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Metabolic and Behavioral Effects of Ractopamine at Continuous Low Levels in Rats under Stress

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ABSTRACT

This study aimed at evaluating the effect of ractopamine (RAC) on metabolism, zootechnical performance, body composition, and behavior in Wistar rats submitted to acute and chronic restrain stress. The oral dose of 5 mg/kg of RAC was administered in periods of 0, 7, 14, 21, and 28 days. The elevated plus-maze test (EPMT) was used for behavioral assessment. Blood, carcass and viscera characteristics were evaluated. Insulin-dependent glucose transporters (GLUT-4) were semi-quantified by Western Blot in epididymal adipocytes. RAC periods associated with chronic stress increased the GLUT-4 protein expression in adipose tissue in a time-dependent manner (P=0.01), i.e., the longer the RAC addition period, the higher the GLUT-4 concentration in chronically stressed animals (0=1.42; 7=1.19; 14=2.03; 21=1.59; 28=2.35). The stress periods combined with RAC increased the time spent in the opened arms of the maze (Chronic stress: 0=10.6; 7=8.7; 14=5.9; 21=12.3; 28=4.0; Acute stress 0=3.1; 7= 4.7; 14=7.5; 21=0.0; 28=2.8) (P=0.04). Chronic (entries on the closed arms [ECA]=3.60) and acute (ECA=3.80) stress reduced locomotive activity in the maze (P=0.03). The results suggested that stress could negatively affect the possible benefits offered by the RAC, mainly impairing the adipose tissue metabolism and behavior in the animals.

Key words: β-adrenergic agonist, Ractopamine, zootechnical performance, Metabolism, Elevated Plus-Maze Tests

INTRODUCTION

β–adrenergic agonists (BAAs) have usually been used in human and veterinary therapeutics either as bronchodilators or tocolytics. Recently, they have been used as powerful lean growth promoters and fat deposition inhibitors. Among BAAs, clenbuterol, salbutamol, and particularly ractopamine are the most studied. However, Ractopamine (RAC) with its lower toxicity, low plasma protein bonding, and easy biotransformation, emerged as a feasible carcass modifier through its nutrient partitioning effects (Smith 1998).

Agents like RAC promote their effects through ligation to β-adrenergic receptors and consequent activation of adenylyl cyclase and protein kinase A systems. This mechanism is identical to that triggered by endogenous adrenergic agonists: adrenalin and noradrenalin. However, prolonged

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exposure of the cells to these agonists can generate down-regulation of those receptors (Liu et al. 1994), a mechanism, which induces a diminished tissue response. Considering adrenaline is liberated during the stress, and that β-adrenergic receptors can be desensitized by constant presence of its ligands, it can be hypothesized that stress conditions could interfere in the pharcokinetics of RAC. Studies with low doses of RAC over increasing periods are scarce. Moreover, production animals are constantly submitted both to chronic stress by confinement and handling, such as acute stress by transport and slaughter (Marchant-Forde et al. 2003), which could make the results of this study important.

Information regarding the potential use of RAC on metabolic parameters has been highlighted in several studies, but information about the behavior is still infrequent. Studies with pigs fed with RAC demonstrated changes in pig's behavior (Marchant-Forde et al. 2003; Poletto et al. 2010). Other studies have demonstrated the influence of beta-adrenergic agonists on rodent's behavior (Flicker and Geyer 1982; Gorman and Dunn 1993). However, no study was found using RAC and stress. Considering that the experimental models could contribute to physiological, molecular, and behavioral evaluations in the laboratory animals, this knowledge could be extrapolated to production animals.

The aim of this study was to verify the effects of RAC on the metabolism, growth performance, corporal composition and behavior of the rats submitted to different sources of stress by acute and chronic immobilization. The main purpose was to improve the RAC supply period knowledge in order to obtain better dose-response effects on the animals subjected to stress. In an attempt to assess the effects of increasing the administration time of RAC and avoid losing receptor sensibility, a low dose of RAC was chosen.

MATERIAL AND METHODS

The animals used in this study were provided by the Physiology and Pharmacology Sector of Veterinary Medicine Department of the Federal University of Lavras – UFLA, Brazil. Procedures used in the experiment were approved by the Animal Experimentation Ethics Committee (NINTEC) of UFLA, according to rules of the Brazilian Laboratory Animal Science Society (SBCAL) under protocol 004/2010.

Animals and Experimental Procedures

Seventy-five male rats (Wistar), approximately 60 days old and weighing 220-260g were used. The animals were divided into 15 experimental groups, matching five different RAC time periods (0, 7, 14, 21 and 28 days), with three different stress levels (without stress, acute stress, and chronic stress). One control group (without stress and without RAC) was also included. Each group consisted of five randomly chosen animals. The animals were kept in individual cages (material: polypropylene, dimensions: 30 cm long, 20 cm wide and 13 cm high), covered with wood shavings and kept under a controlled environment (temperature and humidity) with 12/12h light/dark cycle. The rats received age appropriate feeding in accordance to the metabolic weight (diet Nuvilab®, Brazil) and water *ad libitum*. The experimental period lasted 54 days and started after a seven-day adaptation period (before this period the animals were kept in collective cages) (Fig. 1).

All the experimental procedures were performed in the morning, between 8:00 am and 12:00 am. All the animals, including the control group, were handled every day for weighing and every three days for cleaning the cages.

Growth Performance

Average daily weight gain of the animals was calculated through registered data collected during the experimental period. Feed consumption was assessed by subtracting the remaining fragments in the cage from the total amount provided.

Elevated Plus-Maze Test (EPMT)

On the last day of experimental period for each group, the animals fasted for 8 h (for blood collection and metabolic parameters analyses). They were submitted to the last stress session, and immediately after restraining, they were taken to the elevated plus-maze (EPMT). The maze was constructed according to Rocha et al. (2007), with wood painted black and consisted of two open arms, positioned opposite of each other, and intersected at a 90° angle by two other arms, which were closed. This behavioral test was done in a room with artificial light (Lamp 100w), and acoustic and thermal insulation. Each animal was submitted only once to this test, being put in the

center of the maze facing one of the closed arms. Their behavior was recorded for five min, and the film was viewed and assessed by three trained investigators who did not know, which treatment the animal was subjected, (i.e., blind experiment) (Rocha et al. 2007).

The results were converted to: 1) Percentage of opened arm exploration time (%OAT); 2) Percentage of entries on opened arms (%EOA); 3) Number of entries on closed arms (ECA) (Cruz et al., 1994). Other two parameters were also assessed: number of times final edge of opened arms and number of times the animal walked towards the center of the maze, but did not enter another arm (Cruz et al. 1994; Rocha et al. 2007).

Blood Assessment

After the EPMT, animals were killed by decapitation using an appropriate guillotine (EB-271, Insight®, Brazil). Blood was collected in a tube immediately after decapitation, centrifuged, and the serum was stored at -20°C. Colorimetric kits were used to assess glucose, triacylglycerol, total cholesterol, High Density Lipoprotein (HDL) (Labtest Diagnóstica S.A., Brazil). Low Density Lipoprotein and Very Low Density Lipoprotein (LDL + VLDL) fraction was calculated through the equation: total cholesterol - HDL = LDL + VLDL. Corticosterone and insulin were determined using ELISA commercial kits (Arbor Assays and Millipore Corporation for corticosterone and insulin, respectively).

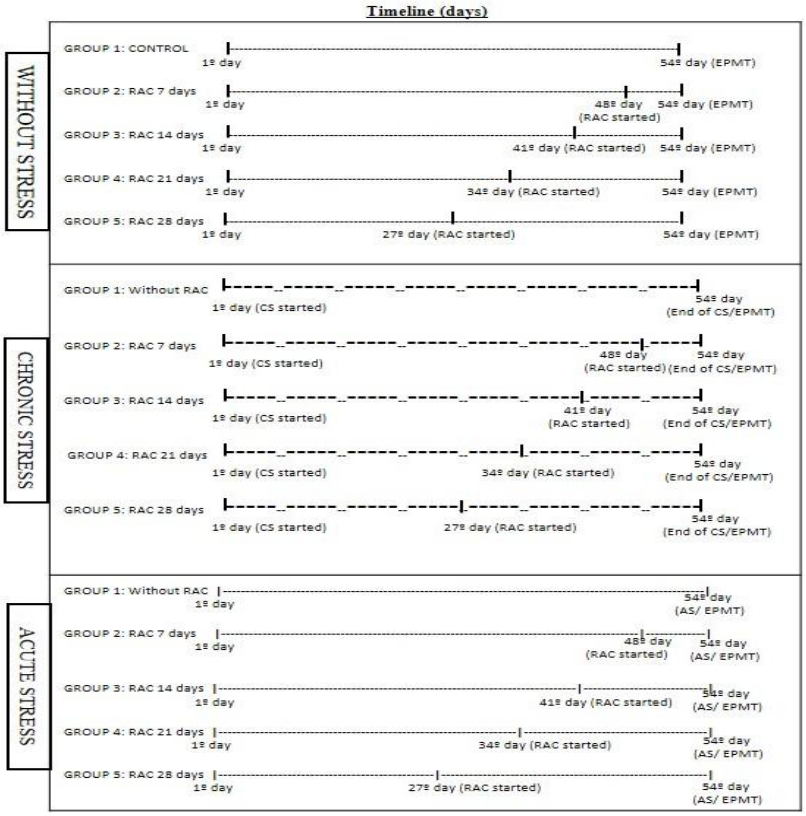

Figure 1 - Timeline of Ractopamine treatment procedures and exposure to stress. On the first day of the experiment all animals were approximately 67 days of age, on the 27th day were 94 days old, on the 34th had 101 days, on the 41th had 108, on the 48th had 115 days and on the 54º had 121 days. Legend: slim hatched= days without stress; Thick hatched= Chronic stress days; AS=Acute stress; CS= chronic stress; EPMT= Elevated plus-maze test; RAC= Ractopamine.

Carcass, Viscera and Muscle Assessments

The animals were dissected and their carcass, muscle, and viscera weights were evaluated. Viscera consisted of intestinal tract, spleen, heart, liver, and reproductive tract. Carcass consisted of dorsal portion, bones, tail, legs, forelimbs, and muscle. Besides the weight of whole carcass, *Gastrocnemius*, *Biceps femoris* and *Longissimus*

dorsi muscles were also taken. The nitrogen content of the carcass was determined using the Kjedahl method according to the Association of Official Analytical Chemists - AOAC (1990). Ether extract concentration was measured according Silva and Queiroz (2002).

Western Blots for GLUT – 4

Quantification of the GLUT-4 protein was performed via Western blots from total protein extract of epididymal adipose tissue of the rats (Malfitano et al. 2010). These analyses were performed on the tissues extracted from the animals of the control group and chronic stress group. The exclusion of the acute stress group was made because the time between the single stress session and the euthanasia (approximately 5 min) would not be sufficient to interfere in protein synthesis in adipose tissue.

Statistical Analysis

The statistical design was a completely randomized trial in a 3 x 5 factorial scheme (three levels of stress – without stress, acute stress, and chronic stress and five treatment periods with RAC – 0, 7, 14, 21, and 28 days). The analyses were performed on Statistical Analysis System - SAS Institute (1996). Data were submitted to normality test (Shapiro Wilk), and then ANOVA. When the F test was significant, comparison between the means was conducted by Student-Newman-Keuls test (SNK) and regression models. The coefficient of variation (CV) was calculated for the variables evaluated.

Behavior variables were performed using R 2.12.2 software (R Development Core Team, 2011). Deviance analysis was performed considering General Linear Models. Significant interactions were compared by different regression models. For those variables whose interaction between the stress and RAC days were significant, the common intercept model presented the best adjustment to the data. The level of significance was $p < 0.05$.

RESULTS

Low levels of RAC inclusion for increased periods in the animal diets did not interfere ($P>0.05$) on the performance (feed intake, weight gain, carcass yield), carcass characteristics (crude protein, ether extract, gastrocnemius, *Biceps femoris*, *Longissimus dorsi*) and blood parameters (glucose, triacylglycerol, cholesterol total, HDL, VLDL+LDL, Insulin, corticosterone). The interactions between the RAC treatment periods and different stress levels were not significant for those variables ($P>0.05$). Chronic stress promoted less weight gain (1.02 g/day) in the animals when compared to the controls (1.46 g/day) or those that were under acute stress (1.56 g/ day) ($P=0.00$). Visceral relative weight was not influenced by the RAC and there was no interaction with stress either ($P>0.05$). However, gastrointestinal tract (GIT – stomach and intestine) ($P=0.02$) and liver weights ($P=0.02$) were lower in the animals under acute stress (GIT=5.53%; liver =2.47%) in relation to unstressed (GIT=6.01%; liver =2.58%) or under chronic stress animals (GIT=5.98%; liver =2.60%) ($P<0.05$).

Biochemical blood parameters such as glucose, triacylglycerol, cholesterol, HDL, and VLDL+LDL fractions were not affected by the stress levels ($P>0.05$). However, it was observed that acute stress did affect the hormonal parameters. Animals in this group showed higher plasma corticosterone levels (358.3 ng/mL) when compared to those from the control (274.93 ng/mL) or chronic stress (278.1 ng/mL) groups ($P=0.00$).

The amount of GLUT-4 protein in epididymal adipose tissue was affected simultaneously by the RAC addition in the diet and by the chronic stress ($P<0.05$). Therefore, there was an interaction between these two factors ($P<0.05$). Thus, the results demonstrated a higher amount of GLUT-4 in the animals that received RAC for 14 and 28 days and submitted to chronic stress ($P<0.05$) (Table 1). These results indicated high GLUT-4 expression in adipose tissue of stressed animals compared to those not stressed in the referred periods. It was also observed that the amount of GLUT-4 was proportional to the RAC treatment in the stressed groups (i.e., the longer the RAC addition period, the higher the GLUT-4 concentration in adipose tissue of chronic stressed animals) (Figs. 2 A, B).

Regarding rat behavior, the EPMT indicated interaction among the different RAC administration periods and stress levels relative to the time spent on the opened arms ($P<0.05$) (Table 2). As the RAC administration periods were lengthened, the percentage of entries into open arms was not affected (Table 3). Conversely, stress increased the percentage of entries into opened arms (Fig. 3 A). It was also observed that the

animals under acute stress spent more time in opened arms (Fig. 3 B). On the other hand, the animals that were not submitted to stress had less entries and spent less time in these arms proportionally to the increase in RAC consumption intervals (Fig. 3 B). Thus, RAC supply decreased anxiety behavior in the stressed rats and increased anxiety behavior in unstressed rats.

Table 1 - GLUT-4 protein quantification in adipose tissue of rats (n=5) receiving rations with 5 ppm ractopamine and submitted to chronic stress.

Stress	***Ractopamine treatment period (days)***				
	0	**7**	**14**	**21**	**28**
			- GLUT- 4 -		
Without	1.46	1.72	1.07**a**	1.41	1.37**a**
Chronic[1]	1.42	1.19	2.03**b**	1.59	2.35**b**
P value	0.01				
*CV (%)**	16.88				

[a,b] Means followed by different letter in a column differ by F test (P<0.05).
[1]Significant linear regression (P<0.05). Legend:*CV = coefficient of variation

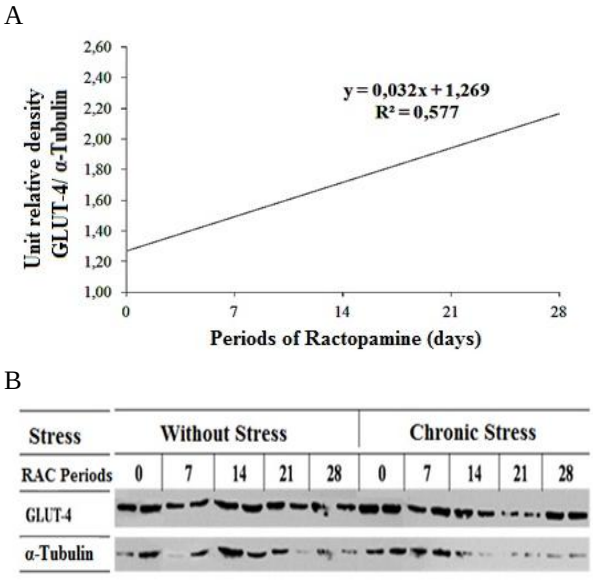

Figure 2 - Quantification of GLUT-4 protein in epididymal adipose tissue of rats receiving 5 ppmractopamine (RAC) and submitted to stress: **A -** variation in GLUT-4 amount in the different periods of treatment with RAC for animals submitted to chronic stress**. B -** Western blotting representative autoradiogram for GLUT-4 and α-tubulin.

Table 2 - Behavior parameters in the elevated-plus maze of rats (n=5) receiving rations with 5 ppm ractopamine and submitted to different levels of stress.

Stress level	***Ractopamine period (days)***				
	0	**7**	**14**	**21**	**28**
		- % of time spent in opened arms [1] *-*			
Without stress	6.1	13.2	7.5	0.8	24.7
Chronic stress	10.6	8.7	5.9	12.3	4.0
Acute stress	3.1	4.7	7.5	0.0	2.8
P value	0.04				

[1]Significant interaction by Deviance analysis (P<0.05). Regression model adjusted: common intercept.

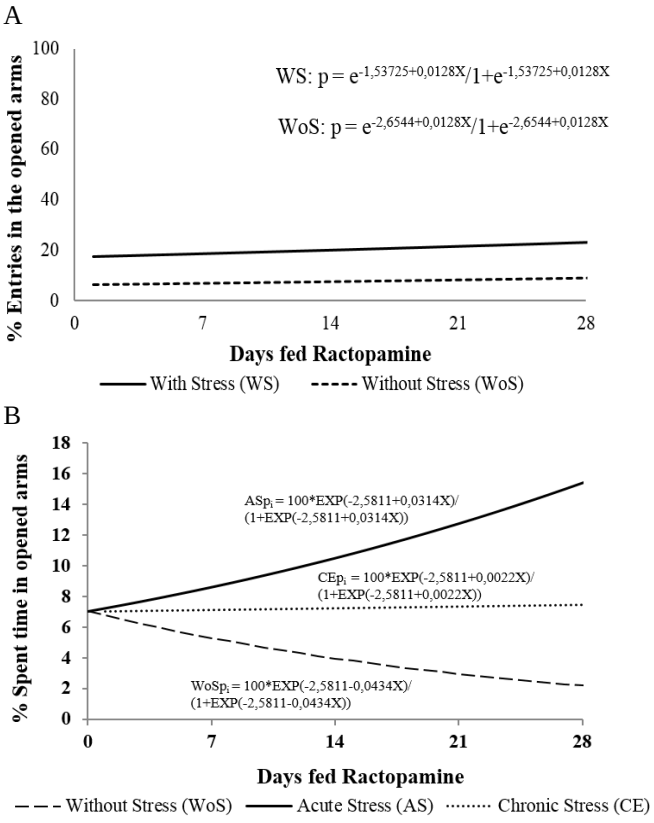

Figure 3 - Elevated-plus maze behavior according to different periods feed additive ractopamine for non-stressed, acute stress and chronic stress groups. a) Variations of entry percentage in opened arms; acute and chronic groups adjusted to a single equation by Deviance analysis;b) Variations of time spent in opened arms.

The number of entries into the closed arms was not influenced by the RAC periods in the diets (P>0.05), but by the stress (P<0.05). Thus, those animals submitted to stress situations (acute or chronic) entered into closed arms less often. Other behavioral variables, such as the number of times

animals reached the center of the maze without crossing it (P=0.93), and the number of times they reached the final edge of opened arms (P=0.43), were not affected by the tested treatments (P>0.05) (Table 3).

Table 3 - Behavior parameters of rats (n=5) receiving rations with 5 ppm ractopamine and submitted to different levels of stress, in the elevated-plus maze (EPM)*.

Parameters	*Ractopamine period (days)*[1]					*Stress level*			
	0	**7**	**14**	**21**	**28**	**NS**	**AS**	**CS**	
				- Locomotion on EPM -					*P value*
% of entries OA	14.89	15.27	13.70	17.46	15.63	9.64**a**	18.00**b**	18.53**b**	0.04
Number of entries CA	4.47	3.33	5.60	3.80	4.53	5.64 **b**	3.80 **a**	3.60 **a**	0.03

[1] Non-significant by F test, by Deviance analysis (P>0.05).
[a,b] Means followed by different letters ina row differ by Wald X^2 test (P<0.05). Legend: NS = animals from non-stressed group; AC = animals form acute stress group; CS = animals from chronic stress group; OA = opened arms; CA = closed arms. *As there was no interaction results are arranged to show only the averages of each group related to stress and ractopamine separately.

DISCUSSION

Oral administration was adopted in this study in order to extrapolate the results to swine production since parenteral administration in the pigs is impracticable. Despite the dose in the present study being considered low, the evaluation of the cumulative effects of increasing administration periods was a primary aim. The 5 ppm dosage was based on the previous work (Ferreira et al. 2011) with the pigs, where authors estimated the optimal value for the RAC treatment between 4.09 and 5.14 ppm, promoting a 6.09% increase in the bonus index, an economic variable related to benefits to the producers for leaner carcasses.

Exposure to stress promoted significant changes in the animal growth and relative viscera's weights. The low weight gain observed in the chronic stressed animals agreed with a study conducted by Ricart-Jané et al. (2002). The authors reported that stress induced a decrease in feed-intake, resulting in decreased rat growth. However, in this study the animals under the stress ingested the same amount of food, suggesting that the weight gain reduction was due to other metabolic processes. According to McEwen (2000), high catecholamine and glucocorticoid levels tend to mobilize and re-establish energy reserves needed by the brain and challenged body functions.

In contrast from previously discussed variables, the GLUT-4 concentration in epididymal adipose tissue of the animals changed due to RAC administration and concomitant stress treatments. These findings indicated some interference of stress on RAC activity related to fat metabolism. Other studies have shown that BAA use could reduce adipose tissue and increase fat cell apoptosis (Eadara et al. 1989). Further, in pigs, RAC effects were observed in the AMPc pathway genes, such as reduction of FAS and GLUT-4 in adipose tissue (Halsey et al. 2011). However, in this study, probably due to the stress, an opposite effect was observed related to that expected from the RAC, suggesting increased lipogenesis. Probably the increase in epinephrine secretion during stress might have promoted a reduction of RAC binding in adrenergic receptors, promoting a higher GLUT-4 concentration in those tissues.

Plasma insulin concentrations were not changed in this study. Some agonists, such as clenbuterol and cimaterol did not affect insulin levels too (Eadara et al. 1989). Another hormone level, corticosterone, was not changed by the RAC in this study, as reported for the cimaterol previously (Eadara et al. 1989). These results were also observed in the pigs treated with RAC, in which plasma cortisol did not differ among the treatments (Marchant-Forde et al. 2003). However, this hormone levels were different according to stress protocols, as observed previously (Gameiro et al. 2006). Despite stress induction being a potential cause of changes in some blood parameters (Ricart-Jané et al. 2002), no changes were observed in glucose, triglycerol, cholesterol and its fractions, HDL and VLDL + LDL levels. The absence of different responses to acute stress corroborate with previous findings (Ricart-Jané et al. 2002), in which stress was not enough to change lipoprotein parameters such as cholesterol and HDL either.

The longer the animal stay in open arms and the larger the number of entries in these arms, their anxiety would be lower (Cruz et al. 1994). On the other hand, the larger the number of entries in both

the arms (open and closed), the greater would be their locomotor activity (Cruz et al. 1994; Rocha et al. 2007). The EPMT of this study pointed to the changes in rat response due to an interaction between the RAC and stress, as increased entries and time spent in open arms. This change was likely linked to brain monoamine profiling of a deficient serotonergic signaling, indicated by reduced serotonin concentrations in the raphe nuclei and frontal cortex, reduced 5-hydroxyindoleacetic acid concentrations in the amygdale, and an enhanced dopamine release ratio in the amygdale (Poletto et al. 2010).

Gorman and Dunn (1993) reported that the animals submitted to restraint stress remained less time inside the chambers (galvanized cylindrical tubes) and spent more time in open areas, presenting a reduction in defense behavior related to the environment. This finding corroborated with the present study, in which the higher percentage of entries into opened arms and longer periods of time spent in them also suggested a less defensive behavior and increasing expansive behavior when submitted to stress. Poletto et al. (2010) suggested an increase in aggressive behavior of the pigs receiving RAC, especially gilts, which were subjected to an intruder pig (unfamiliar) test, which was a stressor. According to Marchant-Forde et al. (2003), pigs fed RAC and subjected to stressful situations, such as handling and transport, showed higher activity in the first weeks of the trial.

However, no interaction effects between the RAC and stress were observed in those animals under chronic stress regarding time in open arms. Animals from this group responded to stress, but not to different RAC periods. According to Nomura et al. (1981), induced chronic stress in the rats increased intrasynaptic levels of norepinephrine, and could promote a sub-sensitivity of β-adrenergic receptors in the brain cortex due to its decreased concentration. According to Marchant-Forde et al. (2003), concentration of catecholamines were greater at the end of a RAC feeding trial in the pigs fed the compound at a constant dose of 10 mg/kg of diet for 4 week. This has importance on this study because the increase of endogenous catecholamines, induced by the stress, could be responsible for the neutralization of RAC effects in stressed animals.

CONCLUSION

RAC periods associated with the chronic stress interfered in a period-dependent manner in GLUT-4 protein expression in adipose tissue. RAC administration combined with stress increased maze exploration in a period-dependent manner, causing an opposite effect observed when RAC was supplied to unstressed animals. These results suggested that RAC influenced the effects of stress in the rats. When extrapolating animal production, the stress could negatively affect the possible benefits offered by the RAC, mainly impairing the adipose tissue metabolism and behavior in the animals.

REFERENCES

Association of Official Analytical Chemists. Official methods of analysis. 15th ed. Arlington; 1990.

Buynitsky T, Mostofsky DI. Restraint stress in biobehavioral research: recent developments. *Neurosci Biobehav R*. 2009; 33: 1089-1098.

Cruz APM, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacol Biochem Behav.* 1994; 49:171-176.

Eadara JK, Dalrymple RH, Delay RL, Ricks CA, Romsos DR. Effects of cimaterol, a β-Adrenergic agonist, on protein metabolism in rats. *Metabolis.* 1989; 38: 883-890.

Ferreira MSS, Sousa RV, Silva VO, Zangeronimo MG, Amaral NO. Cloridrato de ractopamina em dietas para suínos em terminação. *Acta Sci Anim Sci*. 2011; 33: 25-32. (in Portuguese).

Flicker C, Geyer MA. Behavior during hippocampal microinfusions: I., norepinephrine and diversive exploration. *Brain Res*. 1982; 4: 79-103.

Gameiro GH, Andrade AS, Castro M, Pereira LF, Tambelli CH, Veiga MC. Effects of restraint stress on nociceptive responses induced by formalin injected in rat's TMJ. *Pharmacology*. 2005; 82: 338-344.

Gameiro GH, Gameiro PH, Andrade AS, Pereira LF, Arthuri MT, Marcondes FK, et. al. Nociception- and anxiety-like behavior in rats submitted to different periods of restraint stress. *Physiol Behav.* 2006; 87:643-649.

Gorman AL, Dunn AJ. Beta-adrenergic receptors are involved in stress-related behavioral changes. *Pharmacol Biochem Behav*. 1993; 45:1-7.

Halsey CHC, Weber PS, Reiter SS, Stronach BN, Bartosh JL, Bergen WG. The effect of ractopamine hydrochloride on gene expression in adipose tissues of finishing pigs. *J Anim Sci*. 2011; 89: 1011-1019.

Liu CY, Grant AL, Kim KH, Ji SQ, Hancock DL, Anderson DB, et al. Limitations of ractopamine to affect adipose tissue metabolism in swine. *J Anim Sci*. 1994; 72: 62-67.

Malfitano C, Loureiro TCA, Rodrigues B, Sirvente R, Salemi VMC, Rabechi NB, et al. Hyperglycaemia protects the heart after myocardial infarction: aspects of programmed cell survival and cell death. *Eur J Heart Fail*. 2010; 12: 659-667.

Marchant-Forde J.N, Lay DCJR, Pajor EA, Richert BT, Schinckel AP. The effects of ractopamine on the behavior and physiology of finishing pigs. *J Anim Sci.* 2003; 81: 416-422.

Mcewen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.* 2000; 886: 172-189.

Mitchell AD, Solomon MB, Steele NC. Influence of level of dietary protein or energy on effects of ractopamine in finishing swine. *J Anim Sci*. 1991; 69: 4487-4495.

Nomura S, Watanabe M, Ukei N, Nakazawa T. Stress and β-adrenergic receptor binding in the rat's brain. *Brain Res*. 1981; 224: 199-203.

Poletto R, Cheng HW, Meisel RL Garner JP, Richert BT, Marchant-Forde JN. Aggressiveness and brain amine concentration in dominant and subordinate finishing pigs fed the beta-adrenoreceptor agonist ractopamine. *J Anim Sci*. 2010; 88: 3107-3120.

Reeves PG, Nielsen FH, Fahey Junior GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *The J Nut.* 1993; 123: 1939-1951.

Ricart-Jané D, Rodriguez-Sureda V, Benavides A, Peinado-Onsurbe J, López-Tejero MD, Llobera M. Immobilization stress alters intermediate metabolism and circulating lipoproteins in the rat. *Metabolis*. 2002; 51: 925-931.

Rocha VM, Calil CM, Ferreira R, Moura MJ, Marcondes FK. Influence of anabolic steroid on anxiety levels in sedentary male rats. *Stress.* 2007; 10: 326-331.

Silva DJ, Queiroz AC. Análises de alimentos: métodos químicos e biológicos. Viçosa: UFV; 2002. (in Portuguese)

Smith DJ. The pharmacokinetics, metabolism, and tissue residues of beta-adrenergic agonists in livestock. *J Anim Sci*. 1998; 76: 173-194.

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