

## Características químicas, microbiológicas e sensoriais do produto curado preparado com carne de suínos alimentados com dietas contendo beta adrenérgico e antioxidantes naturais

Chemical, Microbiological and Sensory Characteristics of Cured Product Prepared with Pork Fed Diets Enriched with Beta Adrenergic and Natural Antioxidants

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### ABSTRACT

**Background:** Through individual properties and synergistic action of its active principles, adding antioxidants (AN) in diet can indirectly influence the meat properties and chemical characteristics, microbiological and sensory of the salami. This work evaluated the chemical, microbiological and sensory product made with meat from finishing pigs fed diets containing ractopamine (RAC) and natural AN.

**Materials, Methods & Results:** One hundred and eight pigs were used in a completely randomized, blocked by sex and distributed in nine treatments with 0, 10 and 20 ppm of RAC; 0, 250 and 500 ppm of AN and their interactions. The animals were slaughtered in slaughter house fridge with federal inspection. After that, for laboratory analysis and preparation of salami, the *Longissimus dorsi* muscle was removed from the right half carcass. Two duplicates of each treatment were separated and vacuum packed for the laboratory analyzes. In the sensory analysis evaluated the acceptability of Milano salami on a seven-point hedonic scale. The moisture percentage of the salami prepared with meat from animals that received 10 RAC+500 AN in the diet had an average 7.5% higher ( $P < 0.05$ ) than control, 20 ppm for RAC and levels of AN. The ash content in the salami produced from the animals that received 10 RAC+500 AN in the diet had an average 9.5% higher ( $P < 0.05$ ) than control, 20 RAC+250 AN and 250 AN. The protein content in the salami elaborated with animal received 10 RAC+250 AN in the diet was 10.2% higher ( $P < 0.05$ ) than other treatments. The lipid content of the control group salami were 16.6% higher ( $P < 0.05$ ) than other treatments. In the color evaluation of salami prepared with meat from females, the level of inclusion 10 RAC+250 AN showed better acceptability ( $P < 0.05$ ) compared to the other treatments. In relation to the characteristic odor, females' salami with 500 AN from the treatment showed better acceptability ( $P < 0.05$ ) compared to the other treatments. The flavor of the sausages made with males' meat and females' from the treatment with 500 AN showed better acceptability ( $P < 0.05$ ) compared to the other treatments. The texture from the treatment with 20 RAC+250 AN, showed better acceptability ( $P < 0.05$ ) compared to the other treatments. The salami prepared with females' meat that received 20 RAC in the diet had lower sensory acceptability, compared to treatment with 500 AN and 250 AN + interactions with RAC.

**Discussion:** The salami prepared with pork that received RAC and AN in the diet presents high protein and low lipid levels. As the RAC acts on nutrient partitioning increasing protein deposition in the muscle, there is a stimulus to deposition of water in muscle that does not occur with the deposition of fat, which absorbs low amount of water. Thus, with the inclusion of RAC in the diet, protein deposition occurs and greater moisture content in muscle. Thus, with the inclusion of RAC in the diet, protein deposition occurs and greater moisture content in muscle. These effects were observed, with emphasis on treatments with interactions between AN and RAC, which showed higher levels of moisture and protein, but low in lipids. Is likely that the addition of RAC in the diet increases the moisture and protein deposition in muscle. The salami prepared with higher levels of RAC in the diet has a lower acceptability in sensory analysis. Is likely that loss of moisture during maturation of salami does not occur gradually, which leads to formation of roughness, excessive dryness of skin and loosening of the wrapper. Changes occur in the product texture and in enzymatic reactions catalyzed by tissue enzymes and microbial, which originate substances contributing to the flavor and smell of the product. This phenomenon may has influenced the results of acceptability of the product produced.

**Keywords:** ascorbic acid, beta-adrenergic agonist, bioflavonoid, sausage.

**Descritores:** ácido ascórbico, agonista beta adrenérgico, bioflavonoides, salame.

## INTRODUCTION

The production of salami in Brazil represents a significant percentage of the products in the meal market. The main mechanisms that can compromise the quality of meat products by losing nutrient and reducing their shelf life are the protein and lipid oxidation. The oxidation processes can be diminished by most of the synthetic antioxidants but have toxic health properties [15,25].

The natural antioxidants (AN) are chemical substances naturally found in the composition of vegetable origins foods [28,29]. The activity of AN is also interesting technological, therefore, these substances make possible to obtain healthier foods that can be included in the group of "functional foods" [37,39]. Among the AN that act in preventing lipid oxidation and decrease of microbial growth are vitamin C (ascorbic acid) and phenolic compounds (bioflavonoids) [4,11,29].

The use of modifying additives in animal metabolism via nutrition, such as ractopamine (RAC) connect themselves to receptors present on most mammalian cells, stimulating the increase of muscle mass development through hypertrophy and reduce the deposition of fat [7,9]. Through individual properties and synergistic action of its active principles, adding AN in diet can indirectly influence the meat properties and chemical characteristics, microbiological and sensory of the salami. Although there are positive informations related to the synergism of the constituents of AN, there aren't reports of the use associated with the RAC yet. This work was conducted, in order to evaluate the chemical, microbiological and sensory product made with pigs' fed diets meat containing RAC and AN in the finishing phase.

## MATERIALS AND METHODS

The work was performed at the Swine sector (Department of Animal Science) in University of Santa Maria, from July to October 2012. In this project were used 108 pigs (54 castrated males and 54 gilts) and paternal half-brothers with initial body weight of 61 kg. The experimental delineation was completely randomized, blocked by sex and with nine treatments: T1. control (C) (0 ppm of RAC and 0 ppm of AN), T2. C+10 RAC (in ppm), T3. C+20 RAC, T4. C+250 AN (in ppm), T5. C+500 AN, T6. C+250 AN+10 RAC, T7. C+250 AN+20 RAC, T8. C+500 AN+10 RAC and T9. C+500 AN+20 RAC. Also

were used two types of gender, two repetitions per gender, totalizing four repetitions. The animals were slaughtered in slaughter house fridge with federal inspection. After that, the carcasses were cooled to 3°C for a period of 24 h. For laboratory analysis and preparation of salami, the *Longissimus dorsi* muscle was removed from the right half carcass. The *Longissimus dorsi* muscle is commonly used in qualitative evaluations meat [14,30,31]. The manufacture of embedded procedure was conducted according to the usual Milano salami proposed by Brazil [21]. The salami remained for 28 days in ripening chamber with controlled temperature (°C) and relative humidity (%) [35,36]. Two duplicates of each treatment were separated and vacuum packed for the laboratory analyzes. The duplicate weighing around 250 g each, were used for chemical and microbiological analyzes. In chemical analysis were evaluated the levels of humidity, ash, protein and lipids as described by the AOAC method [3]. In microbiological analysis was evaluated counting (CFU g<sup>-1</sup>) of Coliforms at 35°C, Coliform at 45°C, *Staphylococcus* Coagulase Positive, *Salmonella* spp. [22] and lactic acid bacteria. In the sensory analysis evaluated the acceptability of Milano salami on a seven-point hedonic scale [10]. A total of 40 persons do not trained in the assessment of color, odor, flavor and texture scale that ranged from "I like very much" to "I extremely dislike". The presupposition of normality of the data was tested by the Ryan-Joiner test (Shapiro-Wilk similar test), and the null hypothesis expresses that the data have a normal distribution. By this assumption, the data of chemical analysis, microbiological and sensory of the salami had been subjected to analysis of variance by the GLM procedure and the possible differences between means were compared by Tukey test at 5% probability. Statistical analyzes were performed with the Minitab statistical program [19].

## RESULTS

This work followed the guidelines of the Ethics Committee in Search of the Federal University of Santa Maria involving human beings, in the sensory analysis of salami, and animals in a field experiment in order to save their integrity, safety and welfare. The count of coliforms, *Staphylococcus* spp. and *Salmonella* spp. was not affected ( $P > 0.05$ ) among treatment groups (data not shown). The data obtained are within specification standards for foods of the National Agency of Sanitary Vigilance (ANVISA) [2].

The results of the count bacteria lactic acid salami elaborated with meat of pigs fed RAC and AN are presented in Table 1. The counting of lactic bacteria of the salami the control group was on average 56% higher ( $P < 0.05$ ) with treatment with levels of RAC, AN and their interactions. The moisture percentage of the salami prepared with meat from animals that received 10 RAC+500 AN in the diet had an average 7.5% higher ( $P < 0.05$ ) than control, 20 ppm for RAC and levels of AN. The ash content in the salami produced from the animals that received 10 RAC+500 AN in the diet had an average 9.5% higher ( $P < 0.05$ ) than control, 20 RAC+250 AN and 250 AN. The protein content in the salami elaborated with animal received 10 RAC+250 AN in the diet was 10.2% higher ( $P < 0.05$ ) than other treatments. The lipid content of the control group salami were 16.6% higher ( $P < 0.05$ ) than other treatments.

The results from our study allow us to estimate that the moisture in the salami it reduced with the inclusion of AN, which can increase the shelf life of the cured product. The protein contents in the salami were higher ( $P < 0.05$ ) in the interactions between 10 RAC+250 and 10 RAC+500 compared to the other treatments. Additionally, RAC and AN used in isolation reduce the protein content in the salami. Since the lipid

content in the control group salami were higher ( $P < 0.05$ ) than other treatments. According to these results it is possible to suggest that the chemical variables of the salami are improved by the inclusion of RAC, AN and the interaction 20 RAC+500 AN. However, more studies are needed to evaluate the mode of action of AN and its interactions with the RAC.

Sensory evaluation of acceptability (hedonic scale of seven points) of Milano salami, made with meat from castrated male and female pigs fed in finishing with RAC and AN are shown in Table 2. In the color evaluation of salami prepared with meat from females, the level of inclusion 10 RAC+250 AN showed better acceptability ( $P < 0.05$ ) compared to the other treatments. In relation to the characteristic odor, females' salami with 500 AN from the treatment showed better acceptability ( $P < 0.05$ ) compared to the other treatments. The flavor of the sausages made with males' meat and females' from the treatment with 500 AN showed better acceptability ( $P < 0.05$ ) compared to the other treatments. The texture from the treatment with 20 RAC+250 AN, showed better acceptability ( $P < 0.05$ ) compared to the other treatments. The salami prepared with females' meat that received 20 RAC in the diet had lower sensory acceptability, compared to treatment with 500 AN and 250 AN + interactions with RAC.

**Table 1.** Chemical evaluation (%) and lactic bacteria (CFU g<sup>-1</sup>) of Milano salami prepared with meat from pigs fed RAC and AN in the final phase.

Treatment (ppm)	Variables				
	Lactic bacteria	Moisture	Ash*	Protein*	Lipids*
Control	9.2x10 <sup>7a</sup>	34.96 <sup>c</sup>	5.62 <sup>b</sup>	27.80 <sup>b</sup>	28.85 <sup>a</sup>
10 RAC	7.3x10 <sup>7ab</sup>	37.45 <sup>ab</sup>	6.07 <sup>a</sup>	32.69 <sup>b</sup>	24.27 <sup>b</sup>
20 RAC	7.0x10 <sup>7b</sup>	36.07 <sup>b</sup>	6.11 <sup>a</sup>	33.25 <sup>b</sup>	23.95 <sup>b</sup>
250 AN	6.1x10 <sup>7b</sup>	35.34 <sup>c</sup>	5.87 <sup>b</sup>	29.84 <sup>b</sup>	26.64 <sup>b</sup>
500 AN	3.2x10 <sup>7c</sup>	37.00 <sup>b</sup>	6.07 <sup>a</sup>	31.33 <sup>b</sup>	26.32 <sup>b</sup>
10 RAC + 250 AN	3.1x10 <sup>7c</sup>	37.45 <sup>ab</sup>	6.03 <sup>a</sup>	34.86 <sup>a</sup>	21.59 <sup>b</sup>
20 RAC + 250 AN	4.0x10 <sup>7c</sup>	37.52 <sup>ab</sup>	5.89 <sup>b</sup>	32.53 <sup>b</sup>	24.73 <sup>b</sup>
10 RAC + 500 AN	2.6x10 <sup>7c</sup>	38.77 <sup>a</sup>	6.40 <sup>a</sup>	34.80 <sup>a</sup>	20.98 <sup>b</sup>
20 RAC + 500 AN	2.4x10 <sup>7c</sup>	37.11 <sup>b</sup>	6.28 <sup>a</sup>	32.11 <sup>b</sup>	23.93 <sup>b</sup>
RSD	254	1.08	0.33	1.48	1.85
Probability					
Treatment (T)	0.01	0.01	0.01	0.01	0.01
Sex (S)	0.01	0.01	0.01	0.01	0.01

RAC: ractopamine; AN: natural antioxidants; \*adjusted Averages (LSM) by humidity, RSD: residual standard deviation; <sup>a, b, c</sup>different letters in the same column differ by Tukey test ( $P < 0.05$ ).

**Table 2.** Sensory evaluation (hedonic scale of seven points) of Milano salami, made with meat from castrated male pigs and female fed in finishing phase with RAC and AN.

Treatment (ppm)	Quality Features							
	Color		Odor		Flavor		Texture	
	M	F	M	F	M	F	M	F
Control	2.6	2.7 <sup>a</sup>	2.8	2.9 <sup>a</sup>	3.0 <sup>a</sup>	3.1 <sup>a</sup>	2.7 <sup>a</sup>	2.8 <sup>a</sup>
10 RAC	2.9	2.8 <sup>a</sup>	2.7	2.7 <sup>a</sup>	2.8 <sup>a</sup>	2.7 <sup>a</sup>	2.7 <sup>a</sup>	2.6 <sup>a</sup>
20 RAC	3.1	3.3 <sup>a</sup>	3.0	3.0 <sup>a</sup>	3.2 <sup>a</sup>	3.3 <sup>a</sup>	3.2 <sup>a</sup>	3.2 <sup>a</sup>
250 AN	2.9	2.9 <sup>a</sup>	2.8	2.8 <sup>a</sup>	3.2 <sup>a</sup>	3.1 <sup>a</sup>	3.1 <sup>a</sup>	3.1 <sup>a</sup>
500 AN	2.8	2.8 <sup>a</sup>	2.5	2.3 <sup>b</sup>	2.5 <sup>b</sup>	2.4 <sup>b</sup>	3.1 <sup>a</sup>	2.8 <sup>a</sup>
250 AN + 10 RAC	2.8	2.6 <sup>b</sup>	2.6	2.6 <sup>a</sup>	3.3 <sup>a</sup>	3.1 <sup>a</sup>	2.6 <sup>a</sup>	2.6 <sup>a</sup>
250 AN + 20 RAC	2.9	2.8 <sup>a</sup>	2.5	2.4 <sup>a</sup>	2.6 <sup>a</sup>	2.6 <sup>a</sup>	2.4 <sup>b</sup>	2.5 <sup>b</sup>
500 AN + 10 RAC	3.1	3.0 <sup>a</sup>	3.1	2.8 <sup>a</sup>	3.1 <sup>a</sup>	2.9 <sup>a</sup>	2.8 <sup>a</sup>	2.8 <sup>a</sup>
500 AN + 20 RAC	3.2	3.2 <sup>a</sup>	3.0	3.0 <sup>a</sup>	3.2 <sup>a</sup>	3.2 <sup>a</sup>	3.1 <sup>a</sup>	3.1 <sup>a</sup>
RSD	0.9	0.8	0.9	0.9	1.0	1.0	1.0	0.1
Probability								
Treatment (T)	0.19	0.01	0.06	0.01	0.01	0.01	0.01	0.01

RAC: ractopamine; AN: natural antioxidants; M: male; F: female; RSD: residual standard deviation; <sup>a,b</sup>different letters in the same column differ by Tukey test ( $P < 0.05$ ). Hedonic scale (1 = I extremely like, 2 = I liked a lot 3 = I like moderately, 4 = I did not like nor disliked, 5 = I dislike moderately, 6 = I dislike very much, 7 = I extremely dislike).

## DISCUSSION

Lactic bacteria are anaerobic microorganisms, facultative anaerobic or microaerophilic, that had better development in media with low oxygen tensions. Lactic bacteria act in the production and preservation of most fermented foods like sausage [6]. It produces organic acids and other substances antagonists such as hydrogen peroxide, free radicals diacetyl, acetaldehyde, D isomers of amino acids, non-protein and bacteriocins small molecules. They reduce microbial contamination, improve palatability and extend the useful life of the cured product, improving product quality. The presence of lactic bacteria decreased in salami manufactured with meat from animals fed RAC and AN, as well as their interactions, so it is estimated that the inclusion of RAC, AN and their interactions can reduce the quality and durability of salami. However, in studies that AN was used directly in the preparation of cured product, the responses were positive regarding the conservation by delaying fading and deterioration as a result of oxidation [5,8].

The variations in the chemical composition of the salami can be explained by the effects of interactions between RAC and AN added to diets. The RAC in swine, increases skeletal muscle by

muscle fiber hypertrophy, specifically white and intermediate fibers [1] and decreases fat deposition in the carcass [18], which can be related to the blocking of lipogenesis [20]. *In vitro* studies have shown that beta-adrenergic agonist increases production of cyclic adenosine monophosphate (cAMP), which activates kinases that phosphorylate the sensitive hormone lipase (SHL) [23]. In activated state, this enzyme breaks down triglycerides and increases the rate of lipolysis. As the RAC acts on nutrient partitioning increasing protein deposition in the muscle [32], there is a stimulus to deposition of water in muscle, that does not occur with the deposition of fat, which absorbs low amount of water [26]. Thus, with the inclusion of RAC in the diet, protein deposition occurs and greater moisture content in muscle. These effects were observed, with emphasis on treatments with interactions between AN and RAC, which showed higher levels of moisture and protein, but low in lipids.

In relation to inclusion of AN in diets, the synergism between the constituents of the formula is related to the reduction of the oxidative damage on the surface of fibroblasts [24]. When the antioxidants abduct free radicals, they help preserve the organoleptic characteristics and meat quality [27]. Studies

have demonstrated improvement in pH, water ability to retain and color (by reduction peroxidation lipid) during storage of pork supplemented with 200 ppm of a commercial product based on bioflavonoids and ascorbic acid in the diet [16].

The AN may alter the metabolism of glucose and glycogen. Specifically, oxalic acid, ascorbic acid metabolite which is considered a glycolytic inhibitor with effects on lactic acid production post mortem [16]. Thus, the rapid pH decline in postmortem is reduced and the deleterious effects on the meat used in the preparation of processed products are minimized.

It's interesting to point out that the effects of RAC on meat quality are controversial, since some studies indicate no significant impact on pork quality, including color, marbling, firmness and final pH values [34]. In relation to the preparation of cured products, the use of RAC in the diet stimulates protein deposition, which is related to the deposition of water in the muscle [26]. During the maturation of sausage occur biochemical, microbiological and physical associated to color processing, disintegration and changes of proteins, fats and carbohydrates [12]. The degradation products are mainly responsible for the odor and flavor of matured raw sausages. In maturation occurs salamis growth of bacterial flora which ferments sugar, and lactic acid produces lowers the pH of meat proteins to their isoelectric point, making it less able to join the water. This phenomenon helps to loss of water during the drying of the product [13,36].

Acidification generated contributes to the increase product consistency, allowing a solid structure conducive to slicing, and contribute in the formation of odor and taste typically of the salami [12]. Then, it is likely that the addition of RAC in the diet increases the moisture and protein deposition in muscle. Thus, it is likely that loss of moisture during maturation of salami does not occur gradually, which leads to formation of roughness, excessive dryness of skin and loosening of the wrapper. Changes occur in the product texture and in enzymatic reactions catalyzed by tissue enzymes and microbial, which originate substances contributing to the flavor and smell of the product. This phenomenon may has influenced the results of acceptability of the product produced, mainly the salami prepared with meat from females,

where the best acceptability for odor and flavor characteristics was observed with addition of 500 AN in diets. The best acceptability of the salami prepared with meat from animals supplemented with AN in is also justified by the antioxidant capacity of preserving the organoleptic characteristics and meat quality [27].

The success of a food product is primarily a result of consumer acceptance. First, the only big concern turns around the quality from the final product. However, currently, there is concern with the whole process of obtaining, adding each of the different aspects that deal the nutritional quality, hygienic, microbiological and environmental. In this case, the RAC contributes positively to higher concentrations of protein over the concentration of lipids in the salami. This aspect is directly related to the demands of the consumer market, which looks for ensuring a satisfactory health, knowing the nutritional value of its components. Nevertheless, the acceptability of salami prepared with meat from animals supplemented with high levels of RAC has a lower rate of acceptability. In relation to AN in as improved meat quality, more studies are necessary to evaluate the levels to be used in diets for finishing pigs, as well as their interactions with beta-adrenergic agonists.

#### CONCLUSION

Therefore, the treatments do not alter the count of coliforms, *Staphylococcus* and *Salmonella* spp., although reduces the count of lactic acid bacteria. The salami prepared with meat from animals that received addition of ractopamine and natural antioxidants in the diet has a higher protein content and lower lipid content. The salami prepared with meat from animals that received the addition of 500 ppm of natural antioxidants in the diet has better acceptability by sensory analysis.

**Ethical approval.** The slaughter was carried out in commercial slaughterhouse with federal inspection, respecting what the law says about the rest time, insensitivity and sangria.

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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