

Effect of Natural Bioactive Compounds versus Ractopamine: B) Characteristics of the Pig Carcass

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Abstract

Background: The withdrawal of ractopamine from swine production, demanded by both export and local markets, represents a challenge for the swine industry. This study evaluated the effect of AO NATURAL FIT PRO[®] as an alternative to ractopamine on the carcass characteristics of finishing 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 were evaluated in all groups. **Results:** The supplemented groups showed significant improvements ($P < 0.05$). Hot carcass weight was higher in the treated groups (77.817, 78.767, and 79.071 kg) compared to the control group (71.333 kg). **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

In recent years, urbanization and globalization—a phenomenon driven by population growth and per capita consumption—have been exacerbated by economic development and rising incomes, which have increased the demand for food products [1]. In this context, pork production is geared towards supplying meat con-

sumption, both locally 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 compromising 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].

Alternatively, 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 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 such as tannins, purple basil, oroval, Indian gooseberry, and garlic. These compounds function as antioxidants that help protect animals from oxidative stress.
- **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.

2.4. Response Variables and Laboratory Analysis

The study was conducted in two phases: 1) Evaluation of carcass characteristics at the slaughterhouse. 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 following humane methods approved by current regulations. At the time of slaughter, the pigs must be healthy and have rested adequately (2 to 4 hours), especially if they have traveled for many hours. It is very important that

the pigs destined for slaughter are properly immobilized before stunning and bleeding; once this is completed, the slaughtering process takes place [23]-[25]. 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 [26]. **Table 1** shows the ingredient and nutrient composition for pigs weighing 50 to 100 kg LW.

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 ^o	Ractopamine	AO NATURAL FIT PRO ^o + 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 ^o	-	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	

Continued

Ether extract (%)	3.29	3.29	3.29	3.29	
Crude fiber (%)	3.64	3.64	3.64	3.64	
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. Carcass Evaluation

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

2.6. Statistical Analysis

The data were analyzed using an analysis of variance (ANOVA) for a completely randomized design, 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. Carcass Characteristics

Table 2 shows the different carcass characteristics. It can be seen that the hot carcass weight (kg) (77.817, 78.767, and 79.071) and the cold carcass weight (kg) (76.252, 76.578, and 77.267) with the AO NATURAL FIT PRO[®], ractopamine, and AO NATURAL FIT PRO[®] + ractopamine treatments, respectively, were higher ($P < 0.05$) compared to the control group. It was also observed that the pigs in the ractopamine group and the AO NATURAL FIT PRO[®] + ractopamine group had the largest leg diameter (72.467 and 74.11, respectively) compared to the pigs in

the other treatments; however, the four treatments were statistically similar in all other parameters.

Table 2. Carcass characteristics of pigs in the growing-finishing stage.

Parameter	Treatment				SEM ¹	P-value
	Control	AO NATURAL FIT PRO [®]	Ractopamine	AO NATURAL FIT PRO [®] + Ractopamine		
Hot carcass weight (kg)	71.333 ^b	77.817 ^a	78.767 ^a	79.071 ^a	1.58	0.006
Cold carcass weight (kg)	69.922 ^b	76.252 ^a	76.578 ^a	77.267 ^a	1.37	0.003
Water loss (kg)	1.411	1.565	2.189	1.804	0.45	0.678
% water loss	1.97	2.01	2.77	2.28	0.563	0.86
Carcass yield (%)	75.35	74.74	75.71	76.23	0.834	0.59
Backfat thickness (mm)	1.88	1.54	1.62	1.17	0.198	0.113
Carcass length (cm)	101.11	104.2	101.233	101.44	2.04	0.628
Hinleg length (cm)	38.03	37.42	36.23	39.92	1.29	0.229
Hinleg circumference (cm)	68.78 ^b	70.62 ^b	72.467 ^a	74.11 ^a	0.74	0.002
Thoracic cavity length (cm)	70.4	74.28	73.9	75.88	2.64	0.503
Hinleg width (cm)	24.58	25.62	24.46	26.5	0.77	0.205
Thoracic depth (cm)	18.4	17.21	17.51	19.28	1.1	0.497
Head weight (kg)	4.633	4.714	4.833	4.885	0.09	0.195
Forelimb and hindlimb weight (kg)	1.833	1.828	1.9	1.885	0.067	0.816

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

3.2. Forelimbs/Shoulders and Hindlimbs/Hams

For the volume and weight variables of the forelimbs, no statistically significant differences were detected between the experimental groups. The numerical data showed no statistically attributable effect to any of the treatments applied compared to the control group. In contrast, highly significant effects were observed in the volume of the hindlimbs: The control group (9133.3 cc) presented a significantly smaller volume compared to the three treated groups: AO NATURAL FIT PRO[®] (10372.3 cc), ractopamine (10562.7 cc), and the combination of both (11621.0 cc). For the weight of the hindlimbs: The results were consistent with those for volume, showing a highly significant difference ($P = 0.005$). The control group (9264.2 g) was statistically lower in weight compared to the groups supplemented with AO NATURAL FIT PRO[®] (10531.4 g), ractopamine (11124.0 g), and the combination (11147.0 g). No significant differences were found between the groups that received the additives (**Table 3**).

Table 3. Weight and volume of legs and arms.

Parameter	Treatment				SEM ¹	P-value
	Control	AO NATURAL FIT PRO [®]	Ractopamine	AO NATURAL FIT PRO [®] + Ractopamine		
Volume of forelimbs (cc)	7020.8	7854.4	7277.5	7761.4	253.7	0.111
Volume of hindlimbs (cc)	9133.3 ^b	10372.3 ^a	10562.7 ^a	11621 ^a	347.49	0.004
Weight of forelimbs (g)	7735.8	8195	7590	8359.3	333.09	0.299
Weight of hindlimbs (g)	9264.2 ^b	10531.4 ^a	11124 ^a	11147 ^a	302.17	0.005

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

3.3. Intestinal Microenvironment and Digestibility

Regarding small intestine pH, statistically significant differences were found for duodenal pH. The control group had the highest value (6.4), followed by AO NATURAL FIT PRO[®] (6.24) and AO NATURAL FIT PRO[®] + ractopamine (6.06). In the jejunum, the treatment containing AO NATURAL FIT PRO[®] had the highest pH (6.48) compared to the other treatments; while in the ileum, the pH values were similar among the treatments (**Table 4**).

Table 4. pH of the small intestine of pigs treated in the growth-finishing stage.

Parameter	Treatment				SEM ¹	P-value
	Control	AO NATURAL FIT PRO [®]	Ractopamine	AO NATURAL FIT PRO [®] + Ractopamine		
Duodenal pH	6.4 ^a	6.24 ^{ab}	5.93 ^b	6.06 ^{ab}	0.104	0.021
Jejunal pH	6.39 ^{ab}	6.48 ^a	6.15 ^b	6.34 ^{ab}	0.082	0.050
Ileal pH	6.64	6.57	6.47	6.63	0.103	0.624

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

4. Discussion

4.1. Carcass Characteristics

Treatments with ractopamine and AO NATURAL FIT PRO[®] + ractopamine significantly improved carcass characteristics. Hot carcass weights were higher in all treated groups (77.817, 78.767, and 79.071 kg for AO NATURAL FIT PRO[®], ractopamine, and the combination, respectively) compared to the control, showing a consistent trend also in cold carcass weights (76.252, 76.578, and 77.267 kg).

These findings are lower than those reported by Ferreira *et al.* [28], who obtained yields of 81.35% with the addition of ractopamine. However, a meta-analysis that evaluated carcass yield characteristics and some meat cuts determined different yield percentages according to the quality and development of the pigs

when ractopamine was administered: high quality 82.23%, medium quality 75.15%, and low quality 73.85% [29]. This places our overall results as medium quality in the case of the treatment with AO NATURAL FIT PRO® + ractopamine, with a yield of 74.80%.

Leg diameter also showed significant improvements in the groups with ractopamine (72.467 cm) and AO NATURAL FIT PRO® + ractopamine (74.11 cm), indicating better muscle development, although not necessarily better carcass quality [29]. The statistical similarity observed in other parameters suggests that these additives enhance specific feed efficiency characteristics without adversely affecting overall growth metrics [29]-[31].

It is important to consider that, although ractopamine improves certain carcass traits, it may not significantly influence meat quality attributes [29] [32], indicating the need to evaluate the balance between growth performance and carcass quality.

4.2. Forelimbs/Shoulders and Hindlimbs/Hams

The volume and weight parameters of the forelimbs showed no statistically significant differences between treatments. In contrast, the volume of the hindlimbs showed highly significant effects ($P < 0.01$). The groups supplemented with AO NATURAL FIT PRO®, ractopamine, and their combination showed larger volumes than the control group, with no statistically significant differences between the supplemented treatments.

The weight of the hind limbs confirmed these findings with significant differences, where the control group was statistically lower compared to all supplemented treatments. This differential response between anatomical regions suggests that the anabolic effects of ractopamine are predominantly manifested in the muscles of the hind limbs, which is consistent with previous studies documenting preferential muscle deposition induced by β -adrenergic agonists and fat reduction [29] [33].

Ractopamine, as a β -adrenergic agonist, stimulates muscle protein synthesis and reduces lipogenesis, resulting in higher lean meat yield and improved carcass conformation [34] [35]. The absence of evident synergistic effects, similar to that observed in production parameters, suggests that AO NATURAL FIT PRO® may exert its effects through complementary mechanisms, potentially improving nutrient absorption and providing antioxidant effects that reduce lipid peroxidation [36] and, therefore, improve the sensory quality of the meat [37].

It is important to consider that, although these additives improve the quantitative characteristics of the carcass, their impact on meat quality can be limited or even adverse. Many of the sources used may contain toxic and antinutritional factors and have negative effects if used in large quantities [37], as evidenced in the color parameters [29]. This divergence between performance improvements and potential compromises in meat quality requires a comprehensive evaluation to optimize nutritional strategies in commercial swine production.

4.3. Intestinal Microenvironment and Digestibility

Intestinal pH analysis revealed significant variations between segments of the small intestine. In the duodenum, the control group had the highest pH (6.4), followed by AO NATURAL FIT PRO® and the combination with ractopamine. In the jejunum, the AO NATURAL FIT PRO® treatment showed the highest pH (6.48) compared to the other groups, while in the ileum, no significant differences were observed between treatments. A better understanding of phytogetic compounds in the three components of the gastrointestinal ecosystem (intestinal microbiota, intestinal physiology and immunology) and of the underlying mechanisms will possibly allow us to optimize the use of phytogetic substances for economically efficient and sustainable animal production [38].

These findings have important physiological implications for nutrient digestibility and absorption. The elevated pH in the duodenum of the control group may favor pancreatic enzyme activity and nutrient absorption [39], which could partially explain the good production results observed in all groups.

The higher jejunal pH observed with AO NATURAL FIT PRO® suggests optimized digestive conditions that could promote nutrient utilization and improved carcass conformation [34] [35]. The stability of ileal pH across treatments indicates that absorption capacity in this terminal segment is not significantly affected by dietary interventions, maintaining a stable environment for the absorption of specific nutrients such as bile salts and vitamin B12 [40] [41].

The correlation between optimized intestinal pH and improved carcass characteristics has been documented in studies evaluating dietary restrictions and energy levels [42] [43]. However, it should be considered that elevated gastrointestinal pH can promote undesirable fermentations that compromise meat quality [44], indicating the existence of specific optimum ranges to maximize both productive performance and the quality of the final product.

5. Conclusions

The results demonstrate that supplementation with AO NATURAL FIT PRO®, ractopamine, and their combination produces differentiated effects on carcass characteristics during the growth-finishing phase. During transport and pre-slaughter rest, the supplemented groups maintained higher weights, with the AO NATURAL FIT PRO® + ractopamine combination showing the least weight loss during transport (1.81%), suggesting a protective effect against transport stress. The treatments significantly improved carcass characteristics, including hot and cold carcass weights, as well as hind limb diameter. Morphometric analysis revealed differentiated effects by anatomical region, with significant improvements in hind limb volume and weight, but not in the forelimbs, suggesting that the anabolic effects are predominantly manifested in the hind limb muscles.

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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