in the diet. Regarding GLUT4 in skeletal muscles, there was a negative quadratic effect ($P < 0.01$) as the levels of RAC in the diet

Table 2. Lipoprotein lipase total activity (LPL) on subcutaneous adipose tissue (SAT), retroperitoneal adipose tissue (RPAT), and muscle tissue (MT) and muscle glycogen content and biochemical parameters of finishing pigs, barrows and gilts, fed with different levels of ractopamine (RAC).

	RAC (ppm)					CV[1]	Regression	P
	0	5	10	15	20			
LPL activity[2]								
SAT	0.024	0.023	0.021	0.019	0.018	22.39	-	0.114
RPAT	0.025	0.023	0.023	0.022	0.022	13.00	-	0.197
MT	0.026	0.028	0.029	0.029	0.029	13.48	-	0.542
Muscle glycogen content								
Longissimus dorsi[3]	87.40	84.84	84.00	80.97	81.08	6.23	Linear	**
Biochemical parameters								
GLU (mg dL^{-1})	83.80	92.24	98.11	99.46	99.90	11.88	Linear	*
TC (mg dL^{-1})	68.85	77.33	81.17	87.29	90.50	12.95	Linear	**
HDL-c (mg dL^{-1})	35.34	38.00	40.82	42.29	43.23	7.08	Linear	***
TAG (mg dL^{-1})	50.07	56.27	56.69	61.41	63.89	11.25	Linear	**
IN (ng mL^{-1})	0.116	0.107	0.106	0.100	0.094	33.03	-	0.766

GLU = Serum glucose; TC = total cholesterol; HDL-c = HDL cholesterol; TAG = triacylglycerol; IN = insulin.
[1]Coefficient of variation (%).
[2]Moles of fatty acid released per milligram of tissue per minute.
[3]Milligrams of glycogen per gram of tissue.

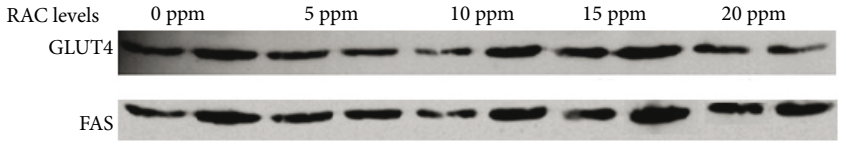

Figure 2. Western blotting of proteins GLUT4 and FAS in subcutaneous adipose tissue of commercial hybrid barrows fed with different levels of ractopamine (RAC).

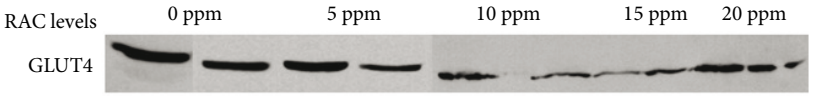

Figure 3. Western blotting of protein GLUT4 in muscle tissue (*longissimus dorsi*) of commercial hybrid barrows fed with different levels of ractopamine (RAC).

Table 3. Semiquantitative analysis by western blotting of the insulin-dependent glucose transporter (GLUT4) and of the enzyme fatty acid synthase (FAS) on subcutaneous adipose tissue (SAT) and muscle tissue (*longissimus dorsi*) of commercial hybrid barrows fed with different levels of ractopamine (RAC).

	RAC (ppm)					CV[1]	Regression	P
	0	5	10	15	20			
SAT								
GLUT4[2]	0.780	0.765	0.696	0.655	0.595	11.57	-	0.263
FAS[3]	0.495	0.680	0.660	0.610	0.655	16.21	-	0.450
Longissimus dorsi								
GLUT4[2]	1.785	1.325	1.025	1.020	1.010	8.91	Quadratic	**

[1]Coefficient of variation (%).
[2]Ratio GLUT4/α-tubulin; units are relative density.
[3]Ratio FAS/α-tubulin; units are relative density.

increased; thus, the smallest amount of GLUT4 (0.959) was estimated for 15.5 ppm of RAC in the diet (Figure 4).

RAC did not affect serum levels of IN (Table 2). GLU, TC, HDL-c, and TAG showed linear increases (P <0.05, P <0.01, P <0.001, and P <0.01, respectively) as RAC levels increased (Figure 5).

4. Discussion

LPL is an essential enzyme acting on the direct control of triacylglycerol between muscle and adipose tissue, increasing lipid storage or providing energy as fatty acids for muscle growth. Evaluating the activity of this enzyme can be a valid tool for the study of the anabolic fat pathway, especially in adipose tissue (8). Based on the effects of catecholamines on LPL activity in adipose and muscle tissue, with a decrease and increase, respectively,

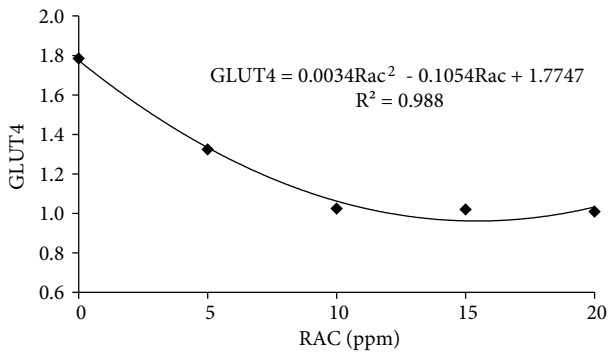

Figure 4. Semiquantitative analyses by western blotting of the insulin-dependent glucose transporter (GLUT4) on muscle tissue (*longissimus dorsi*) of commercial hybrid barrows fed different levels of ractopamine (RAC). Results presented as ratio of GLUT4/α-tubulin; units are relative density.

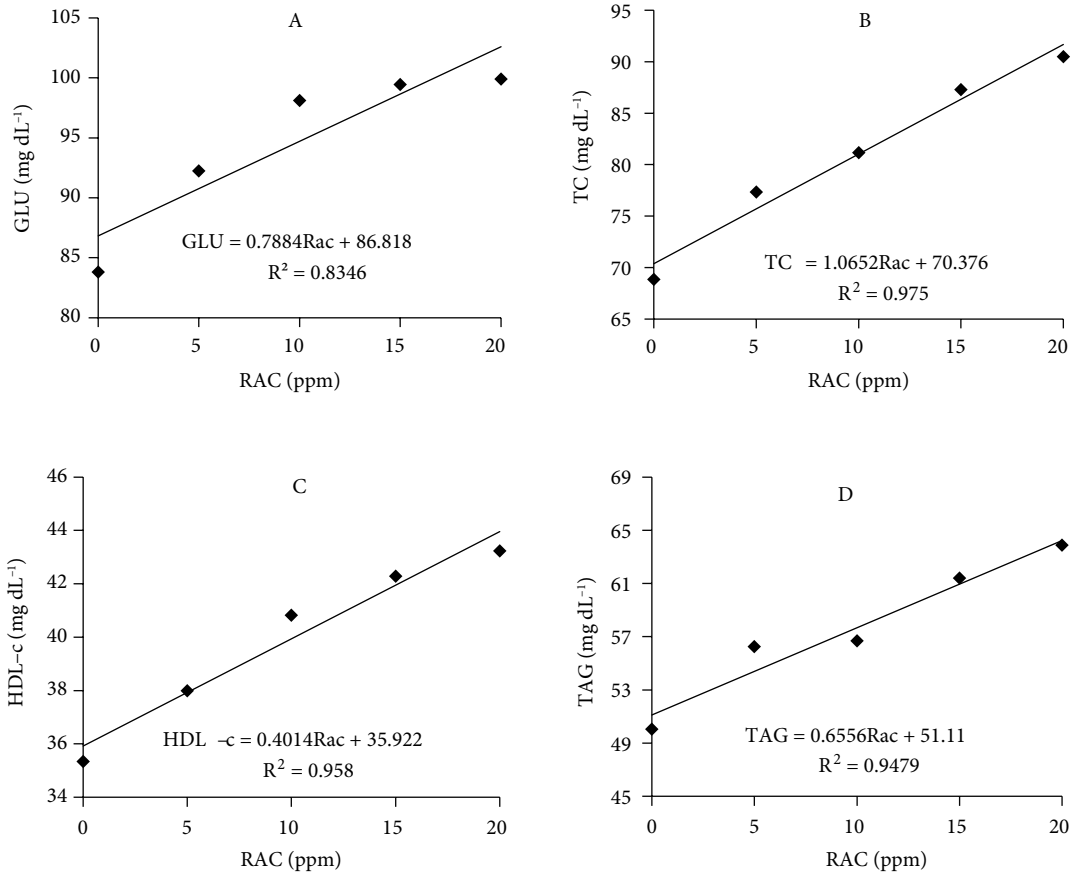

Figure 5. Serum glucose (GLU) (a), total cholesterol (TC) (b), HDL cholesterol (HDL-c) (c), and triacylglycerol (d) versus levels of ractopamine (RAC) in diets of commercial hybrid finishing pigs, barrows and gilts.

found by Fonseca-Alaniz et al. (9) and Goldberg et al. (10), it was expected that swine LPL activity after RAC supplementation could also provide the same change; however, this effect was not observed, probably because RAC does not act directly on the LPL activity of pigs.

Another valid parameter for the study of the anabolic pathway, FAS, was also evaluated in this experiment. As previously reported, RAC did not alter the amount of FAS present in the pigs' subcutaneous fatty tissue; however, a reduction in the amount of FAS mRNA in the same tissue was observed in pigs supplemented with 20 ppm RAC for 28 days (4) as well as for 52 days (3). None of the work addressed methodologically resembles this experiment; therefore, it must be taken into account that the answers herein obtained may have varied from the others due to the animals used, time of exposure to agonist, and level administered.

Therefore, it is thought that supplementation of pigs with RAC alters the lipid metabolism of the animals, resulting in the stimulation of lipolysis instead of blocking lipogenesis, although the adipose tissue of these animals has a lower concentration of lipolytic β3-adrenergic receptors (11). This may be a likely explanation for the decrease in fat deposition in the carcass of pigs supplemented with the additive found by several authors (2,12). In fact, Peterla and Scanes (13) found in vitro that adipocytes from pigs treated with RAC showed an increased release of glycerol. According to Adeola et al. (14), when RAC improves performance and carcass composition of pigs and also induces change in the metabolism of the animals, this increases the utilization of fat for oxidation and enhances the responsiveness of the adipose cells to lipolytic stimuli.

Changes in lipid metabolism at different levels have a direct impact on serum levels of triacylglycerol-rich lipoproteins and cholesterol-rich lipoproteins (15). Hoshi et al. (16) found that pigs supplemented with 20 ppm of RAC showed a linear increase of TC, TAG, and HDL-c. Similarly, the same parameters were found to be increased in this study. Probably as a result of other mechanisms not identified with this research, RAC may have caused a decrease in the uptake of chylomicron remnants and circulating lipoproteins, as well as cholesterol uptake by

the liver, which may have affected the levels of serum TAG and TC, respectively, as well as HDL-c levels (greater fraction present in the pig).

While RAC is able to activate the lipolytic pathway in the adipose tissue of pigs, insulin has a lipogenic role in this tissue (17). Although Dunshea and King (18) found that pigs supplemented with RAC presented decreased basal insulin levels, this effect was not demonstrated in this experiment. It is possible that RAC may have decreased sensitivity to insulin and/or insulin binding to adipocytes of supplemented pigs, which is similar to results found previously in in vitro studies (19). Additionally, the liver, in response to stimulation by the agonist, may present reduced removal of insulin, which could have helped with the maintenance of high levels of this hormone (20).

Measuring the concentration of a metabolite in plasma does not necessarily indicate a rate of metabolic change that occurs in a specific tissue, since the circulating concentrations of these metabolites depend on several factors such as feed intake, production rate, uptake rate and release rate for plasma, intracellular reuse, blood flow, and the complex interplay of hormones (8,14). Thus, all changes observed in this experiment must not be uniquely suggested as the result of a single event, as they may also have arisen from metabolic complexes and integrated events within lipid metabolism of the animal, such as, for example, in response to changes in carbohydrate metabolism.

In addition to acting in important metabolic pathways, RAC is known to be related to lipid and protein metabolism; it has also been shown to have an effect on carbohydrate metabolism of supplemented animals, and thus integrated redirects the nutrients present in the diet favoring the synthesis of proteins at the expense of fat deposition in the carcass (12,21). As with catecholamines, the use of synthetic β-agonists may cause increased concentrations of glucose and nonesterified fatty acids in the plasma of supplemented animals; these changes have already been reported (14,22).

Therefore, hyperglycemia can be considered a mark of metabolic response due to the use of catecholamines and their similarities to β-adrenergic agonists, which is in contrast to the observations of Dunshea and King (18); this effect was demonstrated in the current experiment. The pigs supplemented with RAC showed a maximum increase in serum glucose of approximately 19%. These results are similar to those obtained by Mersmann (8), whose experiment, which was designed to evaluate the acute metabolic effects of supplementation with the same agonist in the diet of pigs, showed an increase in volume as a result of glucose by 24%; Vandenberg et al. (23) also found similar results when working with fish. It is believed that the stimulation of hepatic β2-adrenergic receptors by RAC may have resulted in increased catabolism of glycogen (glycogenolysis and/or gluconeogenesis, indirectly), providing a higher plasma level of glucose (24), presumably to support the increase verified by several authors in protein deposition that occurs in the skeletal muscle of these animals during supplementation with RAC (2,12).

The glycogenic effect previously discussed following the administration of catecholamines can also be analyzed in the skeletal muscle (21); this work showed a decrease in the glycogen content of pigs supplemented with RAC. The results demonstrated here for serum glucose and muscle glycogen content can provide support for the changes found by Gunawan et al. (21) with regards to the expression of glycogen synthase in their experiment with pigs. Initially, the increasing availability of glucose into the circulation due to the mobilization of energy reserves from the liver stimulates the expression of glycogen synthase (GS). With the chronic administration of RAC, the need for energy associated with muscle growth increases and is continuously stimulated by the additive. From this moment, it would no longer be necessary to maintain high levels of GS in muscle cells that suffered down-regulation of its gene with the concomitant necessity of breaking the muscle glycogen stores by the action of the enzyme glycogen phosphorylase. Further studies in this field are encouraged in order to clarify this hypothesis.

Supplementation with RAC increases lipolysis by binding β-adrenergic receptors present in the adipocyte membrane, with the subsequent activation of a signaling cascade that involves the synthesis of cAMP and the subsequent activation of hormone-sensitive lipases by protein kinase A (17). Additionally, it was shown that protein kinase A, through increased serine phosphorylation, shows inhibitory effects on several reactions involved in the process of converting glucose to triacylglycerol and the subsequent storage (25). As proposed by Liu et al. (25), it was expected that pigs supplemented with RAC would show a decreased lipogenesis rate with a consequent decrease in the activity of enzymes involved in lipid synthesis and the glucose transporter GLUT4, which participates in the metabolism of lipids and carbohydrates. However, in contrast to the results of Halsey et al. (4) and Reiter et al. (3), this result was obtained only with the amount of GLUT4 present in the skeletal muscle tissue of animals (Figure 4). Halsey et al. (4) found that 28 days of supplementation with RAC was able to decrease the amount of GLUT4 mRNA present in the adipose tissue of the animals. Reiter et al. (3), while increasing the time of exposure to the agonist (52 days), were able to obtain similar results for GLUT4 mRNA present in the adipose tissue and further proved that the same effect (decrease) was also found in skeletal muscle tissue.

The fact that the amount of GLUT4 and FAS in adipose tissue of pigs did not vary between treatments does not indicate that RAC cannot be an agonist in the adipose tissue of these animals. The characteristic effects of RAC are already well demonstrated in both in vitro and in vivo studies (19,26). It is probable that RAC supplementation for 28 days induced the down-regulation of β-adrenergic receptors present in adipose tissue of the animals, causing a decrease in the β-adrenergic response. It is well established that the long-acting agonist can induce desensitization of β-adrenergic receptors, with the subsequent sequestration of the agonist-receptor complex to the intracellular environment and the appearance of the down-regulation phenomenon (27). All of these processes cause a reduction in the amount of β1, β2, and β3 receptors on the membrane (28). According to Spurlock et al. (29), either desensitization does not occur in muscle tissue or down-regulation processes have little influence on this tissue, since the number of β-receptors in the skeletal muscle of pigs fed RAC for 28 days did not decrease in their experiment. The results obtained from this study confirm the hypothesis of the authors, given that there was a decrease in the amount of glucose transporters in the supplemented animals.

Additionally, it is known that both genes and lipogenic enzymes are extremely sensitive to the energy status of the animal (4). Before slaughter in this experiment, the animals were fasted for 12 h. Thus, SAT, RPAT, and MT were obtained after slaughter and may not reflect the actual scenario of the amount of active enzymes in the adipose tissue of living animals. In the works of Halsey et al. (4) and Reiter et al. (3), pigs were slaughtered without any period of food deprivation, which may be reflected in the results obtained by these authors. Liu et al. (25), however, did not explain whether the animals in their experiment were fasted or not, but their results showed that RAC did not affect the amount of GLUT4 mRNA present in the adipose tissue of the animals.

Changes in blood metabolites and lipid and carbohydrate metabolism observed in the present study and discussed based on observations previously published by other authors show that some of the components of the breakdown of nutrients caused by RAC result in fat reduction and an increase in muscle mass and production efficiency. It is concluded that RAC, when administered to swine in the finishing phase, acts on adipose tissue metabolism in order to stimulate lipolysis, and that there are also changes in carbohydrate metabolism that will support the mass lean growth in supplemented animals.

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