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Stress susceptibility in pigs supplemented with ractopamine[1]

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ABSTRACT: Ractopamine is a β-adrenergic agonist used as an energy repartitioning agent in the diets of finishing pigs. Most ractopamine studies are limited to evaluations of growth performance and meat quality, and there is little information on the effects of this additive on the behavior and welfare of pigs. Therefore, the objective of this study was to evaluate various indicators of stress caused by feeding diets containing ractopamine. One hundred seventy barrows and 170 gilts weighing 107.3 kg were allocated to 30 pens with 10 to 12 barrows or gilts per pen. Pigs were offered 1 of the 3 dietary treatments (0, 5, or 10 mg ractopamine/kg) for 28 d with 5 barrow pens and 5 gilt pens per treatment. Pigs were evaluated for behavior 3 d per week 1 wk before the initiation of the experiment and throughout the experiment. Each pig was classified into 1 of the 13 activities (drinking water, lying alone, lying in clusters, standing, nosing pig, sitting, feeding, biting pig, walking, exploring, running away, playing, and mounting pen mates) and also grouped into 1 of the 3 categories (calm, moving, and feeding themselves) based on those activities. At the end of the experiment, 3 pigs from each pen were slaughtered, and blood samples were collected during exsanguination to determine physiological indicators of stress (cortisol, lactate, and creatine-kinase enzymes). The incidence of skin and carcass lesions was determined at shoulder, loin, and ham. Ractopamine had no effect ($P > 0.05$) on pig behavior, total number of skin and carcass lesions, or blood concentrations of cortisol or lactate. However, there was an increase ($P < 0.05$) of creatine kinase concentrations in pigs receiving ractopamine-supplemented feed. This finding is consistent with the concept that ractopamine may cause muscular disorders, and this warrants further investigation.

Key words: behavior, beta-adrenergic agonist, creatine phosphokinase, cortisol, lactate, skin lesions

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INTRODUCTION

Ractopamine, a β-adrenergic agonist used as an energy repartitioning agent, has been widely studied in swine (Watkins et al., 1990; Sanches et al., 2010), sheep (Baker et al., 1984) and cattle (Ricks et al., 1984). Most studies conducted with swine are restricted to evaluations of growth performance (Sanches et al., 2010), carcass characteristics (Cantarelli et al., 2009), and meat quality (Patience et al., 2009). There is little information in the literature showing the effects of this feed additive on pig behavior and welfare (Schaefer et al., 1992; Marchant-Forde et al., 2003).

According to Moberg (2000), stress threatens homeostasis and animals respond with physiological or behavioral adjustments needed for adaptation to the adverse situation. This adaptation involves neuroendocrine and behavioral responses that aim to keep the balance of vital functions (Von-Borell, 1995).

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Table 1. Dietary composition (as-fed basis)

	Ractopamine, mg/kg		
Item	0	5	10
Ingredient, %			
Corn	65.24	65.22	65.19
Soybean meal	29.60	29.60	29.60
Soybean oil	2.30	2.30	2.30
Vitamin and trace mineral premix[1]	1.50	1.50	1.50
Dicalcium phosphate	0.80	0.80	0.80
Limestone	0.49	0.49	0.49
L-Lys · HCl	0.07	0.07	0.07
Ractopamine[2]	0.00	0.025	0.05
Total	100.00	100.00	100.00
Calculated composition[3]			
Ca, %	0.47	0.47	0.47
ME, kcal/kg	3306	3306	3305
CP, %	18.87	18.86	18.86
STTD P, %	0.22	0.22	0.22
SID Lys, %	0.94	0.94	0.94
SID Met + Cys:Lys	0.55	0.55	0.55
SID Thr:Lys	0.63	0.63	0.63
SID Trp:Lys	0.20	0.20	0.20
Ractopamine, mg/kg	0.00	0.025	0.05

[1]Provided per kilogram of complete diet: 60 mg of Mn ($MnSO_4.H_2O$); 150 mg of Zn (ZnO); 96 mg of Fe ($FeSO_4$.7H2O); 160 mg of Cu ($CuSO_4.5H_2O$); 1.20 mg of I (calcium iodate); 0.25 mg of Se (Na_2SeO_3); 4765 IU of vitamin A (trans-retinyl acetate); 955 IU of vitamin D_3 (cholecalciferol); 16.4 mg of vitamin E (all-rac-tocopherol); 1.00 mg of vitamin K (bisulphate menadione complex); 1.00 mg of thiamine; 3.28 mg of vitamin B_2 (riboflavin); 1.00 mg vitamin of B_6 (pyridoxal phosphate); 12.34 µg of vitamin B_{12} (cyanocobalamin); 0.31 mg of folic acid (folate); 13.08 mg of pantothenic acid (D-Ca pantothenate); 16.8 mg of niacin (nicotinic acid); and 35.0 mg of biotin.

[2]Paylean (Elanco Animal Health, Speke, UK).

[3]According to Rostagno et al. (2000); SID = standardized ileal digestible; and STTD = standardized total digestible.

Research conducted by Schaefer et al. (1992) shows that there is little effect of ractopamine on the behavior of finishing pigs as no abnormal, stereotypic, or aggressive behaviors were observed. However, Marchant-Forde et al. (2003) assessed ractopamine effects on pig behaviors observed during handling and transport and concluded that ractopamine affected the behavior, heart rate, and concentrations of catecholamine of the finishing pigs, making them more difficult to handle. The present study was conducted to evaluate various indicators of stress in barrows and gilts fed ractopamine in commercial production conditions.

MATERIALS AND METHODS

The experiment was conducted according to the ethical principles of animal experimentation established by the Committee of Ethics on Animal Use of the Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu/SP, Brazil (Protocol Number 64/2008).

Location, Animals, and Farm Management

The experiment was conducted in the winter at a commercial operation (Alto Bela Vista, SC, Brazil). Outside temperatures ranged from 8.8 to 20.5°C, with an average of 15°C. Average daily relative humidity and rainfall were 83.5% and 5.7 mm, respectively.

Three hundred forty crossbred finishing pigs (Camborough 25 × AGPIC 337; 170 barrows and 170 gilts) with an average BW of 107.3 kg were used and distributed in 30 pens. Pens were 3.40 m wide and 3.65 m long with solid masonry walls, concrete floors, and a nipple drinker and linear trough situated parallel to the center aisle. Bedding was not used. Manure was handled by a shallow gutter in the rear of the pens. Ten to 12 pigs were kept in single-sex pens (1.03 to 1.24 m^2/pig), and this variation in the number of pigs per pen was balanced across treatments.

The average BW was calculated for each pen after all pigs were weighed and identified using ear tags. Pigs were assigned to treatments based on BW with 10 pens per treatment (5 with barrows and 5 with gilts for each treatment), and pigs with average light, medium, and heavy BW were included in in all treatments.

Feeding

Ractopamine-supplemented diets (5 and 10 mg/kg; Paylean; Elanco Animal Health, Speke, UK) were provided for 28 d in a controlled manner and was divided into 3 daily portions. The control group received the same diet as the other group but without ractopamine. Experimental diets were formulated to meet or exceed the published requirements by Rostagno et al. (2000) and are shown in Table 1.

Preslaughter Handling

Pigs were weighed and subjected to a 4-h fast. Before shipping, 30 pigs from each treatment (the lightest, the medium, and the heaviest in each pen) received another ear tag to distinguish those pigs from others at the abattoir. Pigs were transported to a slaughterhouse (Chapecó, SC, Brazil). The handling and loading of pigs required approximately 31 min per truck, and was accomplished by 5 technicians using sorting boards to direct pigs. Pigs were transported in separated compartments in 4 double-deck trucks each with capacity of 85 pigs. The loading of pigs occurred 1 after the other on the same day, and all trucks contained pigs from all treatments, which were kept separated in the truck. The commercial facility was located approximately 134 km from the slaughterhouse with 8 km of nonpaved road and 126 km of paved road. The total time of transport averaged 3 h and 57 min.

After arrival at the slaughterhouse, the pigs were kept in lairage pens for 6 h before stunning. The total fast period, from the commercial facility to the slaughterhouse, was 14 h. Pigs had no access to feed, but had ad libitum access to water both at the facility and the slaughterhouse. A 5-m long conveyor belt restrained pigs by the sides until electrocuted (700V and 1.25 amp; Valhalla, Stork RMS b.v., Lichtenvoorde, the Netherlands). Pigs were immediately exsanguinated as they were laterally recumbent, shackled, and then hoisted onto the processing line. After evisceration, carcasses were maintained in a refrigerator at temperatures that ranged from 1 to 4°C for 24 h.

Stress Evaluation

Behavior and Welfare. Scan sampling (Altmann, 1974), with some adaptations, was used 1 wk before ractopamine addition and weekly (Monday, Wednesday, and Friday) throughout the experiment, totaling 15 d of evaluations to identify any changes in the pig activity because of dietary ractopamine. The observations were repeated 6 times on each of 3 d/wk for 5 wk. Observation times were 0900, 1000, 1100, 1300, 1400, and 1500 h. Each pig was classified in 1 of the 13 exclusive categories: drinking water, lying alone, lying in clusters, standing, nosing pig, sitting, feeding, biting pig, walking, exploring, running away, playing, and mounting pen mates.

Using the 13 described behaviors, these items were calculated: the number of quiet pigs (the sum of the number of animals lying in clusters, lying alone, standing and sitting), number of pigs moving (nosing pig, biting pig, walking, exploring, running away, playing, and mounting), and the number of pigs feeding themselves (eating feed and drinking water). Then, the percentage of pigs in each category was calculated for each pen.

Skin and Carcass Lesions. The incidence of skin and carcass lesions was evaluated at 3 locations (shoulder, loin, and ham) on the left side of the alive and slaughtered pig, respectively. The skin lesions evaluation was done before the loading, after unloading, and during the lairage period at the slaughterhouse. Twenty-four hours after slaughter, the number of lesions on the left side of the carcass was recorded to determine frequency of carcass lesions.

Physiological Measures. Physiological measurements of cortisol, lactate, and creatine-kinase (**CK**) were assessed in blood collected during exsanguinations. Blood samples (10 mL) were collected from a jugular vein using a disposable plastic tube and transferred to 2 centrifuge tubes. One of the tubes contained 10 drops of heparin (25,000 IU/5 mL) to collect plasma and the other had no heparin, allowing collection of serum. All samples were gently mixed and were centrifuged at 1,500 × *g* for 10 min at room temperature using a portable centrifuge (Excelsa Baby II-206-BL, Fanem, São Paulo, SP, Brazil). After centrifugation, 2-mL aliquots of plasma and serum were transferred to separate cryogenic tubes and stored at –80°C until analyses were conducted.

Plasma cortisol was determined using a competitive binding RIA kit (Coat-A-Count Cortisol Kit; DPC-Diagnostic Products Corporation, Los Angeles, CA), as validated previously (Haussmann et al., 2000). All samples were done in a single batch, and the intraassay CV was 15.74%. Plasma samples were analyzed for lactate (Lactate Kit; Katal Biotecnológica Ind. Com. Ltd., Minas Gerais, MG, Brazil), and all samples were assayed in a single batch with an intra-assay CV of 13.71%. Serum samples were used for CK analyses [CK-total and CK-muscle and brain type subunits (**CK-MB**) Kit; Katal Biotecnológica Ind. Com. Ltd.]. All samples for CK were assayed in a single batch; the CK-MB intra-assay CV was 5.13%, and CK-total intra-assay CV was 11.11%. The concentration of lactate and enzymatic activity of CK were measured by wavelength (546 nm and 340 nm, respectively), using a spectrophotometer (RA-XT; Technicon Instrument Corp., Tarrytown, NY).

Statistical Analysis

Data were analyzed as a randomized block design (blocked by BW) with a 2 × 3 factorial arrangement of sex (barrows and gilts) and ractopamine (0, 5, and 10 mg/kg) using the pen as the experimental unit. The PROC GLM procedure (SAS Inst. Inc., Cary, NC) was used with an ANOVA model that included sex, ractopamine, and sex × ractopamine as possible sources of variation. For the pig behavior data, observation time or hour (0900, 1000, 1100, 1300, 1400, and 1500 h) was also included in the model. Least squares means were separated using PDIFF when F-test was significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

Behavior and Welfare

Ractopamine addition to the diet had no effect on the 13 evaluated behaviors when animals were grouped into calm, moving, or feeding themselves categories (Table 2). This same table illustrates the dynamics of the routine of the pigs during the experiment based on 6 daily evaluations. It was found that the behavior of the animals occurred as a function of the feed provision times. Most of the pigs were calmer (71.13%) until the next feeding. This was observed mainly in the evaluations from 0900 to 1100 h and at 1500 h, and this behavior was less intense from 1300 to 1400 h. At the time of the second feeding (1300 h), it was observed that all the pigs stood up and went toward the feeders. Soon after the feed intake, most of the animals drank water. Also, it is observed that the movement of the animals was less frequent (14.86%) at 1000 h.

Table 2. Dietary ractopamine (RAC) supplementation and pig behavior categories (percentage of calm, moving, and feeding)[1]

Pig behavior	RAC, mg/kg 0	5	10	Average	SEM	P-value, RAC × hour
	——— % ———					
Calm						0.57
0900 h	77.10	75.36	77.15	76.54[ab]	1.00	
1000 h	78.92	81.16	79.05	79.71[a]	0.67	
1100 h	80.89	76.98	80.30	79.39[a]	1.08	
1300 h	36.54	38.47	33.65	36.22[c]	2.08	
1400 h	76.21	72.90	77.15	75.42[b]	1.19	
1500 h	78.60	77.89	82.07	79.52[a]	1.06	
Average	71.38	70.46	71.56			
SEM	1.13	1.23	1.10			
Moving						0.65
0900 h	17.19	17.52	18.36	17.69[c]	0.72	
1000 h	15.26	14.46	14.86	14.86[d]	0.85	
1100 h	16.96	19.52	17.82	18.10[bc]	0.94	
1300 h	18.96	22.45	21.67	21.03[a]	1.18	
1400 h	20.59	22.99	18.77	20.79[ab]	1.04	
1500 h	19.98	20.42	16.13	18.85[abc]	1.02	
Average	18.16	19.56	17.94			
SEM	0.67	0.78	0.72			
Feeding themselves						0.67
0900 h	5.71	7.12	4.48	5.77[b]	0.55	
1000 h	5.82	4.38	6.09	5.43[bc]	0.48	
1100 h	2.14	3.50	1.88	2.51[cd]	0.37	
1300 h	44.50	39.07	44.68	42.75[a]	2.22	
1400 h	3.20	4.11	4.08	3.80[bcd]	0.39	
1500 h	1.42	1.68	1.80	1.63[d]	0.19	
Average	10.46	9.98	10.50			
SEM	0.34	0.35	0.34			

[a–d]Averages followed by distinct letter within a column (evaluation time) differ ($P < 0.05$).

[1]Based on 5 barrow pens and 5 gilt pens/treatment. Ractopamine = Paylean; Elanco Animal Health, Speke, UK.

Among the 13 evaluated behaviors, only 4 (standing, nosing pig, playing, and drinking water) were influenced ($P < 0.05$) by ractopamine supplementation in the diet (Table 3). The percentage of pigs nosing pig increased when 5 mg/kg of ractopamine was added. However, Schaefer et al. (1992) did not find a difference of this variable in pigs that received ractopamine in feed. The inclusion of 5 mg/kg of ractopamine in the diet reduced ($P < 0.04$) the average percentage of pigs playing (0.06%) when compared with the ones that did not receive ractopamine-supplemented diet (0.26%). There was a difference ($P < 0.05$) in the percentage of pigs drinking water only between the control group pigs (1.58%) and the ones that received 5 mg/kg of ractopamine in the feed (2.06%). This information suggests that a small effect of ractopamine was found on the animal behavior.

Schaefer et al. (1992) also found similar effects on pig behavior using ractopamine supplementation levels of 0, 10, 15, and 20 mg/kg. Those authors reported that, after 6-mo ractopamine supplementation, the pigs started spending more time lying in clusters and less time walking. However, they did not observe abnormal, stereotypic, agonistic, or aggressive behavior, and suggested that ractopamine does not cause substantial changes in the behavior of the pigs. However, Marchant-Forde et al. (2003) found results that contradicted the finding in the present study. They evaluated ractopamine effects (0 and 10 mg/kg) on the behavior and physiology of pigs during handling and transport, and observed that the pigs that received ractopamine spent more time being active, were more alert, and spent less time in side position. Moreover, the authors concluded that ractopamine affects behaviors because after the fourth week, the pigs became more difficult to handle. According to Stella (2007), breed, sex, and ractopamine level can interact and alter pig behavior in an open field test. The beneficial effects of a constant level of ractopamine addition in the diet on pig production are minimal after the fifth week of supplementation (Schinckel et al., 2002a, 2002b). Thus, Marchant-Forde et al. (2003) suggested that a lack of behavioral effects detected in that study could have been due to the long period (6 mo) of ractopamine supplementation.

Skin and Carcass Lesions

Lesions located on the skin surface and detected in carcasses after slaughter may interfere in carcass classification and, consequently, decrease its value. There was no interaction ($P < 0.05$) among the ractopamine levels and the gender condition for the assessed variables (Table 4). Moreover, there was no difference ($P < 0.05$) in the total number of evaluated skin and carcass lesions in ractopamine-supplemented pigs when compared with the control group. However, there were several studies that observed an increase in the number of skin and carcass lesions of animals from different species because of stressful situations. Gallo et al. (1998) found a greater number of lesions in cattle transported continuously for 36 h. Barton Gade et al. (1996) stated that there is a correlation between carcass lesion scores and inadequate handling procedures in pig carcasses. Dalla Costa (2005) evaluated the presence of skin and carcass lesions before the loading on the farm (56.8%), during loading (78.1%), unloading (89.1%), and lairage at the slaughterhouse (95.8%).

Physiological Measures

The concentrations of lactate, cortisol, and creatine phosphokinase (total and MB fraction) are presented in Table 5. There was no effect of ractopamine × gender for any of the evaluated physiological measures.

Table 3. Dietery ractopamine (RAC) supplementation and pig behaviors[1]

Pig behavior	RAC, mg/kg 0	5	10	Average	SEM	*P*-value, RAC × hour
	%					
Standing						0.18
0900 h	1.32	1.72	2.86	1.97[a]	0.27	
1000 h	0.63	0.91	1.13	0.89[bc]	0.16	
1100 h	0.69	0.46	0.90	0.68[c]	0.19	
1300 h	1.05	0.54	2.10	1.23[b]	0.14	
1400 h	0.69	0.58	1.02	0.76[bc]	0.19	
1500 h	0.73	0.46	0.35	0.51[c]	0.10	
Average	0.85[y]	0.78[y]	1.39[x]			
SEM	0.10	0.14	0.13			
Nosing pig						0.79
0900 h	6.79	7.26	8.15	7.40[a]	0.33	
1000 h	5.27	5.67	5.26	5.40[cd]	0.45	
1100 h	5.93	7.31	5.88	6.37[abc]	0.46	
1300 h	3.97	5.80	4.65	4.81[d]	0.39	
1400 h	6.25	7.38	6.47	6.70[ab]	0.42	
1500 h	5.51	6.97	4.91	5.80[bcd]	0.39	
Average	5.62[y]	6.73[x]	5.89[y]			
SEM	0.53	0.33	0.63			
Playing						0.10
0900 h	0.48	0.12	0.12	0.24[ab]	0.09	
1000 h	0.00	0.00	0.11	0.04[b]	0.00	
1100 h	0.23	0.00	0.00	0.08[b]	0.00	
1300 h	0.55	0.00	0.40	0.32[a]	0.00	
1400 h	0.31	0.00	0.46	0.26[ab]	0.00	
1500 h	0.00	0.24	0.00	0.08[b]	0.00	
Average	0.26[x]	0.06[y]	0.18[xy]			
SEM	0.00	0.00	0.00			
Drinking water						0.68
0900 h	1.27	1.40	1.19	1.29[b]	0.15	
1000 h	1.70	1.65	1.72	1.69[b]	0.21	
1100 h	1.16	1.62	1.31	1.36[b]	0.21	
1300 h	2.03	3.20	3.44	2.89[a]	0.25	
1400 h	1.91	2.82	2.17	2.30[a]	0.24	
1500 h	1.42	1.68	1.80	1.63[b]	0.19	
Average	1.58[y]	2.06[x]	1.94[xy]			
SEM	0.13	0.20	0.20			

[a–d]Averages followed by distinct letter within a column (evaluation time) differ ($P < 0.05$).

[x,y]Averages followed by distinct letter within a row (RAC) differ ($P < 0.05$).

[1]Based on 5 barrow pens and 5 gilt pens/treatment. Ractopamine = Paylean; Elanco Animal Health, Speke, UK.

Table 4. Dietery ractopamine (RAC) supplementation and skin and carcass lesions evaluated at shoulder, loin, and ham regions[1]

Lesion type	RAC, mg/kg 0	5	10	SEM	Sex Gilt	Barrow	SEM	*P*-value, RAC × sex
	%							
Skin lesion[2]								
Shoulder	2.47	2.80	3.53	0.52	2.62	3.24	0.73	0.19
Loin	2.02	1.82	2.00	0.51	1.21	2.68	0.38	0.12
Ham	1.95	1.48	1.73	0.47	1.30	2.14	0.44	0.24
Carcass lesions								
Shoulder	2.10	1.93	3.03	0.31	2.24	2.47	0.67	0.62
Loin	4.83	5.28	5.37	0.63	4.66	5.67	0.44	0.38
Ham	3.07	3.33	3.00	0.36	2.78	3.49	0.42	0.32

[1]Based on 5 barrow pens and 5 gilt pens/treatment with 3 pigs per pen. Ractopamine = Paylean; Elanco Animal Health, Speke, UK.

[2]Average of evaluations before the loading, after unloading, and during the lairage period at the slaughter house.

In stress situations, there can be intense degradation of muscle glycogen, forming great quantities of lactic acid that is released into the bloodstream (Shaw and Tume, 1992). In the present study, the evaluated ractopamine levels did not have an effect on this physiological measure (average of 40.29 mmol/L). However, Warriss et al. (1990) found an increase in the levels of lactate for pigs that received Salbutamol, another β-adrenergic agonist used in pig feed. Several studies have also shown an increase of lactate concentration because of stressful situations, such as pigs slaughtered under conditions of stress (Warriss et al., 1998); pigs that had high skin lesions scores (Gispert et al., 2000); pigs with extensive transport time (Warriss et al., 1998; Pèrez et al., 2002); and pigs with electrical stunning (Ludtke et al., 2006).

Pigs have increased plasma concentrations of cortisol in response to psychological stress, allowing the fight or escape reaction (Moberg, 2000). There are several published studies in which animals were submitted to stressful situations during preslaughter management, and as a consequence, displayed an increase in circulating cortisol. Grandin (1994) observed that cortisol values may double or quadruple in swine under situations of extreme stress. Shaw and Trout (1995) evaluated the concentration of plasma cortisol in stressed pigs that

Table 5. Dietery ractopamine (RAC) supplementation and physiological measures[1]

Item	RAC, mg/kg 0	5	10	SEM	Sex Gilt	Barrow	SEM	*P*-value, RAC × sex
Lactate, mmol/L	40.32	40.77	39.78	2.18	39.17	41.41	1.97	0.14
Cortisol, µg/dL	6.35	8.02	7.66	0.47	7.76	6.93	0.49	0.15
Creatine phosphokinase, U/L								
CK-MB	2341[y]	4361[x]	4510[x]	132	3536	3938	408	0.77
CK-total	5811[y]	12,436[x]	10,707[x]	1234	10,392	8910	1348	0.88

[x,y]Averages followed by distinct letter within a row (RAC) differ ($P < 0.05$).

[1]Based on 5 barrow pens and 5 gilt pens/treatment with 3 pigs per pen. Ractopamine = Paylean; Elanco Animal Health, Speke, UK. CK-MB = creatine kinase-muscle and brain type subunits and CK-total = creatine kinase-total.

developed PSE meat, and found increased concentrations (16.63 μg/dL). Brown et al. (1998) established average values of 17.02 μg/dL of cortisol for pigs slaughtered under stressful conditions, and the values were around 7.62 μg/dL for those slaughtered under normal conditions. Ludtke et al. (2006) evaluated animal wellness in preslaughter handling and its influence on pork quality and concluded that pigs handled with electric prods had greater cortisol values, and average cortisol values in a handled group were also greater (16.32 ± 5.50 μg/dL) compared with groups managed with boards (10.76 ± 5.76 μg/dL). Averos et al. (2007) assessed physiological measures in pigs transported to the slaughterhouse in different seasons of the year and noticed that the cortisol concentration increased during the transport and increased during the lairage, with values of 3.47 ± 0.19, 8.52 ± 0.28, and 6.96 ± 0.18 μg/dL at loading, unloading, and exsanguination, respectively. Pigs managed with electrical prods during preslaughter had an increased cortisol concentration compared with resting animals, which indicates that these animals were under stress conditions (Santana et al., 2009).

However, in the present study, ractopamine treatment had no effect on cortisol concentrations after stunning or during exsanguination (average of 7.34 μg/dL; Table 5). This agrees with results found by Marchant-Forde et al. (2003) who evaluated finishing pigs that received the diet supplemented with ractopamine. Those authors evaluated the circulating cortisol concentration before and after pig transport, and concluded that there was no difference among the treatments either before or after the transportation. However, at the end of the fourth week of supplementation, they observed that the ractopamine-supplemented pigs had greater concentrations of circulating catecholamines, and ractopamine interfered with pig behavior, causing greater susceptibility to stress during transport.

In our study, CK-total and CK-MB isoenzyme concentrations doubled in animals fed ractopamine. Creatine kinase increase may occur because of several causes, such as an injection causing tissue irritation; seizure, in which the animal jerks itself and traumatizes skeletal muscles; and possible enzymatic induction by drug uses (Scheffer and González, 2003). Pigs that received salbutamol in the diet also had increased CK concentrations (Warriss et al., 1990). The variation of tissue enzymatic activity is normally associated with an increase of enzyme synthesis in that tissue, a reduction in catabolism, or cell proliferation (Gella, 1994). This can occur because of physiological, pathological, or therapeutic causes (Scheffer and González, 2003). After exercise, there was an increase of the total Creatine Phosphokinase (**CK**), CK-MM, and CK-BB (Isoenzyme CK-MM is found in the skeletal and cardiac muscles, CK-BB is found in the Brain and CK-MB is an isoensyme found mainly in the heart. The enzyme CK-Mt is an mitochondrial enzyme and represent almost 15% of CPK cardiac activity; Doizé et al., 1989) in Landrace pigs. Cattle transported for long periods also had increased CK, which may be a result of the effort to maintain their position in the moving vehicle, resulting in great muscle fatigue and, in some cases, skin lesions (Tadich et al., 2000). Similar results were observed by Warriss et al. (1995), Knowles (1999), and Pèrez et al. (2002). In addition, pentathlon horses had increased CK during exercise (Balogh, 2001).

Creatine kinase activity can vary because of sex, muscle mass, physical activity, and breed (Katirji and Al-Jaberi, 2001). According to Watkins et al. (1990), Uttaro et al. (1993), and Sanches et al. (2010), there is an increase in muscle depth in pigs that received ractopamine in feed, and this increase is caused by a linking of ractopamine β-receptors to the receptors of the membrane that will increase the diameter of muscle fibers, more specifically the white and intermediary fibers (Aalhus et al., 1990). The increase of CK concentration is also found in diseases caused by the rupture of muscle fibers.

Considering all these facts, it is suggested that the increase of CK enzyme concentration of pigs that received ractopamine in the present study may have been increased in 2 ways. The first factor would be based on the increase of muscle fiber diameter, caused by the action of β-adrenergic agonists because micro lesions increase fiber diameter, stimulating the release and activity of CK enzyme. The second factor would be the supposition that pigs that received ractopamine are more susceptible to stress because, according to Schmidt et al. (1974), only an extreme susceptibility to stress increases CK concentrations in pigs. Therefore, the ractopamine-supplemented pigs in this study may had more stress and muscle fatigue during transportation, increasing the membrane permeability, releasing CK in this muscle tissue circulation, and consequently increasing the activity of this enzyme in plasma.

In conclusion, ractopamine supplementation in finishing pig feed did not alter incidence of skin lesions or circulating concentrations of cortisol and lactate. However, this feed additive causes physiological and some behavior alterations, which may warrant further investigation.

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