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Ractopamine effects on swine meat quality depending on gender and castration method

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Abstract: *The modern swine industry has in recent years, advanced into technologies aiming the increase of yield and meat quality, as well as economic performance of the hole segment. Innovations methods of castration and metabolic additives have been evaluated, in special the use of immunocastration and ractopamine addition in the feed. Although the production growth and industrial gains of these technologies are already well discussed, the real impact of employment together or isolated on the technological meat quality and its effect on the biochemical matrix, requires further study. Therefore, this scientific research was directed to evaluate the effects, concentrating on changes in electrophoretic profiles of meat, resulting animals commercially produced concurrently using ractopamine and immunocastration. 48 animals were used commercially created, 8 per treatment (castrates - CC, immunocastrated - IM, and female - F) feed diets supplemented with (CR) or without (SR) ractopamine at the final stage of finishing. We evaluated the qualitative characteristics of meat, such as pH, color, water-holding capacity (drip loss, cooking loss - PCOC, and loss defrosting - PDESC) and objective tenderness (shear force). The combined use of immunocastration with ractopamine influence pH 24h swine loin, the lightness (L*) and shear force, and pH, and shear force were higher and lower luminosity in IC-CR in the diet. The drip loss increased when no supplementation with dietary ractopamine. The effect simultaneously immunocastration with the addition of ractopamine in the diet not influence impacts on technological meat quality.*

Keywords: *β-adrenergic agonist, Water-holding capacity,Castration,Tenderness*

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I. Introduction

Brazilian swine industry has grown enormously. From the 70's, consumption of pork meat jumped from 9.20 kg per capita, in 2011 it was 15.01 kg per capita and in 2030 it will be 26.34 kg per capita worldwide[1]. Brazil achieved the fourth place in pork exportation due to investments in sanity, good practices in the farms, integrated systems of production and improving the farmers management [2].

According to ABPA (2018), Brazil produced 3,759 million of tons of swine meat, considered the fourth world producer and fourth place in exportations of swine meat in 2018. In the same year, Brazil achieved the Market of Russia, Hong Kong, China, Singapura, Argentina, Angola, Uruguai, Chile, Georgiaand Emirades, exporting 155 mil tons for those places. [3].

Those numbers explain the technological evolution in the sector, thanks to effort in genetics, nutrition, yield improving final weight, and carcass and meat quality. Technologies came as alternatives to upgrade the production system such as immuncastration rather than surgical castration, in order to eliminate the strong odor present in the whole male, with the supplementation of the diets with ractopamine for swine. Ractopaminechloridrate (β-adrenergic antagonist) is classified as a phenethanolamine, a natural analogous of catecholamine, epinephrine and norepinephrine, acts as an agent redirecting nutrients according to cell function or metabolism, promoting deeper effects on growth and muscle development and fat tissues, directing nutrients once destined to fat tissues formation, now for protein synthesis [4,5, 6, 7].

Wray-Cahen[6] and Marchant-Forde et al. [8] reported the use of ractopamine, approved in the USA (FDA, 2008) for swine in termination phase to increase weight gain, food efficiency, and deposition of lean meat in the carcass.

Several studies demonstrate ractopamine increases meat quality in tenderness, water loss and color and immunocastration is directly related to lean meat in the swine body. Those characteristics are also influenced by the gender, female, male castrated or immunocastrated[10, 11, 12]. Epinephrine could increase daily weight gain and nitrogen retention in swine but since the development of B-agonists in the 80's, the use for these substances were no more interesting. Nowadays ractopamine, cimaterol, L-644-969, salbutamol, clembuterol and zilpaterol are between the most used agonists β-adrenergic to increase protein synthesis [6].

The meat quality is a broad and complex concept, defined by a set of objective and subjective characteristics, which covers physical, nutritional and hygienic aspects [13], while the subjective aspects include the sensorial perception, presentation and exposure of the product. It is dependent on the temperature and cooling rate of muscle tissue after slaughterand can be evaluated by physico-chemical parameters such as pH, color, exudation losses, cooking losses, water retention capacity, intramuscular fat and tenderness, and by sensorial methods that evaluate succulence, meat appearance and mastication resistance [14].

Other factors affect the conversion of muscle to meat, most of them related to animal production: genotype / race, sex, age, castration, nutritional management and weight at slaughter, allied to factors caused by man, particularly those related to stress caused by the handling, transportation and the slaughter process itself, also significantly influence the conversion of muscle to meat, its tenderness and the quality of the meat product [15, 16, 17].

The addition of ractopamine in the pig ration did not significantly affect the quality of the protein evaluated by the water retention capacity and drip loss, according to the work developed by Zagury et al. (2002) [18], but differences were observed in pH and luminosity, however the values obtained correspond to normal meat quality. Differently Møller, Bertelsen and Olsen (1992) [19] and Wood, Wiseman and Cole (1994) [20] verified a pH increase that attributed the reduction of muscle glycogen levels, resulting in darker meat in animals supplemented with ractopamine.

Formighieri [21] investigating the effect of ractopamine on pigs, surgically castrated males, immunocast and females, found no interaction between sex and RAC treatment, concluding that the combination of diet with RAC and immunocastration had no impact on pork quality. The sex of the animals had no significant influence on the properties of pork *in natura*, and the immunoassayed pigs did not differ from castrated males, and the diet containing RAC did not increase the incidence of PSE and RSE (reddish-pink, soft, exudative - reddish, soft pink and exudative), however, regardless of the treatment, 80.0% of all the samples had a CSR meat problem and 10.3% presented PSE meat, suggesting the need for an improvement in pre-slaughter conditions.

In this work the characteristics of meat quality were evaluated in immunoassayed pigs, surgically castrated and female, fed or not with ractopamine.

II. Material and Methods

Study Design andStudy Location: Animals and Slaughtering

Swine were distributed into six treatments: male surgically castrate (SC); male immunocastrated (IC); female (F), all with and without ractopamine. Animals were raised in a commercial pig farm, from the most representative breed raised in Brazil. They leave maternity with 63 days and live weight (LW) 20.6 kg. Swine were then housed in a Terminated Swine Unit, according to parameters stablished in Oliveira (2016) [22]. Surgical Castration occurred during first week of swine's life. Other males were submitted to immunocastration (2.0 ml of Vivax® - Pfizer Animal Health: 1st dose at 8th week and second dose at 4th week before slaughter). Ten days after second dose, animals presented behavior similar to surgically castrated males. To avoid vaccine failures, an inspection was performed, observing behavior signs as penis exposition, mount over other animals, aggressiveness to others, over and for long periods and testicles characteristics (whole male presents testicle reddish and bigger).

During termination phase, female and male received two treatments: diet without ractopamine (NR) and with ractopamine (WR), for 28 days before slaughter, according to fabricants recommendation. Diet basis was corn and soybean with 18.92%of protein; 1.171% of lysine and 3,268.85 kcal/kg of EM) usually used in swine farms for termination phase. Feed was ad libitum at this time.

At end of 164 days, with average LW of 122.5kg for females WR and 114.0kg NR; 131.67kg for immunocastrated male WR and 126.0kg for NR and 123.33kg for the surgically castrated males WR and 114.44kg NR, swine were submitted to a fasting of six hours, boarded and transported for 20 km to the slaughter house.

The Normative and Standard procedures were adopted by the Farming Industry and Slaughter Bressiani (Capivari/SP) according to Brazilian rules [23] and Specific Rules for Swine Slaughtering Technical Procedures [24].

After a resting time, animals were slaughtered by electrical stunning and vertical bleeding. Humanitarian slaughter was performed according to Brazilian laws [23].Homogeneity of the batches and slaughter conditions allowed the random selection of 48 carcasses, following the statistical design (8 swine per treatment) for the measurements and analyses of qualitative characteristics of swine meat.

Procedure methodology:Evaluation of meat quality

pH measurements from Semimembranosus muscle: measurements were obtained from the left half-carcass 45 minutes after slaughter. Intramuscular pH was obtained in deep. pH was then measured after 24 hours from Longissimus dorsi e SM, using a pHmeter Oakton pH300, with a punction electrode Digimed. Three replicates of each were done.

Carcass cooling was performed according to actual legislation, for 24 hours at 2°C. After this period to realize the drip loss, a portion of the Longissimus dorsi (LD) was collected from the 4^{th} vertebra. Samples were maintained refrigerated for further analyses. The half-left carcass was sectioned between 10^{th} and 11^{th} rip, exposing the transversal section of the loin for measurements of pH of LD and objective color.

For cocking losses, freezing losses and tenderness samples from LD, with 2.5 cm of thickness were vacuum packed, frozen in a fast tunnel and taken to a Laboratory of Meat Quality at ESALQ-USP for analyses.

Color: After the exposition of the LD in the left half-carcass, 15 minutes was waited for the concurrency of blooming (time to reduced myoglobin deoximyoglobin, from pale purple – to oximyoglobin – cherry red, what it happens in the presence of oxygen. From this point, three measurements were taken, using a colorimeter Minolta (Chroma Meter, CR-400, Mahwah, New Jersey, USA). Results were expressed in CIE (1978) [25], as: L* (luminosity, a* (intensity of red/green and b* (intensity of yellow and blue). Parameters were calibrated with white china (Y = 93.7, x = 0.3160 and y = 0.3323, with area of 8 mm diameter, observation angle of 10° and illuminant D65.

Drip loss: Two samples of meat from 80 to 100 g each were collected from LD of each animal. Samples were hanged in a insufflated sac, assuring samples had no contact with the sac and stored at 4°C, for 48 hours. Samples were then removed, dried with paper towel and weighted. The % difference in weight was the loss per dripping, as described by Honikel (1998) [26].

Loss per cooking and freezing: Samples were weighted in an analytical scale (Gehaka - model B 2000) and maintained at -4°C for 48 horas. Again, dried in paper towels and weighted.The difference of weights, in % was the loss per freezing, discharging the exudate and the packing. The cooking loss was done according to the methodology described in American Meat Science Association[27]. Meat portions were packed and cooked in water-bath (at 80°C) until achieves internal temperature of 70°C. It was then cooled at 4°C for 24 hours.

Shear force: For shear force, beefs of 2.5 cm of thickness were refrigerated at 5°C for 12 hours and 8 samples of 1.27 cm of diameter was harvested using a metal cylinder from each beef, according to method described by AMSA (1995) [27].Samples were placed with the fibers perpendicularly to the lamina. Disks were sheared using Warner Braztler 3mm (Model TA.XT2i, Texture Analyser, Goldaming, Surrey, England) coupled with a texturometerStable TaTx, com with down speed of 200-250mm/min, probe route distance of 30mm from the base. Average shear force was calculated per sample and expressed in kgf.

Statistical analysis: A random casualized blocks was used, with unfolding liberty grades for treatments in factorial design 2 x 3 (with and without ractopamine X sexual condition, female, immunocastrated male and surgical castrated male).In order to evaluate the associated characteristics, all analyses were done with the PROC MIXED Statistical Analysis System, version9.1.3 (SAS Institute Inc., Cary, NC, USA).

III. Result

Meat quality*in natura*

The pH from Semimembranosus muscle (SM) did not show difference significant between gender and in treatments done 45 minutes post mortem. pH obtained after 24 hours of cooling for the same muscle also did not show significant difference ($P < 0.05$) between genders which received ractopamine in the diet. Therefore, groups with no ractopamine (NR) were significate different. Female meat pH was 2% higher in comparison to immunocastrated males and surgically castrated males and females with ractopamine supplementation presented higher pH than females with no ractopamine (Table 1).

Final values of pH (pH $LD_{24horas}$) from muscle LD (Table 1) differ from treatments and gender. Again, males presented pH values significantly lower than females. Males with ractopamine also presented final pH (pH $LD_{24horas}$) lower in comparison to females. Inside the ractopamine-free diet, immunocastrated showed a lower pH than females and surgically castrated. Those last ones, there were no differences on the pH.

Differences were also observed between treatments with and without ractopamine for females and immunocastrated ($P < 0.05$).

Table 1 – pH effect in SM (Semimembranosus) and in LD (*Longissimusdorsi*), measuraments of color (L*, a* and b*) of swine from different gender and castration methods (F=female, IM= immunocastrated male, CC= male surgically castrated) with (WR) or without (NR) supplemented ractopamine in the diet.

Variable	Treat	F	IM	CC	SE
pH SM $_{45\ min}$	WR	6.30 $^{a; A}$	6.50 $^{a; A}$	6.40 $^{a; A}$	0.073
	NR	6.50 $^{a; A}$	6.40 $^{a; A}$	6.30 $^{a; A}$	
pH SM $_{24\ hs}$	WR	5.70 $^{a; B}$	5.70 $^{a; A}$	5.70 $^{a; A}$	0.019
	NR	5.80 $^{a; A}$	5.70 $^{b; A}$	5,60 $^{b; A}$	
pH LD $_{24\ hs}$	WR	5.70 $^{a; A}$	5.50 $^{b; A}$	5,50 $^{b; A}$	0,026
	NR	5.50 $^{a; B}$	5.30 $^{b; B}$	5.40 $^{a; A}$	
L*	WR	55.2 $^{a; A}$	54.03 $^{a; B}$	54.76 $^{a; A}$	0.599
	NR	55.03 $^{a; A}$	55.57 $^{a; A}$	56.19 $^{a; A}$	
a*	WR	9.17 $^{a; A}$	9.65 $^{a; A}$	9.57 $^{a; A}$	0.376
	NR	9.67 $^{a; A}$	9.24 $^{a; A}$	10.42 $^{a; A}$	
b*	WR	3.59 $^{a; A}$	3.56 $^{a; A}$	3.54 $^{a; A}$	0.247
	NR	3.34 $^{a; A}$	3.41 $^{a; A}$	3.91 $^{a; A}$	

Averages in the same line, followed by the same letter do not differ among each other, by Tukey test; averages at the same column followed by same capital letter, do not differ among each other, by Tukey test; S.E. = standard error; pH SM 45 min = pH Semimembranosus measured after 45min post mortem; pH SM 24 hs = pH Semimembranosus measured 24hs post mortem; pH LD 24 hs = pH *Longissimusdorsi* measured 24hs post mortem; L*, a* and b* = color measurement on *Longissimusdorsi* with24h.

In Table 1, instrumental color was described (CIE Lab*) from *Longissimus dorsi* (LD) muscle from immunocastrated males with ractopamine, presenting a value 4% lower than L*, in comparison to animals who did not received ractopamine in the diet. For all other color parameters (a* and b*) there were no significant differences between gender and diets.

In relation to qualitative characteristics on Table 2, there were significate differences in the variables studied (gender X diet X castration method) for the shear force.

Table 2 – Effect on the sexual condition (F=female, IM= immunocastrated male, CC= male surgically castrated) with (WR) or without (NR) supplemented ractopamine in the diet on the qualitative characteristics of swine meat.

Variable	Treat.	F	IM	CC	S.E.
drip loss (%)	WR	9.11 $^{a; A}$	8.54 $^{a; A}$	8.64 $^{a; A}$	0.679
	NR	9.02 $^{a; A}$	8.65 $^{a; A}$	9.28 $^{a; A}$	
LFF (%)	WR	8.85 $^{a; A}$	7.04 $^{a; A}$	7.98 $^{a; A}$	0.695
	NR	7.47 $^{a; A}$	8.69 $^{a; A}$	7.99 $^{a; A}$	
LFC (%)	WR	27.07 $^{a; A}$	26.20 $^{a; A}$	27.37 $^{a; A}$	0.685
	NR	25.35 $^{a; A}$	25.53 $^{a; A}$	26.94 $^{a; A}$	
SF (kgf)	WR	3.14 $^{a; A}$	3.34 $^{a; A}$	2.86 $^{a; A}$	0.392
	NR	1.91 $^{a; B}$	2.21 $^{a; B}$	2.24 $^{a; A}$	

Averages in the same line, followed by the same letter do not differ among each other, by Tukey test; averages at the same column followed by same capital letter, do not differ among each other, by Tukey test; S.E. = standard error; LFF = loss for freezing; LFC = loss for coocking; SF = Shear force.

The loin from females and from immunocastrated males, without ractopamine presented higher tenderness (39.18% and 33.83%respectively) in comparison to those supplemented with ractopamine. This difference was not observed on surgically castrated males. For the animals with no ractopamine on their diet, there was no difference between gender or castration method on the qualitative characteristics (Table 2).

IV. Discussion

Meat quality in natura

pH is one of the most important characteristics regarding meat quality, being a parameter widely used to determine the level of meat acidification and alkalinity. In addition, it is one of the most commonly used measurements by industries (practicality and speed) to assist in the verification of meat quality, showing a strong association between the pH measured in the slaughter line and the qualitative characteristics of pig carcasses, such as the ability of water retention, color, tenderness, succulence and taste - properties associated with the solubility of myofibrillar and sarcoplasmic proteins [28].

The evaluation of the pH at the first post-mortem hour in the Semi membranous muscle (pH SM 45 min) is accepted as an important criterion to diagnose the final quality of the meat. The results for the effects of gender difference, castration type and dietary ractopamine supplementation for the SM 45 min pH are within the expected values for normal porcine meat, with an average value of 6.4. This demonstrates that under the conditions proposed in this project, the addition of ractopamine and the use of immunocastration simultaneously did not cause changes at the beginning of the muscle conversion process in meat. It should be noted that other random factors such as pre-slaughtering and room temperature also influence this process. This range of normality is described in the literature with values above 5.8 [29]. In pigs, when the pH reaches levels below 5.8 within 45 minutes' post mortem, there is evidence of PSE meat, according to Hofmann (1988) [30].

The Longissimus dorsi and Semi membranous muscles are the ones used to evaluate the quality of pork through the initial pH and / or reflectance measurements because they are accessible in intact carcasses. The pH values of these muscles, measured at the same time, correlate with each other indicating the pattern of pH decline in one muscle is similar to what is happening in the other muscle. In general, the initial pH of Longissimus dorsi is always slightly lower than Semimembranosus [31].

Regarding pH 24 hours, the lowest pH for animals without ractopamine supplementation was probably due to the absence of the effects of the β-adrenergic agonist, which promotes the stimulation of glycolysis of the Longissimus dorsi muscle (pH LD 24 hs). Such stimulation in the period prior to the slaughter of the animals may lead to a reduction in muscle glycogen stores. As a consequence, the production of lactic acid that leads to post-mortem acidification occurs in a limited way, thus generating a higher pH [32].

It should be emphasized that the results verified in this study corroborate with those described by several authors [33] for meat classified as normal according to pH. However, the pH measured in the immunocompromised animals that received the diet without ractopamine presented values tending to PSE meat (5.3) according to the classification described by Swatland (2008) [34], which allows to conclude that the castration method had an effect on the final pH. According to Cronin et al. (2003) [35] literature data indicate that due to the behavior of male pigs with higher level of aggressiveness and physical activity, muscle glycogen reserves can reduce, affecting the pH and quality of the meat of this type of animal. In the present study, the effect of ractopamine on the production of ractopamine in the treatment of rats was not significant ($P > 0.05$).

Bridi et al. (2006) [7] also found no increase in the incidence of PSE meat when studying the effect of ractopamine addition on the diet of pigs of the heterozygous and homozygous halothane genotype. Similarly, Pauly et al. (2009) [10], Gispert et al. (2010) [12], Škrlep et al. (2010) [36]did not describe differences between the pH of loins (Longissimus dorsi) of females, males surgically castrated and immunocastrated.

Although the results found for the initial pH of the carcasses (pH SM 45min) were not within the range considered PSE, the final pH of the LD (pH LD 24 hs) around 5.5 may have a relation with the values obtained for the drip loss. This exudation is possibly related to the drop in pH and consequently its influence on the integrity of myofibrillar proteins in the post mortem. Garrido et al. (1994) [37] found high correlations of final pH and water retention capacity (WRC) in the Semimembranosus and Longissimus thoracis muscles.

Ruiz (2007) [38], among others, affirmed that the most important effect of post-mortem denaturation of muscle proteins is loss of WRC, being that the point of least water retention capacity of the main proteins in the muscle of the isoelectric point, when the meats reach the final pH around 5.4-5.5. These proteins, at this pH, because they have positive and negative charges in equal quantity, are with the maximum approximation of the coarse and fine filaments, causing in the decrease or until the disappearance of the space between them. Such a condition makes it impossible to bind these molecules with water, reducing their capacity and water retention stability. And, it is generally accepted that the lower the final pH in the muscle, the water retention capacity of the muscle also decreases, resulting in an increased surface of muscle exudate or loss by dripping.

Although the assessment of water retention capacity as measured by drip loss% verified in this study did not demonstrate significant differences between genders, ractopamine supplementation and castration methods, the values obtained were within a range considered acceptable as proposed by Warriss and Brown (1993) [39], all results being close to the 10% normal meat limit proposed by the authors.

However, other values for the percentage of drip loss are found in the literature, and the range of 2.0 [40] is also used for the classification of meat as normal, at <5.0 [21], accepting up to 6.7 [41].

As in this study, other authors have shown that the % of drip loss was not influenced by ractopamine supplementation, by genders, or castration method [42, 43]. Lowe (2013) [44] evaluating the interaction of immunocastration and ractopamine supplementation also did not observe changes in drip loss when ractopamine levels increased in the diet, as did Patience et al. (2009) [45] studying the inclusion of 5ppm in the diet, and Athayde et al. (2012) [43], who also found no effect of the inclusion of ractopamine (0.5 and 10ppm) on the back of surgically castrated males and females.

On the other hand, other studies found a positive effect, reducing drip loss with ractopamine supplementation [47, 48]. Hinson et al. (2014) [48], providing 7.4 ppm of ractopamine, obtained lower drip loss when compared to control. Garbossa et al. (2013) [49] evaluating the effect of different inclusion levels of ractopamine (0, 5, 10, 15 and 20 ppm) on surgically castrated males and females found that the loss of drip water decreased linearly as ractopamine dose increased in the diet. Rocha et al. (2013) [50] investigating the effect of ractopamine supplementation, castration method concluded that the supply of ractopamine decreased drip losses. The results showed higher drip loss when compared to other studies [51, 52].

Drip losses depend on the shortening of sarcomeres, which is regulated by the interaction of muscle temperature and development of rigor mortis. Thus, the cooling conditions are very important. However, the main point is the speed and extent of the pH drop after slaughter. All factors that influence the occurrence of quality deviations such as PSE, DFD, acidic meat, CSR, NFP will inevitably affect the degree of drip loss as well. In a study by Fisher (2007) [53], one-third of the loins with drip loss greater than average were red rather than pale, and a third of the loins with a higher than average brightness was somewhat exudative. There was a slight tendency for more drip loss when the live weight at slaughter is increased from 110 to 160 kg. This may be the effect of a slower cooling rate, probably due to the thicker fat layer.

Color is one of the most important sensory attributes that influences the purchase decision, and the instrumental luminosity (L*) is one of the parameters used to classify pork in normal RFN (normal meat, without anomalies), PSE (pale, soft and exudative meat), RSE (normal, soft and exudative color) or DFD (hard, dark and firm flesh) measured using a colorimeter. According to Marchiori and Felicio (2003) [54], the verification of the instrumental analysis of meat color in meat samples of different species is used to determine the luminosity (L*), red intensity (a*) and yellow intensity (b*) operating in the Commission Internationale de l'Éclairage - CIE (L*, a*, b*) system. It should be emphasized that in evaluating pork quality it is interesting to associate the results of two or more quality characteristics to a safer estimate [55].

The American Meat Science Association (AMSA) admits L * values between 49 and 60 within the normal standards of meat quality, which was the benchmark adopted in this study.

Silveira et al. (2006) [56] obtained a statistical difference in the instrumental color (L *, a * and b *) between meat from surgically castrated and immunocastrated males, as well as Tonietti (2008) [57] verified that immunocastration influenced L *, a * and b * Longissimus dorsi muscle. Already, Caldara et al. (2013) [58] obtained lower values of L* for the immunocastrated, without verifying differences for the valuesof a* and b*. Škrlep et al. (2012) [51]verified differences between the L*, a* and b* values of whole, male, surgically castrated and immunocastrated males.

However, Pauly et al. (2009) [10], Gispert et al. (2010) [12] and Athayde et al. (2010) [59] (L*, a* and b*) in the Longissimus dorsi muscle, as well as Gökdal et al. (2010) [60] reported that there were no significant differences between pigs, females and castrated pigs physically for objective color values (L*, a* and b*). When evaluating the swine of immunoassayed pigs, did not find any effect of the immunocastration in the parameters of objective color. Škrlep et al. (2010) [36] only found a difference in the values ofb*, noting significant differences in the other color values (L* and a*) among immature and physically castrated pig loins and b* between genders (surgically castrated males, immunocastrated and females). Lowe (2013) [44] found difference between the genders (females, males immunocastrated and surgically castrated) only in the values of b*.

Regarding ractopamine supplementation, different from what was found in this scientific investigation, Carr et al. (2005, 2009) [47, 61], Gonzalez et al. (2010) [62], Hinson et al. (2014) [48] and Formighieri (2012) [21] found no significant difference in pig meat coloration supplemented with ractopamine. However, Leal et al. (2014) [63] studied different levels of ractopamine in the diet (0,3,6,9,12 and 15ppm) verified a quadratic effect for the parameters a* and b*. Uttaro et al. (1993) [64]also reported alterations in a * and b * in loin and in pork ham supplemented with 20ppm ractopamine. Patience et al. (2009) [45] found that supplementation with 5 and

10 ppm of ractopamine did not alter L*, a* and b*, but supplementation with 20 ppm resulted in changes in meat color.

Hinson et al. (2014) [48] found lower values of b* in loins of animals treated with RAC. However, Stites et al. (1994) [65] and Watanabe (2009) [11] found no significant differences in color values (L*, a* and b*) for the animals treated with RAC.

However, for the classification of pork quality, there is in the literature discrepancy between the values of luminosity for these standards. Warner, Kauffman and Greaser (1997) [66] classified as a function of the luminosity (L*) of the loin PSE 54.9 - 56.1, RSE 46.6 - 48, RFN 44.8 - 46.2 and DFD 37.6 - 39. Van Laack and Kauffman (1999) [67] adopted the values of L* 55.9 ± 0.5 for PSE meat, 47.2 ± 0.4 for CSR and 45.1 ± 0.5 for RFN. Joo et al. (1999) [68] defined the L* values of 54.0 for PSE, 46.1 RSE, 44-46.5 for RFN and 41.7 for DFD. Ramos and Gomide (2009) [69] describe the values of L* for normal meat to be within the range of 45 to 53.

The values of the instrumental tenderness expressed by the shear force, measured in a texturometer, obtained in this study showed that there was a difference between genders, castration method and ractopamine supplementation. The supply of ractopamine in the diet negatively influenced the lean tenderness of the male and female pigs when compared to the animals of the same genus that did not receive supplementation and the surgically castrated males. However, in surgically castrated males such an effect has not been verified.

In the literature several references of objective texture and subjective (sensory) tenderness as well as the correlation between them are found as parameters for the classification of tenderness and hardness ranging from 2.24 to 3.01kgf [55], 3.88kgf (38N) [70], 4.28kgf (42N) [71] to 6.0kgf, in this study, the reference value adopted was 3.2kgf as the limit between tenderness and hardness for meat matured for seven days [72]. Hamill et al. (2012) [73] reported that in laboratory studies with sensory panels, indicated values of shear force of pork above 4.69kgf are considered "hard". Therefore, it is possible to classify as tender the meat of all the treatments, since the highest value for the shear force was 3.34kgf without maturation.

The value obtained for the shear force in the meat of the animals, which did not receive supplementation with ractopamine, immunocastrated male, surgically castrated and females, in this study was lower than that verified by several authors such as Pauly et al. (2009) [10] who found a significant effect of immunocastration on meat tenderness. The use of immunocastration resulted in significantly lower values (3.45 kgf) of shear force in the Longissimus dorsi muscle when in comparison to castrated males (3.70 kgf) and whole males (3.77 kgf).

Other authors also reported higher shear force values such as Tonietti (2008) [57], which obtained shear force values of 4.10 and 4.22 for surgically castrated and immunocastrated males respectively, and Formighieri (2012) [21], who obtained 3.42 kgf for immunoassayed males, 3.30 kgf for surgically castrated males and 3.29 kgf for females. Caldara et al. (2013) [58], who studied the characteristics of the carcass and meat quality of female pigs, immunoscastrated and surgically castrated, obtained values of 3.61, 4.17 and 3.63 kgf, respectively, and considered the values adequate for swine meat tenderness. Li et al. (2015) [74] found that immunization against GnRF slightly increased the shear force of the loin.

Differently from that verified by Silveira et al. (2006) [56], who found no difference in the shear force of the surgically castrated and immunocastrated males, and Athayde et al. (2012) [43], who did not observe differences in the objective texture of the loins of surgically castrated males and female, found the value of 5.20 kgf.

Elsbernd, Patience and Prusa (2016) [75] also did not verify differences in shear force, color, pH, cooking loss, when they compared the quality and sensorial characteristics of the pork meat of different genera and type of castration, and found no differences in sensory (odor and taste), physical and chemical characteristics among females, males immunocastrated and surgically castrated, except for marbling in frozen samples. The results showed that porcine meat derived from immunologically castrated males is similar to the meat of physically castrated males in terms of sensory quality characteristics and between fresh and frozen products.

One of the causes of the greater shear force in β-adrenergic fed pigs may be related to the increase of the diameter of the muscular fibers and reduction of the post-mortem proteolysis. According to Walker et al. (1989) [76] the shear force is dependent on the inclusion of beta-agonist, that is, the higher the level of inclusion, the harder the flesh. Studies have reported an inverse relationship between meat tenderness and dietary supplementation with ractopamine [77, 78].

The results of the shear force of this study, for supplementation with 10ppm of ractopamine for females (3.14kgf), males surgically castrated (2.86kgf) and immunocastrated (3.34kgf) when compared to the ractopamine group, corroborate with those verified by Formighieri (2012) [21], who found no difference between the control group (3.15kgf) and that supplemented with 7.5ppm ractopamine (3.51kgf).

Leal et al. (2014) [63] observed a linear increase ($P < 0.05$) in the shear force of the LD muscle of the pigs as the levels of ractopamine increased in the diet, that is, every 1.0ppm of ractopamine occurs a variation of

235.789 grams in the force of shear. Amin (2013) [80] found that supplementation of 20ppm of ractopamine for 28 and 35 days increases the shear force of pork by studying the effect of different levels and time of supplementation with ractopamine on pork quality. Silva (2013) [79] also reported a 13% increase in meat texture of supplemented animals when compared to those not supplemented, as did Athayde et al. (2012) [43], who also found a higher shear force (5.90 kgf) for supplementation with 10ppm of ractopamine when compared to 5ppm (5.04kgf) and no supplementation (4.56kgf).

Cooking and thawing losses are also indicative of muscle water retention capacity as well as protein integrity. According to Filho, Braga and Mata et al. (2002) [81] when muscles contract during freezing, there is a danger of breaking down the cellular structures that will only be noticed during the defrosting in the form of exudate, which is caused by the rupture of the meat cells. Defrost losses can reach 47% of the total weight, due to the severe contraction that occurs during the process.

The same factors that act on exudation weight loss also influence the weight loss of cooked meat, since the relative differences remain after heating. Baking loss is due to the shrinkage of myofibrils during the process, and may vary over time and cooking temperature, since elevated temperatures can cause protein denaturation and intermuscular fat loss [58].

Gender, castration method and ractopamine supplementation did not influence the water losses due to thawing and cooking ascertained in this scientific work. These results are similar to those verified by several authors who did not observe significant differences in the percentages of weight loss in cooking, whether in ractopamine supplemented or not, of different genera or castration methods [82].

Therefore, authors reported reduction in % of cooking losses when animals were supplemented with ractopamine. Oliveira (2016) [22] obtained a reduction of 17.3% in cooking loss with the use of ractopamine, even though it was perceived by Betts (2011) [82] when compared to the control of 5% and 10ppm of ractopamine. Leal et al. (2014) [63] verified the loss of liquid in the thawing of the loin, with linear effect of ractopamine supplementation in the diets. The increase in the amount of ractopamine had a reduction in the rate of liquidity without thawing, reaching 5.30% with a dose of 15ppm, that is, since it increased the amount of ractopamine decreased the losses by thawing and, in the losses by cooking, 9ppm of ractopamine showed the lowest percentage of loss (5.29%).

The percentages of loss by capture of consciousness in the research were similar to those reported by Škrlep et al. (2012) [51], Caldara et al. (2013) [57], and Silva (2013) [79]. Lower than those verified by Leal et al. (2014) [63], and higher than the results obtained by Formighieri (2012) [21]. And, the losses by thawing were lower than those described by Silva (2013) [79] and, higher than those observed by Leal et al. (2014) [63].

V. Conclusion

Although values for drip loss were high, other characters used as retention water capacity – loss for cooking and thawing were inside normal average range. Ractopamine addition in the diet and the castration method did not interfere negatively on the meat quality parameters analyzed. Gender also did not show significant differences with the use of ractopamine.

Acknowledgements

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