MEAT QUALITY IN PIGS FED DIETS WITH GRADUAL RACTOPAMINE SUPPLEMENTATION AND NUTRITIONAL ADJUSTMENTS[1]

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ABSTRACT: This study evaluated the effects that gradual ractopamine supplementation in diets with nutritional adjustments on pig meat. Were used 80 finishing crossbred barrows in a randomized block design with a 2×5 factorial arrangement (two diets: with and without nutritional adjustment; five levels of ractopamine supplementation: 5-5, 10-10, 20-20, 5-10 and 10-20 ppm) in the 14 initial and 14 final study days, four replicates with two animals by experimental unit. Higher shear force values (P<0.05) were obtained using the 5-5 and 10-20 ppm ractopamine supplementation plans in the diets without nutritional adjustment. With nutritionally adjusted diets, the 5-10 ppm of ractopamine supplementation plan yielded higher shear force values (P<0.05). Water retention capacity was higher (P<0.05) for animals fed adjusted diets and 5-5 and 10-20 ppm of ractopamine plans. In the 10-20 ppm of ractopamine supplementation plan, meat pH was higher (P<0.05) for diets without nutritional adjustment, whereas in the 20-20 ppm of supplementation plan, pH was higher for adjusted diets.

Keywords: agonist, amino acids, β-adrenergic, nutrition, protein.

SUPLEMENTAÇÃO DE RACTOPAMINA E AJUSTES NUTRICIONAIS DAS DIETAS NA QUALIDADE DA CARNE SUÍNA

RESUMO: O objetivo do estudo foi avaliar a qualidade da carne de suínos alimentados com dietas suplementadas gradualmente com ractopamina e ajustadas nutricionalmente. Foram utilizados 80 suínos, machos castrados, em terminação. O delineamento experimental foi o de blocos ao acaso em esquema fatorial 2x5 (dietas com e sem ajustes nutricionais; e cinco suplementações de ractopamina: 5-5; 10-10; 20-20; 5-10; 10-20 ppm, respectivamente nos 14 dias iniciais e 14 dias finais do experimento) e quatro repetições com dois animais por unidade experimental. Constatou-se maior força de cisalhamento (P<0,05) nos planos de suplementação de 5-5 e 10-20 ppm de ractopamina para as dietas sem ajustes nutricionais. Nas dietas com ajustes nutricionais, o plano de suplementação de 5-10 ppm apresentou maior força de cisalhamento (P<0,05) e os planos com suplementação de 5-5 e 10-20 ppm apresentaram maior capacidade de retenção de água (P<0,05) em relação aos demais. Observou-se aumento do pH (P<0,05) com a suplementação de 10-20 ppm de ractopamina em dietas não ajustadas nutricionalmente. Na suplementação de 20-20 ppm de ractopamina o pH foi maior (P<0,05) em relação ao plano com suplementação de ractopamina em dietas não ajustadas nutricionalmente.

Palavras-chave: agonistas, aminoácidos, β-adrenérgico, nutrição, proteína.

INTRODUCTION

Consumers are increasingly concerned about the nutritional value of meat, in view of recommendations to reduce intakes of saturated fatty acids to avoid the occurrence of heart disease (Jakobsen *et al*., 2009; Marçal *et al*., 2016). In this way, it is possible to manipulate the meat quality according to the type and quantity of feed provided (Melo *et al*., 2014).

Among the technologies available to improve carcass and meat quality in pigs, ractopamine has improved the performance and meat production, as well as reducing the fat content of the carcasses, an important factor for the acceptance of pork by the consumer (Silva and Silva, 2015). Nonetheless, despite the benefits in performance and carcass characteristics accrued from this class of growth promoter (Cantarelli *et al*., 2009), questions on optimal dosages and suitable dietary schedules remain unanswered.

In swine, metabolic changes caused by ractopamine may interfere with their nutritional requirements (Andretta *et al*., 2011). Increased daily protein deposition in the carcasses of pigs fed a ractopamine-enriched diet is known to increase their daily requirement for amino acids. Lysine deficiency concurrent with ractopamine supplementation can limit the animal's response to diet, since lysine is crucial for protein synthesis and carcass quality. Because dietary lysine levels affect the extent to which pigs respond to ractopamine (Apple *et al*., 2004), nutritional adjustments are required to compensate for the addition of the latter compound (Corassa *et al*., 2013).

Considering the scarcity of data on the relationship between nutritional adjustments and ractopamine supplementation plans, this investigation evaluated the effects that gradual dietary ractopamine supplementation with and without nutritional adjustment have on the meat pH, color, water retention capacity, shear force and oxidation of meat in finishing pigs.

MATERIALS AND METHODS

The investigation complied with ethical standards and was approved by the Ethics Committee on Animal Use (permit 425/2012) of the Universidade Federal de Mato Grosso do Sul.

Eighty commercial crossbred barrows were used with 68.55±0.33 kg and 93.68±2.11 kg of initial and final weight, respectively. The animals were allocated in a randomized block design with a 2×5 factorial arrangement (two diets: with and without nutritional adjustments; five levels of ractopamine supplementation: 5-5, 10-10, 20-20, 5-10 and 10-20 ppm) in the 14 initial and 14 final study days, four replicates with two animals by experimental unit. Definition of blocks took into account the initial weight of animals.

The diets (Table 1) were prepared to meet the nutritional requirements for barrows of high genetic potential and superior performance weighing 70 to 100 kg proposed by Rostagno *et al*. (2011). In the nutritionally adjusted diets, adjustment of amino acid levels was based on the concept of ideal protein. The calcium-to-phosphorus ratio was maintained at 2.4:1 in the adjusted diets. Ractopamine replaced kaolin. Feed were provided *ad libitum* throughout the 28-day study period.

At the end of the study, the animals were fasted from ration for 8 h and subsequently truck-transported to the abattoir, where they were housed in collective cages with free access to water for 6 h. The carcasses were halved lengthwise and half-carcasses were washed. *Longissimus dorsi* muscle samples were collected from the left half-carcass at the 12th rib and slow-chilled for subsequent freezing. The samples were thawed in the meat laboratories of Embrapa Beef Cattle. The variables evaluated were color, water retention capacity, meat pH, shear force, and oxidation. All laboratory procedures were performed in a refrigerated environment.

Shear force was measured with a texturometer calibrated to 2.0 kg. The samples were boiled at 72-75°C, cooled in an ice bath, and refrigerated at 5°C for 12 h. Three parallelepiped samples measuring 1 cm (height) × 1 cm (width) × 4 cm (length) were taken and placed on a slide with the muscle fibers oriented perpendicularly to the blade. Shear force results were expressed as kgf/g.

Oxidation was evaluated by adding a 10 g sample to 25 mL of 75% TCA. Homogenization was performed in a Stomacher device for 1 min, followed by filtration. A 4 mL aliquot of filtrate was placed in a test tube containing 1 mL of 7.5% TCA and 5 mL of 0.02 M TBA, heated for 40 m, and read in a spectrophotometer at 538 nm. Intramuscular pH was measured in deep tissue using a DMPH-2 pH-meter (Digimed, São Paulo) calibrated for pH 4.0 and 7.0, equipped with a DME-CF1 electrode. Intramuscular pH and color were measured in triplicate before the other tests. Measurements of color L^*, a^* and b^* were made at six points on each side of the sample using a Minolta colorimeter.

Table 1. Percentage and nutritional compositions of experimental diets

Ingredient	Diet					
	Without nutritional adjustment			With nutritional adjustment		
	5	10	20	5	10	20
Corn	81.40	81.40	81.40	74.17	74.17	74.17
Soybean meal, 46.1%	15.96	15.96	15.96	20.87	20.87	20.87
Soybean oil	0.020	0.020	0.020	1.140	1.140	1.140
Dicalcium phosphate	0.899	0.899	0.899	0.985	1.005	1.055
Calcitic lime	0.608	0.608	0.608	0.650	0.656	0.674
[1]Premix	0.100	0.100	0.100	0.100	0.100	0.100
Salt	0.354	0.354	0.354	0.355	0.355	0.355
L-lysine HCl	0.359	0.359	0.359	0.346	0.370	0.414
DL-methionine	0.078	0.078	0.078	0.104	0.115	0.135
L-threonine	0.103	0.103	0.103	0.114	0.127	0.151
L-tryptophan	0.018	0.018	0.018	0.013	0.016	0.022
Ractopamine	0.025	0.050	0.100	0.025	0.050	0.100
Kaolin (inert)	0.075	0.050	0.00	1.128	1.026	0.814
[2]Calculated nutritional values						
Crude protein, %	14.09	14.09	14.09	15.74	15.78	15.85
Met. energy, Kcal/kg	3,230	3,230	3,230	3,230	3,230	3,230
Net energy, Kcal/kg	2,465	2,465	2,465	2,465	2,465	2,465
Digestible lysine, %	0.829	0.829	0.829	0.932	0.950	0.984
Digestible meth+cyst, %	0.497	0.497	0.497	0.559	0.570	0.590
Digestible threonine, %	0.555	0.555	0.555	0.624	0.636	0.659
Digestible tryptophan, %	0.149	0.149	0.149	0.168	0.171	0.176
Digestible valine, %	0.572	0.572	0.572	0.646	0.646	0.646
Calcium, %	0.512	0.512	0.512	0.558	0.565	0.584
Available phosphorus, %	0.250	0.250	0.250	0.272	0.276	0.285
Sodium, %	0.160	0.160	0.160	0.160	0.160	0.160

[1]Contents per kg: iron: 100 g; copper: 10 g; cobalt: 0.2 g; manganese: 30 g; zinc: 100 g; iodine: 1.0 g; selenium: 0.3 g; vit. A: 6000000 IU; vit. D_3: 1000000 IU; vit. E: 12000 IU; vit. B_1: 0.5 g; vit. B_2: 2.6 g; vit. B_6: 0.7 g; pantothenic acid: 10 g; vit. K_3: 1.5 g; nicotinic acid: 22 g; vit. B_{12}: 0.015 g; folic acid: 0.2 g; biotin: 0.05 g; choline: 100 g; excipient q.s.p. 1000 g.
[2]Diets formulated to meet the nutritional requirements of barrows of high genetic potential, as proposed by Rostagno *et al*. (2011).

The results were submitted to ANOVA analysis using the SAS statistical software adopting the level of 5% of probability. The interactions between ractopamine supplementation and dietary adjustment were evaluated. The interactions were subsequently broken down, and mean dietary adjustment factors were evaluated using the F-test. Mean values obtained from ractopamine supplementation plans were analised using Tukey's test.

RESULTS AND DISCUSSION

Was observed interaction (P<0.05) between nutritional adjustment and ractopamine supplementation plans, with effects on shear force, water retention capacity and pH (Table 2). Interaction breakdown revealed that ractopamine plans with 5-5 and 10-20 ppm and adjusted diets reduced (P<0.05) shear force than non-adjusted diets. For other supplementation plans, nutritional adjustment did not affect (P>0.05) shear force.

As for non-adjusted diets, none of the ractopamine supplementation plans (P>0.05) influenced shear force. With adjusted diets, in contrast, shear force was higher (P<0.05) for the 5-10 ppm plan. Warriss *et al*. (1990) and Wood *et al*. (1994) found that pigs

Table 2. Ractopamine supplementation in diets with and without nutritional adjustment given to finishing barrows and its effect on shear force, water retention capacity, pH, and oxidation

Variable	R (ppm) 14+14 days	Diet		Mean	P value			CV, %
		Without nutritional adjustment	With nutritional adjustment		R	D	R x D	
Shear force, kgf/g	5-5	5.18 Aa	3.35 Bb	4.27				
	5-10	4.80 Aa	5.14 Aa	4.97				
	10-10	3.70 Aa	2.92 Ab	3.31				
	10-20	4.88 Aa	2.95 Bb	3.92				
	20-20	4.04 Aa	3.22 Ab	3.63				
	Mean	4.52	3.51		<0.001	<0.001	0.050	37.75
Water retention capacity, %	5-5	46.23 Ba	50.26 Aa	48.25				
	5-10	48.00 Aa	49.50 Aa	48.75				
	10-10	48.99 Aa	50.17 Aa	49.58				
	10-20	48.83 Ba	50.60 Aa	49.72				
	20-20	45.34 Aa	52.71 Aa	49.02				
	Mean	40.46	50.65		0.565	<0.001	0.038	8.51
Oxidation, mg/kg	5-5	0.16	0.14	0.15				
	5-10	0.18	0.18	0.18				
	10-10	0.23	0.15	0.19				
	10-20	0.18	0.12	0.15				
	20-20	0.17	0.13	0.15				
	Mean	0.18	0.14		0.850	0.177	0.914	59.11
pH	5-5	5.69 Ab	5.66 Ab	5.68				
	5-10	5.66 Ab	5.75 Ab	5.70				
	10-10	5.79 Aab	5.69 Ab	5.74				
	10-20	5.85 Aab	5.70 Bb	5.77				
	20-20	5.73 Bab	5.93 Aa	5.83				
	Mean	5.74	5.74		0.002	0.950	<0.001	2.76

[1]R: ractopamine; D: diet.
Different uppercase letters in same row indicate significant differences (P<0.05; F-test).
Different lowercase letters in same column indicate significant differences (P<0.05; Tukey's test).

receiving dietary ractopamine developed more fibrous meat (highest shear force values) due to the increased diameter of muscle fibers or possibly as a result of diminished activity of calpain (a proteolytic enzyme), which is lowered by increasing lean growth efficiency, resulting in lower *post mortem* degradation of myofibrillar protein.

Although increased shear force has been reported as a function of ractopamine supplemention, this effect was not observed in the present investigation. Furthermore, the adjusted diets were expected to increase carcass protein deposition, and hence increase shear force, an effect not found in this study, possibly because non-adjusted diets met the nutritional requirements of animals.

Interaction breakdown also revealed that both 5-5 and 10-20 ppm ractopamine plans with non-adjusted diets reduced (P<0.05) water retention capacity than with adjusted diets. For other ractopamine plans, there were no differences (P>0.05) due to nutritional adjustments diets.

Comparisons across ractopamine supplementation plans revealed that diet adjustment had no effect (P>0.05) on water retention capacity. Lower water retention capacity translates to more pronounced exudate release, with consequent losses in nutritional value, resulting in drier, less tender meat (Fernandes *et al*., 2011). Water retention capacity tends to be higher in animals given ractopamine (Warriss *et al*., 1990), an effect of lower fat deposition and increased protein deposition, which increases water retention (Crome *et al*., 1996).

Warriss *et al*. (1990) and Wood *et al*. (1994) observed that animals treated with salbutamol (a β-adrenergic) exhibited lower muscle glycogen concentration, with decreased pH and protein denaturation, resulting in increased water retention capacity and reduced meat with incidence of pale, soft, exudative (PSE).

There was no interaction (P>0.05) between nutritional adjustment and ractopamine supplementation. Also, oxidation was not changed (P>0.05) by ractopamine supplementation plans or nutritional adjustments.

In pigs, ractopamine supplementation promotes reduction of body fat, particular subcutaneous and intermuscular (Carr *et al*., 2005). Decreased fat deposition can reduce lipid oxidation rates, improving pork quality (Gregory *et al*., 2011). In the present experiment, however, the treatments did not alter lipid oxidation, corroborating with the results obtained by Amin *et al*. (2015) who used 20 ppm of ractopamine at different periods of supplementation and found no effects on meat oxidation. Other studies such as Leick *et al*. (2010) evaluating 0 and 5 ppm of ractopamine and Apple *et al*. (2008) using 0 and 10 ppm also found no significant effects on lipid oxidation. Garbossa *et al*. (2013) demonstrated that meat from pigs fed supplemented ractopamine can be stored for 90 days without qualitative losses in terms of lipid oxidation.

In the present study, interaction also revealed that use of the 10-20 ppm ractopamine plan with non-adjusted diets increased (P<0.05) pH than with adjusted diets. Use of the 20-20 ppm plan with adjusted diets increased (P<0.05) pH than with non-adjusted diets. For other ractopamine plans, there were no differences (P>0.05) between adjusted and non-adjusted diets. In adjusted diets, meat pH was highest for the 20-20 ppm plan.

Meat pH values are crucial for muscle contraction during *rigor mortis*. In live muscles, pH is approximately 7.2, while in pork it can range from 5.2 to 7.0. Pronounced drops in pH and temperature during chilling cause sarcomeres to shorten, resulting in less tender meat and lower water retention. Glycolysis-accelerating factors are therefore to be prevented, given their ability to diminish meat pH values (Cesar and Souza, 2007).

According to Garbossa *et al*. (2013), final meat pH is higher for pigs fed diets supplemented with a β-adrenergic agonist, since these animals consume muscle glycogen, which decreases lactic acid production and accumulation in the carcass after slaughter, thus hindering pH reduction.

There was no interaction (P>0.05) between nutritional adjustment and ractopamine supplementation was observed on lightness (*L**), redness (*a**), and yellowness (*b**) (Table 3). Nutritional adjustment led to higher *L** and lower *a** values (P<0.05) than non-adjusted diets. Yellowness, however, was not affected (P>0.05) by either nutritional adjustment or ractopamine supplementation.

Published *L** values for regular pork vary widely (Caldara *et al*., 2012). *L** values from 49 to 60 meet pork quality standards established by the American Meat Science Association (AMSA, 2001). However, a narrower range, from 45 to 53, is advocated by Ramos and Gomide (2007).

In the present investigation, increased *L** values resulting from adjusted diets may be related to the low final pH of meat of animals with a characteristic rapid consumption of glycogen anaerobic, which leads to higher lactic acid generation. Lactic acid accumulation causes meat protein denaturation, resulting in water loss and higher reflectivity, giving meat it a paler hue (Garbossa *et al*., 2013). Few studies have related meat color to ractopamine supplementation or nutritional adjustment. Armstrong *et al*. (2004) and Bridi *et al*. (2006) found no effect of dietary ractopamine levels ranging from 2.5 to 30 ppm on fresh pig meat coloration. Similarly Almeida *et al.* (2010), for instance, did not obtain effects of ractopamine intake on *L** values in pork.

In the present study, mean *a** values remained within the 5.50-5.94 bracket advocated by Silveira (1997). Higher *a** values can be explained by increased iron and potassium levels in tissues (Juncher *et al*., 2001), that are associated with myoglobin levels in muscles. Dietary ractopamine has been shown to promote significant reduction in oxygenated myoglobin levels, decreasing meat redness (Almeida *et al*., 2010).

In the present experiment, mean *b** value was higher than those from 5.80 to 6.53 obtained by Silveira (1997). Van der Wal *et al*. (1988), however, reported a *b** value of 13.7. Broadly, *b** values are indicative of carotenoid deposition in fat, decreasing linearly with ractopamine intake (Garbossa *et al*., 2013).

Table 3. Ractopamine supplementation in diets with and without nutritional adjustment given to finishing barrows and its effect on meat lightness (*L), redness (*a**), and yellowness (*b**)**

Variable	R (ppm) 14+14 days	Diet		Mean	[1]P value			CV, %
		Without nutritional adjustment	With nutritional adjustment		R	D	R x D	
*L**	5-5	48.15	51.43	49.79				
	5-10	48.86	49.58	49.22				
	10-10	48.49	49.66	49.08				
	10-20	47.79	51.85	49.82				
	20-20	49.06	50.16	49.61				
	Mean	48.47 B	50.55 A		0.989	0.025	0.709	14.28
*a**	5-5	5.52	5.32	5.42				
	5-10	6.32	5.11	5.71				
	10-10	6.58	5.62	6.10				
	10-20	5.01	4.63	4.82				
	20-20	5.66	4.69	5.18				
	Mean	5.83 A	5.07 B		0.163	0.039	0.881	49.22
*b**	5-5	13.24	12.85	13.06				
	5-10	11.60	13.01	12.30				
	10-10	12.63	13.51	13.07				
	10-20	11.64	12.60	12.12				
	20-20	12.52	12.44	12.48				
	Mean	12.33	12.88		0.426	0.182	0.619	25.42

[1]R: ractopamine; D: diet.
Different letters in same row indicate significant differences (P<0.05; F-test).

CONCLUSION

Higher shear force values were obtained using the 5-5 and 10-20 ppm ractopamine supplementation plans combined with diets without nutritional adjustment. With nutritionally adjusted diets, the 5-10 ppm supplementation plan yielded higher shear force values. Water retention capacity was higher for animals fed adjusted diets and 5-5 ppm and 10-20 ppm ractopamine plans. In ractopamine supplementation plan of 10-20 ppm, meat pH was greater for diets without nutritional adjustment, while in 20-20 ppm supplementation plan, pH was higher for adjusted diets.

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