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Effect of ractopamine on the chemical and physical characteristics of pacu (*Piaractus mesopotamicus*) steaks

[*Efeito da ractopamina sobre as características físicas e químicas de filés de pacu (Piaractus mesopotamicus)*]

L.M.F.S. *Oliveira*, R.S. *Leal*, T.C. *Mesquita*, M.E.S.G. *Pimenta*[*], M.G. *Zangeronimo*, R.V. *Sousa*, R.R. *Alvarenga*

Universidade Federal de Lavras – UFLA – Lavras, MG

ABSTRACT

The objective was to evaluate the use of ractopamine (RAC) in the diet for pacu (*Piaractus mesopotaminus*) in the finishing phase on some quality parameters of the fillets. Thirty-five animals weighing 0.868±0.168kg were distributed in a completely randomised design with five treatments (0.0 - control; 11.25, 22.50, 33.75 and 45 ppm of RAC) and seven replicates with two fillets obtained from the same animal. The diets were isocaloric and isoprotein and experimental time was 90 days. RAC did not affect (P>0.05) the initial pH or ph after 24 hours of the fillets. Compared to the control, RAC increased (P<0.05) the moisture content of the fillets *in natura* and lipid oxidation of samples stored for 12 days in the refrigerator or freezer for 60 days. The RAC in 11.25 ppm reduced (P<0.05) the lipid content, while 45 ppm reduced (P<0.05) the crude protein in the fillets. Considering only RAC, there was a linear increase (P<0.05) in the lipid content (P<0.05) and a linear reduction in crude protein and weight loss after cooking the fillets. There was a quadratic effect (P<0.05) on the ash content, weight loss and lipid oxidation in fillets stored in the refrigerator or freezer. A RAC dose of 33.75 ppm resulted in a lower lipid oxidation index. In conclusion, ractopamine at 11.25 ppm is effective for reducing the fat content in fillets of pacu, although it increases the formation of peroxides in samples kept in the freezer for longer than 60 days. At 33.75 ppm, ractopamine is effective in reducing the effect of oxidation during storage in the refrigerator or freezer.

Keywords: fish, lipid oxidation, animal production, fillet quality

RESUMO

O objetivo foi avaliar a influência do uso de ractopamina na alimentação de pacus (Piaractus mesopotaminus) *na fase de terminação sobre alguns parâmetros de qualidade dos filés. Trinta e cinco animais com peso de 0,868±0,168kg foram distribuídos em delineamento inteiramente ao acaso com cinco tratamentos (0,0 – controle; 11,25; 22,50; 33,75 e 45ppm de ractopamina) e sete repetições, sendo a parcela representada por dois filés provenientes de um animal. As rações foram isoenergéticas e isoproteicas, e o tempo experimental foi de 90 dias. A ractopamina não influenciou (P>0,05) o pH inicial dos filés e nem após 24 horas. Comparado ao controle, a RAC aumentou (P<0,05) a umidade dos filés* in natura *e a oxidação lipídica dos mesmos quando armazenados por 12 dias em geladeira ou 60 dias em freezer. Na dose de 11,25 reduziu (P<0,05) o teor de extrato etéreo e, na dose de 45 ppm, reduziu (P<0,05) o de proteína bruta dos filés. Considerando somente a RAC, houve aumento linear (P<0,05) do teor de gordura e redução linear (P<0,05) da proteína bruta e perda de peso por cocção. Houve efeito quadrático (P<0,05) sobre o teor de cinzas, perda de peso por descongelamento e oxidação lipídica para os filés armazenados em geladeira ou em freezer, sendo 33,75 ppm o nível de RAC que resultou em menor índice de oxidação lipídica. Conclui-se que a ractopamina a 11,5 ppm é efetiva em reduzir o teor de lipídeos em filés de pacu, embora aumente a formação de peróxidos em amostras mantidas em freezer*

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*Autor para correspondência (*corresponding author*)
E-mail: maria.emilia@dca.ufla.br

após 60 dias. A 33,75 ppm a ractopamina é efetiva em reduzir os efeitos da oxidação durante o armazenamento em geladeira ou freezer.

Palavras-chave: peixe, oxidação lipídica, piscicultura, qualidade de filé

INTRODUCTION

Aquaculture represents an important activity in food production in Brazil. The necessity of increasing productivity in terms of quantity and quality (foods with lower fat levels, for example) has fostered intense growth in aquaculture. In this context, farmers seek to invest in species with high productivity and low fat deposition in the carcass.

The pacu (*Piaractus mesopotamicus*) is an omnivorous and migratory fish originating in the Paraguay and Parana rivers. This native species has been adopted by fish farmers due to its acceptance, reproducibility and adaptation to the conditions of captivity. However, the meat has a high fat content. Studies show that the highest deposition occurs in the finishing phase (Povh *et al.*, 2009; Bicudo *et al.*, 2010).

In the search for improved meat quality and production performance, additives have been used to modify metabolism in several species. Ractopamine is a β-adrenergic agonist that alters nutrient metabolism, redirecting nutrients to protein rather than lipid anabolism, which helps to improve production indices and meat quality (Mersmann, 1998; Van den Berg and Moccia, 1998; Haji-Abadi *et al.*, 2010).

In fish, administration of ractopamine was previously reported with some positive results in terms of increasing the protein content and reducing lipid levels in the meat (Van den Berg and Moccia, 1998; Van den Berg *et al.* 1998). The results were less obvious than those observed in mammals; however, this additive can alter other characteristics of meat due to alterations in *post mortem* metabolism.

Studies conducted with different species of fish used variable doses ranging from 10 mg/kg (Haji-Abadi *et al.*, 2010) to 100mg/kg (Mustin and Lovell, 1993). Thus, further studies are needed to determine the most appropriate dose to use in pacus under Brazilian conditions.

This study aimed to evaluate aspects related to meat quality in pacus fed diets with different levels of ractopamine in the finishing phase.

MATERIAL AND METHODS

The experiment was conducted from September 2010 to March 2011 at the Laboratory of Fish Metabolism of Fish Culture Station of the Federal University of Lavras, Minas Gerais, Brazil. The geographical coordinates are -21°14'43" latitude and -44°59'59" longitude. The altitude is 919m.

Thirty-five animals weighing 0.868±0.168kg were randomly divided into individual experimental aquaria with a capacity of 100L and equipped with an automated circulation system, biological and physical filters. The water temperature was maintained between 25 and 28°C.

A completely randomised design was used with four treatments (ractopamine levels) plus a control with seven replicates of two steaks per plot. For the assessment of malonic dialdehyde concentrations, samples were kept in the refrigerator or freezer and the same design was used, but subdivided in time (four time points).

The experimental diets (Table 1) were isocaloric and isonutrient, and were comprised of corn, soybean meal, fish meal and wheat bran and supplemented with vitamins, minerals and amino acids to meet the requirements of the species in accordance with Boscolo *et al.* (2011), with the exception of protein, which was increased to 32% due to increased protein synthesis in animals fed with diets containing ractopamine (Mitchell *et al.*, 1990).

The experimental period was 90 days. Initially, two weeks were used for the adjustment of the animals to the experimental conditions. During this time, animals were fed a basal diet without the addition of ractopamine. The fish were fed *ad libitum* twice a day (8:00 and 16:00) throughout the experimental period.

At the end of the experiment, after fasting for 12 hours, the animals were slaughtered for carcass evaluations. After stunning the fish with a benzocaine solution (50mg/L), the animals were submitted to spinal section. After this, they were gutted and filleted. The fillets were taken to the Central Analytical Laboratory of the Department of Food Science of the Federal University of Lavras, where the pH was measured using an electrode (Bernauer portable digital pH meter model F-1002, Blumenau, Brazil) placed in three different points of each sample fillet. The average value was considered as the result. This procedure was repeated 24 hours later in samples stored under refrigeration at 4°C.

Table 1. Experimental diets composition

Ingredients (%)	Ractopamine (ppm)				
	0.00	11.25	22.50	33.75	45.00
Soybean meal 45%	50.00	50.00	50.00	50.00	50.00
Fish flour 74%	8.00	8.00	8.00	8.00	8.00
Corn	24.90	24.90	24.90	24.90	24.90
Wheat flour	8.00	8.00	8.00	8.00	8.00
L-lysine HCl 78%	0.19	0.19	0.19	0.19	0.19
DL-methionine 98%	0.28	0.28	0.28	0.28	0.28
L-threonine 99%	0.05	0.05	0.05	0.05	0.05
Soybean oil	4.00	4.00	4.00	4.00	4.00
Bicalcium phosphate	3.80	3.80	3.80	3.80	3.80
Acorbic acid	0.06	0.06	0.06	0.06	0.06
Salt	0.20	0.20	0.20	0.20	0.20
Premix[1]	0.50	0.50	0.50	0.50	0.50
Butil hydroxy toluene	0.02	0.02	0.02	0.02	0.02
Ractopamine hydrochloride 2.05%	0.00	0.1125	0.2250	0.3375	0.4500
Calculated composition[3]					
Digestible energy (Kcal)	3203	3203	3203	3203	3203
Crude protein (%)	32.26	32.26	32.26	32.26	32.26
Digestible protein (%)	27.56	27.56	27.56	27.56	27.56
Crude fiber (%)	4.19	4.19	4.19	4.19	4.19
Ether extract (%)	7.06	7.06	7.06	7.06	7.06
Calcium (%)	1.48	1.48	1.48	1.48	1.48
Available phosphorus (%)	0.81	0.81	0.81	0.81	0.81
Methionine (%)	0.62	0.62	0.62	0.62	0.62
Lysine (%)	1.95	1.95	1.95	1.95	1.95
Triptophan (%)	0.38	0.38	0.38	0.38	0.38
Threonine (%)	1.13	1.13	1.13	1.13	1.13

[1]Composition per kg of product: 1500 UI of vitamin A, 20mg of vitamin B1, 15mg of vitamin B2, 1000 UI of vitamin B3, 10 mcg of vitamin B12, 25mg of vitamin E, 120mg of vitamin PP, 2,000mg of choline, 80mg of calcium pantothenate, 2.0mg of folic acid, 80mg of manganese, 24mg of iron, 50mg of zinc, 8.0mg of cupper, 3.0mg of iodine, 0.10mg of selenium and 170mg of butil hydroxy toluene.
[2]According Furuya *et al.* (2004).

Weight loss by thawing and cooking was determined by subtracting the weight of the fresh samples taken from the fillets before and after cooling and heat treatment, using the same methodology proposed by Moreira (2005) for broilers.

Proximate analysis of the steak samples was done using the methodology proposed by AOAC (Association..., 1995). All analyses were performed in triplicate. The analysis of moisture and ash were performed on cooled fresh fillets and the analysis of lipids and protein were conducted on lyophilised samples of fillets (Freeze-Liobras Model L202, San Carlos, Brazil). The moisture content was determined by a gravimetric method after oven drying to a constant weight. The lipid content was assessed by the Soxhlet method and crude protein by the Microkjeldhal method in free fat samples. The

ash content was determined by a gravimetric method using a muffle furnace at 550°C.

For the assessment of lipid oxidation, the TBARS (thiobarbituric acid reactive substances) test was used, according Tarladgis *et al.* (1960). The initial analysis was performed immediately after the slaughter of the animals (time zero) and on the 4th, 8th and 12th days of storage in the refrigerator (~ 4°C) and on the 15th, 30th and 60th days of storage in the freezer (~ -10°C). All analyses were performed in triplicate using a spectrophotometer (Varian, Inc.; Cary Model 50 UV-Vis Spectrophotometer, Porto Alegre, Brazil), adjusted to 531 nm. The absorbance values were multiplied by a constant (7.38) to estimate the amount of malonic dialdehyde per kg of sample (Tarladgis *et al.* 1960).

Data was subjected to analysis of variance after a normality test (Shapiro-Wilk). The values obtained with ractopamine treatment were submitted to regression analysis. The Dunnett test was used to compare the control with each level of ractopamine. For the ash, ether extract, crude protein, weight loss by thawing or cooking and malonic dialdehyde concentration in fillet samples, the square root transformation of data was used. To compare lipid oxidation over time, the SNK test at 5% was used. All statistical analyses were performed using the statistical Sisvar package version 5.3.

RESULTS AND DISCUSSION

Among the analysed variables, ractopamine had no effect (P>0.05) on the initial pH or on the pH 24 hours after slaughter (Table 2). The analysis of these indices aims to determine the reduction in pH in meat during and after the *rigor mortis* process. This pH reduction is important to the quality of the meat. In fish, these values should remain below 6.45 (Batista *et al.*, 2004). In the present work, all the pH values after slaughter were close to 7.0, as expected, and showed a significant decrease after 24h. This acidification prevents bacterial deterioration of the meat. No studies in the literature were found relating the use of ractopamine in fish and its effect on pH values. In the present work, no correlation between this variable and ractopamine dose was observed.

Table 2. Chemical composition, pH and weight lost by thawing and by cooking of fillets of pacus (*Piaractus mesopotamicus*) fed diets containing different levels of ractopamine during finishing phase

Variable	Ractopamine (ppm)				Control	Coefficient of variation (%)	P value
	11.25	22.5	33.75	45.0			
Initial pH	7.45	7.34	7.44	7.40	7.48	2.43	0.64
Final pH	6.14	6.26	6.35	6.25	6.13	3.75	0.40
Humidity (%)	72.3*	70.9*	72.4*	71.8*	65.94	3.24	0.62
Ash (%)[1]	1.27	1.33	1.46	1.20	1.29	7.69	0.04
Ether extract (%)[2]	8.98*	18.21	17.87	21.26	21.49	8.42	<0.01
Crude protein (%)[2]	17.72	18.71	16.94	13.08*	19.55	9.57	0.08
Weight lost							
By thawing (%)[1]	14.32	10.75	13.84	15.48	14.90	12.44	0.031
By cooking (%)[2]	2.07*	2.35*	1.18	0.86	1.03	23.29	<0.01

* Differ of control by Dunnett test (P<0.05)
[1] Quadratic regression (P<0.05)
[2] Linear regression (P<0.05)

Regarding the moisture content in fillets, ractopamine increased (P<0.05) the values compared to control; however, no differences (P>0.05) were observed between doses. Ractopamine has the effect of increasing the water holding capacity (WHC), which would cause an increase in the moisture content in fillets (Rosenvold and Andersen, 2003). The WHC and moisture are important for several reasons, such as the fact that meat exudation occurs as a result of a lower capacity for water retention. This compromises the appearance of the meat and leads to yield losses of products. Thus, moisture and the WHC influence the perception of the texture of fresh meat after cooking (Rosenvold and Andersen, 2003). In a study conducted by Moccia *et al.* (1998) with rainbow trout, the authors did not observe an effect on the moisture content in fish meat using the same levels of ractopamine.

The levels of ractopamine had a quadratic effect (P<0.05) on the ash content, with a gradual increase until 29 ppm (Figure 1). In assessing the proximate composition of pacus fillets, ash values were within expectations for this species (Bicudo *et al.*, 2010; Tanamati *et al.*, 2009), which is an important nutritional component in food.

The fat content of the fillets increased linearly (P<0.05) with the dose of ractopamine. Compared to the control, only the dose of 11.25 ppm resulted (P<0.05) in a lower fat content. With respect to the protein content, a linear decrease (P<0.05) was observed in relation to ractopamine dose. Compared to the control, the dose of 45 ppm resulted (P<0.05) in a lower protein content in the fillets. It is known that ractopamine is capable of altering lipid and protein metabolism in several species of farm animals (Haji-Abadi *et al.*, 2010). The effects of ractopamine on the composition of the fillets can be explained by the activation of β-adrenergic receptors, leading to the activation of hormone-sensitive lipase by phosphorylation, thus initiating lipolysis in adipocytes (Mersmann, 2002; Ferreira *et al.*, 2011). In response to this lipolysis, fatty acids are released for use as an energy source in other tissues (Mersmann, 2002; Salem *et al.*, 2006; Haji-Abadi *et al.*, 2010).

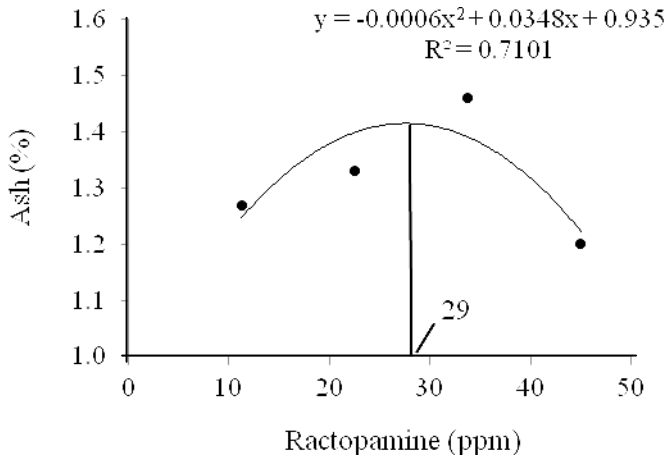


Figure 1. Ash concentration (%) of fillets of pacus (*Piaractus mesopotamicus*) fed diets containing different levels of ractopamine during finishing phase.

The lipolytic action of β-adrenergic receptors in adipose tissue occurs in mammals; however, a study on rainbow trout (*O. mykiss*) (Van Heeswijk *et al.*, 2006) showed that noradrenaline and adrenaline (natural β-agonists) reduce the lipolysis rate in adipocytes. In another study with rainbow trout, the release of free fatty acids in the liver was observed (Haji-Abadi *et al.*, 2010). In catfish (*Ictalurus punctatus* and *I. furcatus*), Mustin and Lovell (1993) used 20mg/kg of ractopamine in a high-protein diet and showed similar results to those found in mammals. But, in this case, they observed a greater effect with an increase in the protein content in the diet.

In another study, Van den Berg and Moccia (1998) evaluated the effect of ractopamine (0, 5, 10, 20 and 40ppm) on trout carcass composition. The authors observed a reduction in the fat content with 10 ppm of ractopamine, while 40 ppm increased the fat content compared to the control group, similar to what was observed in the present work. At high levels of ractopamine, it is believed that desensitisation of adrenergic receptors may occur (Ferreira *et al.*, 2011).

The ractopamine dose influenced weight loss (P<0.05) in a quadratic fashion by thawing; however, no level of this additive differed from control (P>0.05). The optimal level of ractopamine, which resulted in lower thawing weight loss, was 25ppm (Figure 2).

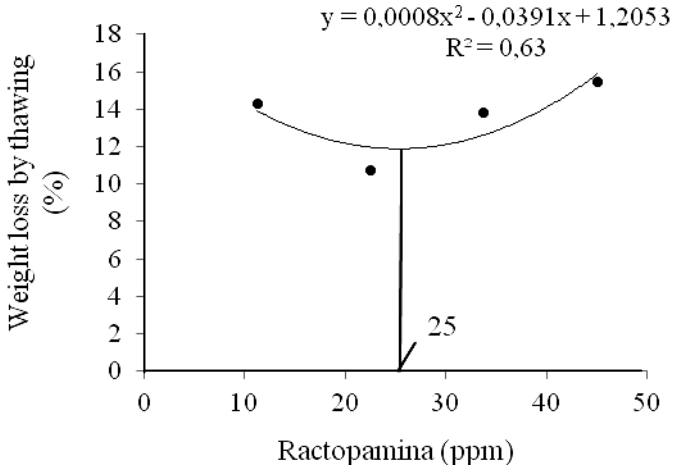


Figure 2. Weight loss by thawing (%) of fillets of pacus (*Piaractus mesopotamicus*) fed diets containing different levels of ractopamine during finishing phase.

Ractopamine linearly reduced (P<0.05) the weight loss by cooking. Compared to the control, the doses of 22.5 and 11.25 ppm of ractopamine resulted (P<0.05) in higher losses. The increase in water retention capacity promotes a reduction in weight loss by thawing and cooking; this capacity can be associated with moisture (Webster *et al.* 1995; Rosenvold and Andersen, 2003), but this effect was not apparent in the present work and no effect was observed on the moisture content.

Compared to the control, ractopamine at a dose of 45 ppm increased (P<0.01) the malonic dialdehyde content in chilled fillets when stored for 12 days in the refrigerator (Table 3). This effect was not observed with lower doses of the additive. A level of 33.75 ppm reduced (P<0.01) the malonic dialdehyde content. Between the ractopamine diets, there was a quadratic effect (P<0.01) in samples stored for 8 and 12 days in the refrigerator, at doses of 30 and 26ppm, respectively; these doses led to lower concentrations of malondialdehyde (Figure 3).

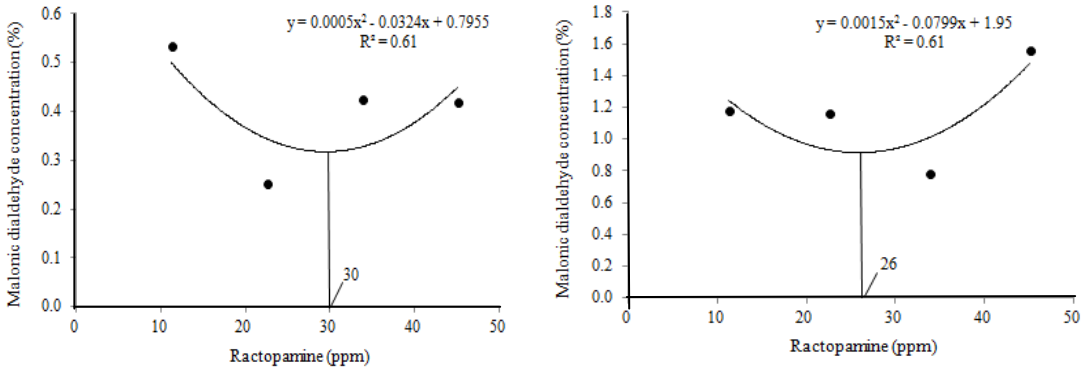
Table 3. Average concentration of malonic dialdehyde (mg/kg) in stored fillets of pacus (*Piaractus mesopotamicus*) fed diets containing different levels of ractopamine during finishing phase

Storage way	Day of evaluation	Ractopamine (ppm)				Control	Coefficient of variation (%)
		11.25	22.5	33.75	45		
Refrigerator (4 ºC)	0	0.132 a	0.137 a	0.140 a	0.149 a	0.144 a	14.36
	4	0.235 a	0.184 a	0.174 a	0.220 a	0.190 a	
	8[1]	0.532 b	0.249 a	0.422 b	0.417 b	0.321 a	
	12[1]	1.166 c	1.157 b	0.783 c*	1.549 c*	1.118 b	
Freezer (-10 ºC)	0	0.132 a	0.137 a	0.153 a	0.149 a	0.144 a	13.91
	15	0.145 a	0.107 a	0.168 a	0.129 a	0.131 a	
	30	0.091 a	0.097 a	0.067 a	0.115 a	0.082 a	
	60[1]	0.806 b*	0.882 b*	0.591 b	1.056 b*	0.652 b	
		P value					
		RAC	Dia	R*D			
		<0.01	<0.01	<0.01			

*Differ of control by Dunnet test (P<0.05)
Means followed by different letters in column differ by SNK test (P<0.05)
[1]Quadratic regression (P<0.05)

As for durability under refrigeration, a ractopamine dose of 33.75 ppm was associated with the lowest concentration of malonic dialdehyde over time. The samples remained stable in relation to lipid oxidation during the entire period of evaluation.

Ractopamine did not affect (P>0.05) the malonic dialdehyde concentration in frozen steaks stored for 30 days. At 60 days, ractopamine increased (P<0.01) the concentration of this substance compared to the control, except when a dose of 33.75 ppm was used. At this dose, the concentration of malonic dialdehyde was similar to the control. A quadratic effect (P<0.05) of ractopamine was observed after 60 days of storage. The optimal dose for reducing the malonic dialdehyde concentration was 24 ppm (Figure 4). As for durability in the freezer, ractopamine had no influence (P>0.05). All steaks were suitable for consumption at the end of the period of analysis.



8th day 12th day

Figure 3. Average malonic dialdehyde concentration (mg/kg) at 8th and 12th day of storage at 4 °C of samples of fillets of pacus fed diets containing different levels of ractopamine during finishing phase.

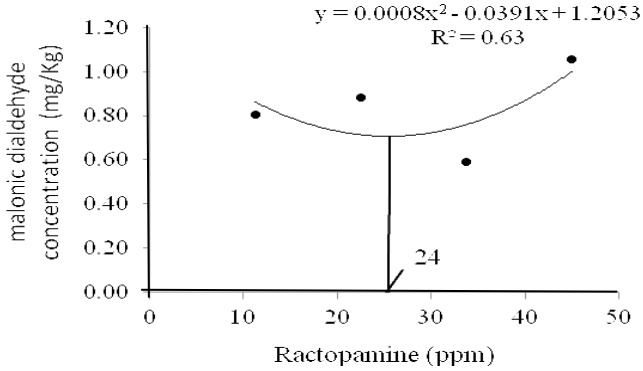


Figure 4. Average concentration of malonic dialdehyde (mg/kg) at 60th day of storage at -10° C of samples of fillets of pacus fed diets containing different levels of ractopamine during finishing phase.

The thiobarbituric acid reactive substances (TBARS) level represents the rate of lipid oxidation that it is measured by the amount of malonic dialdehyde formed from hydroperoxides. Hydroperoxides are products of the initial reaction between polyunsaturated fatty acids with oxygen (Fernandez *et al.*, 1997; Alvarez *et al.*, 2012). The lipid oxidation processes which occur during the storage of foods cause important changes in their sensory properties, such as rancidity. Rancidity is related to the development of unpleasant flavours and odours associated with food spoilage. In this sense, the use of agents with the aim of reducing or delaying this process is common in food technology to minimise the effects of oxidation,

thus maintaining the nutritional quality and extending the shelf life of foods (Alvarez *et al.*, 2012).

Generally, the shelf life of cooled fish fillets is relatively short, compared with other meat products. The shelf life has been estimated at 13 to 16 days (Cakli *et al.* 2007; Kilinc *et al.*, 2007; Alvarez *et al.*, 2008; 2012). This is due to the high content of polyunsaturated fatty acids which makes this product very susceptible to lipid oxidation during storage, a major cause of deterioration (Fraser and Sumar, 1998; Alvarez *et al.*, 2012). In cooled fish meat, the appearance of off-flavours and changes in the colour and texture reduce the sensory quality of the product in a few days, thus shortening the shelf life (Huss, 1995; Alvarez *et al.*, 2012). In this sense, any method used to prolong the shelf life of fish meat would be of great interest to the industry. Considering that one of the effects of ractopamine is a reduction in lipid levels (Haji-Abadi *et al.*, 2010) in meat, it is understood that this substance could also influence the effects of lipid oxidation.

Malonic dialdehyde values of 1-2 mg/kg are considered to be the upper limit above which fish meat is unfit for consumption due to changes in colour and unpleasant flavours (Connell, 1995; Tironi *et al.*, 2010). In the present work, TBARS values were lower than those reported by other authors (Cakli *et al.*, 2007; Kilinc *et al.*, 2007; Kostaki *et al.*, 2009; Alvarez *et al.*, 2012).

In this study, the results indicate that lipid oxidation occurred during storage, as expected, both in the freezer and in the refrigerator. The difference was in the time that elapsed until the product was considered spoiled. The results indicate that a ractopamine dose of 20 or 30 ppm is beneficial in terms of reducing the effect of oxidation. For storage in the refrigerator or freezer, a level of 33.75 ppm was effective.

CONCLUSION

Ractopamine at 11.25 ppm was effective in reducing the fat content in fillets of pacu, although it increased the formation of peroxides in samples kept in the freezer for 60 days. At a dose of 33.75 ppm, ractopamine was effective in reducing the effect of oxidation during storage in the refrigerator or freezer.

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