

Effect of Natural Bioactive Compounds versus Ractopamine: C) Quality of Pork Meat

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Abstract

Background: The removal of ractopamine from pork production, demanded by both export and local markets, represents a challenge for the pork industry. This study evaluated the effect of AO NATURAL FIT PRO[®] as an alternative to ractopamine on meat quality in finished pigs. **Methods:** Thirty-two castrated male pigs (62 ± 2.5 kg initial weight) were randomly assigned to four treatments: control, AO NATURAL FIT PRO[®] for 8 weeks, ractopamine 10 ppm for 5 weeks, and a combination of both additives. Carcass characteristics, meat quality, and fatty acid profile were evaluated. **Results:** Color parameters were affected, especially in the *Longissimus dorsi* muscle, where group T4 showed a lower intensity of red color. No significant differences were observed in water-holding capacity, tenderness, or fatty acid profile. **Conclusions:** AO NATURAL FIT PRO[®], ractopamine, and their combination improved carcass characteristics without compromising the nutritional quality of the meat. The natural additive represents a viable alternative to ractopamine for optimizing pork quality.

Keywords

Pigs, Productivity, Natural Bioactive Compounds, Ractopamine, Meat Quality

1. Introduction

Population growth and per capita consumption have been exacerbated by economic development and rising incomes, as well as by demands for higher quality meat, primarily pork. This has generated a high demand for food products [1]. In this context, pork production is geared towards supplying meat consumption, both lo-

cally and for export. However, the use of inputs and raw materials significantly influences the final price of the product [2].

To optimize the profitability of pig farms, the use of feed additives and supplements, such as antibiotics, hormones, and β -agonists, is common [3]. These allow for the implementation of precision feeding programs that help animals express their maximum genetic potential at different physiological stages, thus improving traceability and farm sustainability [2]. In the livestock industry, certain additives are used to improve feed efficiency, increase growth performance, and decrease carcass fat content without altering quality [4] [5].

Among these additives, ractopamine hydrochloride (RAC) stands out. It is a phenylethanolamine with a structure similar to that of natural β -agonists (epinephrine and norepinephrine) and synthetic ones such as clenbuterol [6]-[8]. Pharmacologically, RAC stimulates β_1 and β_2 adrenergic receptors in skeletal muscle and adipose cells, activating signaling pathways that promote lipolysis and, consequently, increase lean carcass tissue [9]-[11]. Despite its approval in countries such as the United States, Canada, Mexico and Brazil, the use of RAC is prohibited in the European Union, China and other nations [12] [13].

The use of β -agonists has been questioned due to their adverse effects. Animals treated with RAC have been reported to be more susceptible to injury and anxiety during transport, an unavoidable process in intensive farming that itself induces stress and negative physiological changes, such as weight loss, fractures, and reduced muscle glycogen. The latter affects the proper transformation of muscle into meat, causing quality problems (dark cuts or pale, soft, and exudative meat) and economic losses [14]-[16]. Furthermore, the use of these additives has been associated with increased mortality rates and leg injuries in cattle [8] [17]. The presence of residues of these compounds in meat for consumption poses a potential risk to human health, which underlines the need to investigate the mechanisms of protection of the human body against these additives and to obtain scientific evidence on their toxicity [5] [8].

As an alternative, the use of phytogetic additives, bioactive compounds derived from plant extracts rich in antioxidants such as phenols, flavonoids, vitamins, and minerals, has become widespread in the last decade [18]-[20]. These compounds promote the elimination of free radicals and protect the organism from oxidative stress, improving the overall health of animals [18] [21]. The combination of these compounds with methyl group donors and organic acids, along with good management practices, is presented as a key strategy for maximizing productive performance sustainably [18] [22].

The withdrawal of ractopamine, mandated by both export and domestic markets, coupled with the negative effects of stress, presents a challenge for the swine industry in Mexico. This creates a need to find effective substitutes that do not compromise human health and allow unrestricted access to international markets. Consequently, this study was designed to evaluate the effect of the AO NATURAL FIT PRO® supplement as an alternative to ractopamine on carcass characteristics

and meat quality in pigs during the growing-finishing stage.

2. Materials and Methods

2.1. Location and Experimental Conditions

The study was conducted at a commercial farm, in a building designated exclusively for the experiment. This allowed the animals to be kept free from external disturbances and under controlled and standardized environmental and management conditions.

2.2. Animals, Housing, and Management

Thirty-two castrated male pigs from a commercial line, in the growing-finishing stage, with an average initial live weight of 62 ± 2.5 kg, were used. The animals were randomly assigned to four treatments, with eight replicates per treatment. Each pig was housed in an individual 1.2 m² pen, equipped with a hopper feeder and nipple drinker, and was considered the experimental unit. Feed and water were offered *ad libitum* throughout the experimental period. To maximize voluntary intake, 10% more feed was provided than the amount consumed the previous day.

2.3. Experimental Diets and Treatments

The basal diets (**Table 1**) were formulated to meet or exceed the nutritional requirements for pigs in the growing and finishing phases. All treatments were supplemented with the following enzymes: phytase (10 g/100 kg), xylanase (15 g/100 kg), protease (40 g/100 kg), and a mycotoxin adsorbent (Micofix, 150 g/100 kg). The treatments were:

- **Control:** Basal diet without additives (no ractopamine and no AO Natural Fit Pro supplements).
- **AO NATURAL FIT PRO®:** Basal diet supplemented with the additive for the 8 weeks prior to slaughter. AO NATURAL FIT PRO® is a bioactive compound derived from plants containing extracts of purple basil, oroval, Indian gooseberry, and garlic, which function as antioxidants and help protect animals from oxidative stress. Other derivatives include tannins, found in fruits and seeds; these are polyphenols with antioxidant and anti-inflammatory properties used in nutrition for health benefits.
- **Ractopamine:** Basal diet supplemented with 10 ppm of ractopamine for the 5 weeks prior to slaughter.
- **AO NATURAL FIT PRO® + Ractopamine:** Basal diet supplemented with both additives, administered according to the timings of treatments 2 and 3, respectively.

The study was conducted in two phases: 1) Meat quality analysis and laboratory chemical analysis.

At the end of the experimental period, the 32 pigs were transported to the municipal slaughterhouse in Toluca, State of Mexico, where they were slaughtered

using humane methods approved by current regulations. The hot carcass weight (HCW) was obtained, and the relative weights of the carcass and its components were calculated in relation to the final live weight (g/kg LW).

For the chemical analysis of the diets, 250 g samples of feed were collected weekly from each phase (grower and finisher). At the end of the study, the samples from each phase were mixed to form a composite sample, which was ground (1 mm sieve) and analyzed in duplicate to determine the dry matter, ash, crude protein, and ether extract content, according to AOAC methods [23].

Table 1. Composition of ingredients of the basal diet and nutrient composition for pigs from 50 to 100 kg of live weight.

Ingredients (kg)	Treatment				Nutritional requirements (NRC 2012)
	Control	AO NATURAL FIT PRO*	Ractopamine	AO NATURAL FIT PRO* + Ractopamine	
Corn	55.11	54.96	55.06	54.96	
Sorghum	9.2	9.2	9.2	9.2	
Soybean meal	26.51	26.51	26.51	26.51	
Wheat bran	5	5	5	5	
Vegetable oil	1	1	1	1	
Vitamin premix	0.05	0.05	0.05	0.05	
Mineral premix	0.1	0.1	0.1	0.1	
Calcium carbonate	1.3	1.3	1.3	1.3	
Orthophosphate	0.8	0.8	0.8	0.8	
Lysine	0.4	0.4	0.4	0.4	
Threonine	0.3	0.3	0.3	0.3	
Methionine	0.2	0.2	0.2	0.2	
AO NATURAL FIT PRO*	-	0.15	-	0.1	
Ractopamine	-	-	0.05	0.05	
Additives ¹	0.0215	0.0215	0.0215	0.0215	
Total	100	100	100	100	
Calculated nutritional value					
Dry matter (%)	89.1	89.1	89.1	89.1	
Net energy (Mcal/Kg)	2.50	2.50	2.50	2.50	2.47
Crude protein (%)	16	16	16	16	
Ether extract (%)	3.29	3.29	3.29	3.29	
Crude fiber (%)	3.64	3.64	3.64	3.64	

Continued

Calcium (%)	0.76	0.76	0.76	0.76	0.75
Phosphorus (%)	0.63	0.63	0.63	0.63	0.62
Lysine (%)	1.20	1.20	1.20	1.20	1.16
Threonine (%)	0.80	0.80	0.80	0.80	0.74
Methionine (%)	0.38	0.38	0.38	0.38	0.34

¹Included in all groups = Phytase 10 g/100 kg, Xylanase 15 g/100 kg, Protease 40 g/100 kg, Micofix 150 g/100 kg.

2.5. Meat Quality Assessment

The yield of the hot carcass (immediately post-slaughter) and the chilled carcass (24 hours post-slaughter) was determined. Subsequently, the yield of the primal cuts was evaluated.

From the 32 carcasses, samples were taken from the *Longissimus dorsi*, *Semi-membranosus*, and *Biceps* muscles for the following analyses:

1) Chemical composition: Dry matter, moisture, crude protein, ash, and ether extract content were determined following the official AOAC methods [23].

2) Shelf life: pH was measured with a portable potentiometer and temperature with a penetration thermometer at 0, 3, 6, 9, and 12 days post-slaughter.

3) Cooking water loss: This was quantified using gravimetric methods, following the AMSA protocol [24].

4) Cutting force: It was measured using a Warner-Bratzler blade on samples of the *Longissimus dorsi* muscle.

2.6. Color Analysis

The color of the *Longissimus dorsi* muscle was evaluated on days 0, 3, 6, and 9 post-slaughter. One-cm-thick slices were exposed to atmospheric oxygen for 30 minutes to allow oxymyoglobinization. Subsequently, the CIE color coordinates L* (luminosity), a* (red-green index), and b* (yellow-blue index) were measured using a Konica Minolta Chroma Meter CR-300 colorimeter (Konica Minolta, Tokyo, Japan), according to the methodology of the International Commission on Illumination CIE [25]. From these coordinates, the chroma (C*) and hue angle (h°) were calculated.

2.7. Fatty Acid Analysis

Intramuscular fat extraction from the *Longissimus dorsi* muscle was performed according to the method of Bligh and Dyer [26]. Ten grams of meat were homogenized with a chloroform/methanol mixture (2:1) in a 10:1 (v/w) ratio. The solvent was evaporated at 50°C using a rotary evaporator.

Fatty acid methyl esters (FAMES) were prepared and analyzed by gas chromatography. The sample was resuspended in 10 mL of hexane, filtered through 0.22 µm Whatman filter paper, and transferred to vials. An Agilent 6890N gas chro-

matograph coupled to an Agilent 5973 mass spectrometer, with a 7683 series autosampler and an Omega Wax 250 column (Agilent Technologies, Santa Clara, CA, USA), was used, with helium as the carrier gas. FAME identification was performed by comparing retention times with a 37-component reference standard (Supelco, Bellefonte, PA, USA).

2.8. Statistical Analysis

The data were analyzed using an analysis of variance (ANOVA) for a repeated measures design (0, 3, 6, and 9 days post-slaughter), using the GLM procedure of the SAS software [27]. The statistical model used was:

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij},$$

where y_{ij} is the observation value,

μ is the population mean,

τ_i is the treatment effect, and

ε_{ij} is the experimental error.

It was assumed that the experimental error followed a normal distribution. Differences between treatment means were considered significant at a $p < 0.05$ level and were compared using Tukey's test.

3. Results

The pigs used in this study remained clinically healthy throughout the experimental phase.

3.1. Muscle Color

In the *Semimembranosus* muscle, a significant difference ($P = 0.042$) was observed in terms of lightness (L^*). The control group (52.97) was significantly lighter than the other three treatment groups, indicating that the additives, alone or in combination, made the meat of this muscle slightly darker. In the *longissimus dorsi* and *Biceps* muscles, there were no significant differences between treatments. However, in the *Longissimus dorsi* muscle, there was a greater effect: the control group (10.18) and AO NATURAL (10.14) showed a significantly more intense red color (a^*) than the AO NATURAL FIT PRO® + ractopamine group (6.63). The use of ractopamine, and especially its combination with AO NATURAL, significantly decreased the red color of this muscle. The control group (5.69) had a significantly higher b^* hue than the AO NATURAL FIT PRO® + ractopamine group (3.23), indicating that the latter treatment reduced the yellow hue. In the *Longissimus dorsi*, the control (12.29) and AO NATURAL (12.00) groups had a significantly more intense color (C^*) than the AO NATURAL FIT PRO® + ractopamine group (8.02), confirming that the meat treated with the combination has a paler or less saturated color. In the *Biceps* muscle, all treatments resulted in a significantly lower hue angle (H^*) ($P = 0.043$) compared to the control (31.48). This indicates a change in the overall hue of the meat. The information is shown in **Table 2**.

Table 2. Color of the muscles evaluated in pigs in the growth-finishing stage.

Parameter	Muscle	Control	AO NATURAL FIT PRO ^o	Ractopamine	AO NATURAL FIT PRO ^o + Ractopamine	SEM ¹	P-value
L *	<i>Longissimus dorsi</i>	55.07	54.59	53.97	53.73	1.69	0.941
	<i>Semimembranous</i>	52.97 ^a	50.77 ^b	50.25 ^b	50.13 ^b	0.75	0.042
	<i>Biceps</i>	54.14	50.91	52.37	50.8	1.38	0.303
a*	<i>Longissimus dorsi</i>	10.18 ^a	10.14 ^a	8.27 ^{ab}	6.63 ^b	0.66	0.001
	<i>Semimembranous</i>	8.99	9.95	7.38	7.17	0.85	0.856
	<i>Biceps</i>	9.63	10.14	7.99	7.26	0.91	0.109
b*	<i>Longissimus dorsi</i>	6.76	6.42	5.33	4.32	0.7	0.08
	<i>Semimembranous</i>	4.53	4.79	3.55	4.65	0.64	0.459
	<i>Biceps</i>	5.69 ^a	4.70 ^{ab}	4.12 ^{ab}	3.23 ^b	0.55	0.027
C*	<i>Longissimus dorsi</i>	12.29 ^a	12.00 ^a	9.68 ^{ab}	8.02 ^b	0.88	0.005
	<i>Semimembranous</i>	10.13	10.96	8.14	8.75	1.02	0.219
	<i>Biceps</i>	11.26	11.01	9.05	7.98	1.02	0.093
H*	<i>Longissimus dorsi</i>	32.74	32.9	30.43	31.3	2.39	0.864
	<i>Semimembranous</i>	27.56	25.2	24.78	29.03	1.71	0.271
	<i>Biceps</i>	31.48 ^a	24.10 ^b	25.01 ^b	23.79 ^b	2.04	0.043

¹Standard error of the mean. ^aGreater difference, ^bSmaller difference. L* (luminosity), a* (red-green) and b* (yellow-blue), the intensity of color (C*) and the Hue angle (H*).

3.2. Meat Quality Parameters

The analysis of meat quality parameters, specifically water loss and tenderness (measured by shear force), was evaluated in the *Longissimus dorsi*, *Semimembranosus*, and *Biceps* muscles. The Warner-Bratzler (WBSF) method was used to measure shear force in pork, which determines tenderness by measuring the maximum force required to cut through a cylindrical sample of meat. For sample size, it is recommended to extract at least six (6) cores (cylinders) from pork loin or chop to obtain an accurate average. The core diameter should be a standard 1.27 cm (0.5 inches), and the cores should be extracted parallel to the muscle fibers so that the blade cuts perpendicular to them. The thickness of the meat relative to the original chop or loin should be approximately 2.5 to 3.5 cm [28]. The results are presented in **Table 3**. In general terms, the inclusion of the additives in the diet did not generate a statistically significant effect on the variables evaluated in the different muscles; however, some numerical trends were observed.

Table 3. Shear force and water loss of the *Longissimus dorsi*, *Semimembranosus* and *Biceps* muscles.

Parameter	Treatment				SEM ¹	P-value
	Control	AO NATURAL FIT PRO [®]	Ractopamine	AO NATURAL FIT PRO [®] + Ractopamine		
% water loss due to cooking (<i>Longissimus dorsi</i>)	31.17	29.39	32.31	31.90	0.855	0.103
% loss of water by boiling (<i>Semimembranosus</i>)	32.72	33.14	33.15	29.40	1.324	0.156
% water loss due to cooking (<i>Biceps</i>)	32.65	31.11	30.10	28.79	1.370	0.258
Shear strength (<i>Longissimus dorsi</i>) (kg/cm ²)	2.92	2.56	2.77	3.20	0.370	0.673
Shear strength (<i>Semimembranosus</i>) (kg/cm ²)	2.68	2.87	2.58	2.11	0.244	0.176
Shear strength (<i>Biceps</i>) (kg/cm ²)	3.05	2.61	2.36	2.05	0.277	0.097

¹Standard error of the mean.

3.3. Proximate Chemical Composition

Table 4 presents the nutritional content information of the *Longissimus dorsi* muscle of pigs fed with AO NATURAL FIT PRO[®] and Ractopamine in growth-finishing stages, where no significant differences ($P > 0.05$) were observed with respect to % Dry Matter, % Moisture, % Ash, % Crude Protein and % Ether Extract between treatments.

Table 4. Nutritional content of the *Longissimus dorsi* muscle of pigs fed with AO NATURAL FIT PRO[®] and Ractopamine in growth-finishing stages.

Parameter	Treatment				SEM ¹	P-value
	Control	AO NATURAL FIT PRO [®]	Ractopamine	AO NATURAL FIT PRO [®] + Ractopamine		
Dry matter (%)	29.82	28.88	28.82	28.73	0.318	0.076
Moisture (%)	72.22	73.07	73.35	73.13	0.317	0.084
Ash (%)	0.912	1.19	1.11	1.09	0.066	0.108
Crude protein (%)	22.62	22.7	22.99	22.84	0.336	0.795
Ether extract (%)	6.29	4.99	4.72	4.80	0.461	0.0719

¹Standard error of the mean. ^aGreater difference, ^bLesser difference.

3.4. Fatty Acid Profile and Nutritional Stability

No statistically similar differences were found between treatments in the fatty acid content of the *Longissimus dorsi* muscle, indicating that the quality was not affected by the inclusion of AO NATURAL FIT PRO[®] (**Table 5**).

Table 5. Fatty acid content of the *Longissimus dorsi* muscle.

Fatty acids (g/100 g AG)	Treatment				EEM ¹	P-value
	Control	AO NATU- RAL FIT PRO [®]	Ractopamine	AO NATURAL FIT PRO [®] + Ractopamine		
C12:0 (Lauric)	0.045	0.045	0.046	0.050	0.011	0.989
C14:0 (Myristic)	0.985	0.962	0.896	0.920	0.109	0.926
C16:0 (Palmitic)	25.21	25.14	25.91	24.48	0.746	0.644
C16:1 (Palmitoleic)	1.78	1.62	1.37	1.71	0.162	0.384
C18:0 (Stearic)	13.47	13.22	13.63	12.30	0.601	0.436
C18:1n9c (Oleic)	41.96	43.77	41.42	44.00	1.601	0.338
C18:2n6c (Linoleic)	11.33	11.34	12.30	12.56	0.893	0.674
C18:3n3 (Linolenic)	0.246	0.107	0.122	0.116	0.062	0.351
Cis 11 Eicosenoic	0.296	0.244	0.202	0.355	0.079	0.684
C20:2n6 (CIS.11,14-EICOSADIEN)	0.176	0.201	0.160	0.166	0.048	0.928
C20:3n3 (CIS-11,14,17-EICOSAT)	1.286	1.385	1.264	1.251	0.316	0.988
C22:2n6 (CIS-13,16-DOCOHEXADI)	0.022	0.032	0.078	0.023	0.013	0.059
Other fatty acids	1.636	1.174	1.706	1.278	0.291	0.503
AGS2	39.718	39.379	40.496	37.752	1.150	0.435
AGM3	44.710	46.383	43.870	46.850	1.198	0.305
AGP4	13.935	13.064	13.928	14.120	1.220	0.917
AGP n-3 5	1.526	1.488	1.386	1.368	0.306	0.979
AGP n-6 6	12.408	11.576	12.542	12.752	0.985	0.824
n-6/n-3 7	10.438	8.636	10.188	10.827	1.652	0.772

¹Standard error of the mean.

3.5. Evolution of Muscle pH Post-Slaughter

Regarding the pH of the *Longissimus dorsi* muscle, no significant differences were found on days 0, 3, 9 and 12; however, on day 6, statistically significant differences ($P < 0.05$) were determined in the treatment containing AO NATURAL FIT PRO[®] + ractopamine, which had the highest pH value (5.70) compared to the control treatments, AO NATURAL FIT PRO[®] and ractopamine (5.57, 5.53 and 5.63 respectively, **Figure 1**).

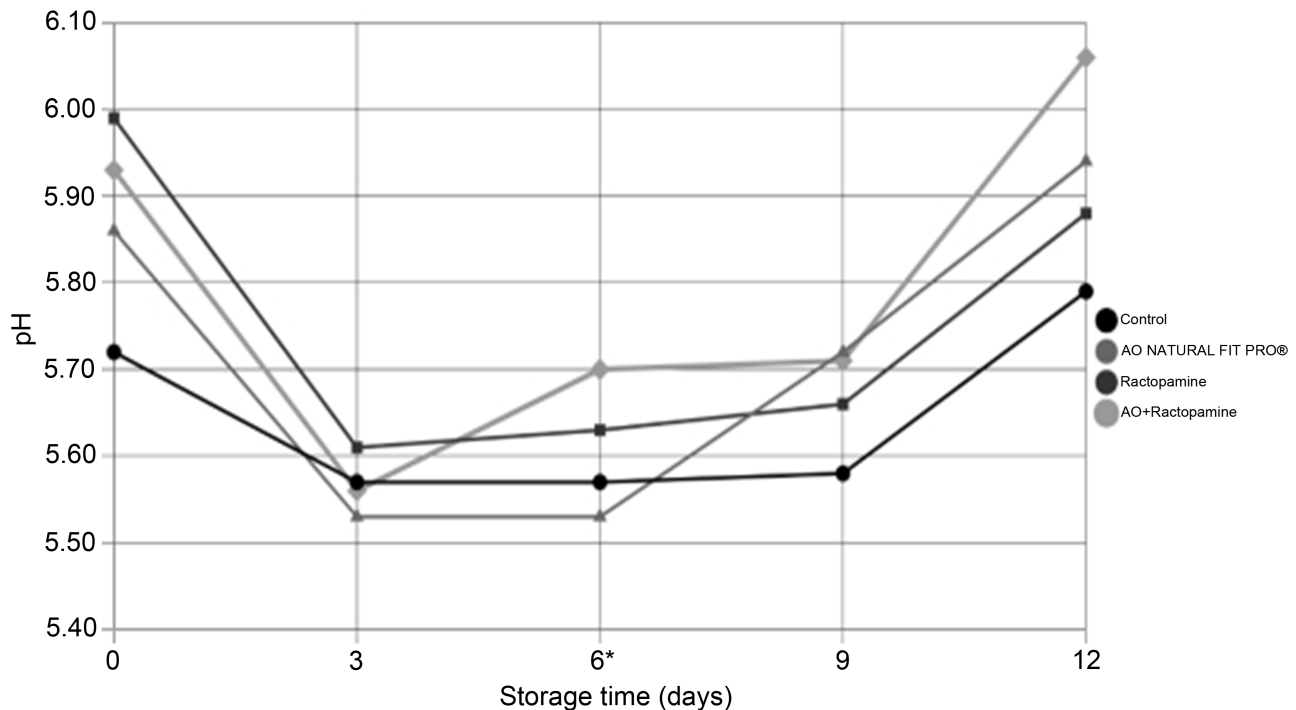


Figure 1. Evolution of pH in the *Longissimus dorsi* muscle during refrigerated storage. Treatments: Control (●), AO NATURAL FIT PRO® (▲), Ractopamine (■), AO NATURAL FIT PRO® + Ractopamine (◆). The asterisk (*) indicates significant differences between treatments on day 6 ($P < 0.01$).

4. Discussion

4.1. Muscle Color

The treatments showed significant effects on color characteristics in different muscles. In the *Semimembranosus* muscle, all treated groups exhibited lower lightness (L^*) compared to the control (52.97), indicating that the additives slightly darkened the meat [29] [30]. It has been documented that the duration of color improves with diets supplemented with ractopamine hydrochloride approximately 26 days before slaughter [30].

In diets with 10 ppm of ractopamine, the color of the *Longissimus dorsi* tends to be lighter and less red [31]. In our study, the *Longissimus dorsi* showed the most pronounced changes: the AO NATURAL FIT PRO® + ractopamine group exhibited less intense red color ($a^* = 6.63$), even compared to the ractopamine group (8.27) versus the control (10.18) and AO NATURAL (10.14). Several studies have observed that the addition of ractopamine to the diet tends to produce less red meat [32] [33].

The lesser yellow hue of the *Biceps* (AO NATURAL FIT PRO® + ractopamine = 3.23 versus 5.69 for the control) and lower overall saturation (AO NATURAL FIT PRO® + ractopamine = 8.02 versus 12.29 for the control) are consistent with Pereira *et al.* [34] and Bergstrom *et al.* [30], who documented the impact of ractopamine on color intensity, although it does not typically affect product quality.

In the *Biceps* muscle, all treatments resulted in lower hue angles (H^*) compared

to the control (31.48), indicating changes in overall color perception. Although ractopamine and other additives can improve lean meat yield and reduce fat [35], these benefits can compromise color quality, a crucial factor for consumer acceptance. Notably,

4.2. Meat Quality Parameters

A key finding of this study is that the positive effects of the additives on carcass yield and characteristics did not translate into significant alterations in the technological quality of the meat. No significant effects were observed on water-holding capacity or tenderness in the *Longissimus dorsi*, *Semimembranosus*, and *Biceps* muscles ($P > 0.05$).

This lack of significant effects suggests that, although the evaluated additives improve quantitative aspects of production, their impact on the technological properties of the meat is limited. The scientific literature presents contradictory results regarding the effect of ractopamine on meat quality. Athayde *et al.* [36] reported increases in shear strength values at high doses (10 mg/kg), indicating a reduction in tenderness, while other studies show variable effects depending on the dose and muscle type [37].

The variability in responses may be influenced by factors such as initial weight, sex, and specific experimental conditions [38]. The divergence between these positive effects observed in productive and morphometric parameters versus the absence of improvements in technological quality agrees with Aalhus [39], who suggests that, although ractopamine enhances growth and carcass characteristics [40] [41], its effects on intrinsic quality are limited.

4.3. Proximate Chemical Composition

The observations indicate that the nutritional content of the *Longissimus dorsi* muscle of pigs fed with AO NATURAL FIT PRO® and Ractopamine in the growth-finishing stages showed no significant differences ($P > 0.05$) with respect to the % of Dry Matter, % of Moisture, % of Ash, % of Crude Protein and % of Ether Extract between the treatments. This helps us understand that the supplements have a similar effect compared to the use of other growth factors used in the growth-finishing stage.

On the other hand, moisture retention in the muscle tissues of the supplemented groups may be related to the effects of ractopamine on protein metabolism and muscle structure [37]. The addition of ractopamine produces most of the desirable effects described in the literature on growth and carcass characteristics, with the exception of dressing percentage and backfat depth [42].

Supplementation with AO NATURAL FIT PRO® provided a similar effect on moisture content, resulting in improved juiciness and palatability of the meat [43]. The absence of significant differences in ash, crude protein, and ether extract between treatments indicates that the evaluated additives did not compromise the intrinsic nutritional value of the muscle, maintaining a constant supply of essen-

tial nutrients.

This consistency in the basic nutritional composition of crude protein and ether extract in *Longissimus dorsi* suggests uniform effects of the treatments on different muscle types [44]. The findings on chemical composition, along with the observed improvements in carcass yield, are consistent with Barbosa *et al.* [45] regarding the positive effects of ractopamine on performance metrics.

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4.4. Fatty Acid Profile and Nutritional Stability

The results of this study confirm that the treatments evaluated (AO NATURAL FIT PRO®, ractopamine, and their combination) did not exert statistically significant effects on most individual fatty acids or on the overall composition of fatty acid groups (saturated, monounsaturated, and polyunsaturated) in the *Longissimus dorsi* muscle.

This absence of lipid modifications is consistent with scientific evidence indicating that ractopamine has a minimal impact on fatty acid profiles [37] [46]. The observed stability in lipid composition is particularly relevant from a nutritional and technological perspective.

Although some studies have documented specific modifications when ractopamine is combined with other additives—with increases of 18% in linoleic acid and 36% in arachidonic acid when using citrus extracts [46], or alterations in the saturated: unsaturated ratio with conjugated linoleic acid [47]—the treatments evaluated in this study did not reproduce these effects. This indicates that conjugated linoleic acid, added fat, and ractopamine act primarily additively to improve pig growth and carcass quality [47]-[49].

This stability of the lipid profile, together with improvements in productive performance and carcass characteristics, strengthens the concept that additives act primarily by optimizing metabolic efficiency and protein deposition without essentially altering the intrinsic nutritional composition of the meat.

4.5. Evolution of Muscle pH Post-Slaughter

Monitoring of the pH of the *Longissimus dorsi* muscle during the post-slaughter period revealed no significant differences between treatments on days 0, 3, 9, and

12. However, on day 6, a statistically significant difference ($P < 0.05$) was observed, with the treatment containing AO NATURAL FIT PRO® + ractopamine showing the highest pH value (5.70) compared to the control (5.57), AO NATURAL FIT PRO® (5.53), and ractopamine (5.63).

This transient difference on day 6 post-slaughter suggests a temporary alteration in the biochemical processes of muscle acidification, possibly related to modifications in glycolytic metabolism or in the buffering capacity of the muscle induced by the combination of additives.

The pH observed in all treatments remained within normal ranges for pork at 45 minutes (6.0 to 6.5) [50] and at 24 hours (5.70 to 6.10), indicating that quality was not compromised [51]. In a similar experiment, the post-slaughter pH at 24 hours of the *Semimembranosus* and *Longissimus* muscles was not affected by diet or sex [52].

It has been documented that during storage periods of 14 to 28 days, regardless of the type of packaging, the pH decreases to a range of 5.5 to 5.8, leading to increased water loss through dripping [53]. It is important to mention that the addition of AO NATURAL FIT PRO® did not show statistically significant differences in water loss or pH changes that would affect meat quality.

The absence of sustained differences in other evaluation periods indicates that the effects of supplementation on post-slaughter acidification are limited and do not significantly alter the normal evolution of muscle pH. These findings are consistent with previous evidence indicating that, although ractopamine can positively influence carcass characteristics such as yield and ribeye area without affecting feed intake [37] [54], its effects on post-slaughter biochemical parameters are generally minimal.

5. Conclusions

The results demonstrate that supplementation with AO NATURAL FIT PRO®, ractopamine, and their combination produces differentiated effects on meat quality in pigs during the growing-finishing stage. The treatments exerted significant effects on color characteristics, particularly in the *Longissimus dorsi* muscle, where the AO NATURAL FIT PRO® + ractopamine combination reduced red color intensity, yellow hue, and overall saturation. Conversely, no significant differences were observed in technological parameters such as drip loss or tenderness.

The proximate chemical composition showed greater moisture retention in the treated groups without affecting ash, crude protein, or ether extract content. The fatty acid profile remained stable, confirming that these additives optimize productive efficiency without substantially altering the nutritional profile of the meat. Post-mortem muscle pH evolution showed transient differences only on day 6, indicating limited effects on the biochemical processes of muscle acidification. The findings reveal a dichotomy between clear production benefits and limited or adverse effects on certain sensory quality attributes. The absence of consistent synergistic effects between AO NATURAL FIT PRO® and ractopamine suggests

complementary mechanisms of action that manifest themselves differentially depending on specific physiological conditions.

The implementation of these additives in commercial swine production systems must carefully consider the balance between improvements in production efficiency and carcass yield versus potential compromises in specific sensory attributes. This comprehensive evaluation is essential to optimize both the profitability of the production system and the acceptability of the final product.

Conflicts of Interest

None of the authors have a conflict of interest.

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