

Article

Acid Silage from Pirarucu (*Arapaima gigas*) By-Products Affects Performance, Physiological Parameters and Meat Quality of Slow-Growing Broilers

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Abstract

The increasing demand for sustainable and cost-effective feed alternatives has stimulated the use of agro-industrial by-products in poultry nutrition. This study evaluated the effects of acid silage from pirarucu (*Arapaima gigas*) by-products (ASP), derived from fish-processing waste, on growth performance, physiological responses, and meat quality of slow-growing broilers. A total of 240 male Label Rouge broilers were assigned to diets containing 0, 1, 2, or 3% ASP from 8 to 56 days of age. Moderate ASP inclusion levels (1–2%) improved ($p \leq 0.05$) growth performance by increasing weight gain and improving feed conversion ratio, particularly during the grower and finisher stages. Hematological analyses demonstrated favorable physiological responses, including increased ($p \leq 0.05$) hemoglobin concentration, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin. Plasma biochemical parameters indicated improved ($p \leq 0.05$) protein metabolism, with higher total protein, albumin, and globulin concentrations, while glucose, cholesterol, and urea remained unaffected ($p > 0.05$). Carcass evaluation revealed improved ($p \leq 0.05$) breast yield at 2% ASP inclusion, and breast composition showed increased ($p \leq 0.05$) protein, lipid, and mineral deposition, accompanied by reduced ($p \leq 0.05$) moisture content. However, the highest inclusion level (3%) did not promote additional productive benefits. These findings demonstrate that moderate ASP inclusion (1–2%) can improve



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productive efficiency and meat nutritional quality while promoting sustainable reuse of aquaculture by-products, supporting circular and environmentally responsible poultry production systems.

Keywords: Amazon; *Arapaima gigas*; breast; fish by-products; fish protein

1. Introduction

The search for sustainable feed resources has intensified in recent years due to the growing need to reduce production costs while minimizing the environmental impacts associated with livestock systems [1]. In this context, agro-industrial by-products have attracted considerable attention because they can replace conventional feed ingredients either partially or completely, while simultaneously adding value to materials that were previously regarded as waste. Besides contributing to waste reduction, these alternative ingredients may improve production efficiency and the quality of animal-derived products [2,3]. Furthermore, these residues may reduce feed costs, which can account for approximately 70% of total production expenses, thereby intensifying this challenge in regions with logistical constraints [4–7].

Aquaculture stands out as one of the agro-industrial production chains that generate the largest amounts of waste, especially due to the high volume produced during fish slaughtering and processing, with by-products such as heads, viscera, scales, skins, and bones often being underutilized or improperly discarded, resulting in considerable environmental, sanitary, and economic impacts [8,9]. According to Cândido et al. [10] and Guimarães et al. [11], it is estimated that 50–70% of the biomass processed by the fish industry is converted into by-products, many of which still possess valuable nutrients that can be recovered through suitable processing technologies.

In this context, the application of silage techniques to fish residues has shown promising potential due to its ability to enhance the nutritional value of these by-products, particularly protein and lipid concentrations [12]. It is important to highlight that the silage process involves reducing the pH of the raw material, either through the addition of organic acids (chemical silage) or microbial fermentation resulting from the enzymatic activity of strategically inoculated microorganisms added to the substrate (biological silage), enabling the efficient transformation of fish by-products into a stable and nutritionally more valuable feed resource [11,13–15].

Among Brazilian freshwater fish species, *Arapaima gigas* (pirarucu) represents one of the most economically important native resources because of its rapid growth, excellent meat quality, and wide distribution throughout the Amazon Basin [14,16]. Also known as the “Amazon codfish”, its highly valued meat, especially in the salted–dried form, has been identified as a potential substitute for cod [16,17]. Nevertheless, the processing of this large-bodied species generates considerable quantities of by-products, including heads, skins, scales, fins, viscera, and bones, which generally have low commercial value despite their nutritional potential [16,18,19].

Based on these premises, this study hypothesized that the inclusion of acid silage from pirarucu by-products (ASP) as a feed additive in slow-growing broiler diets could improve growth performance, optimize carcass characteristics, and promote improvements in physiological parameters of the birds [20]. This hypothesis is primarily supported by previous studies demonstrating that pirarucu by-products may contain concentrations of high biological value nutritional compounds, including high-quality proteins and essential fatty acids, such as omega-3 and omega-6, in addition to bioactive compounds capable of

enhancing energy metabolism, nutrient utilization, and productive performance in animals [18,19,21]. Furthermore, redirecting these residues toward animal feeding contributes to environmental sustainability by reducing waste and adding value to the aquaculture production chain [11,22–24]. Therefore, the objective of this study was to investigate the effects of increasing ASP levels in diets for slow-growing broilers on growth performance, physiological parameters, and meat quality.

2. Materials and Methods

2.1. Processing of Pirarucu By-Products and Silage Production

Residues consisting of the rib and vertebral regions of pirarucu were obtained from a fish processing facility supplied with raw material originating from managed fishing areas in the Amazon region. The residues were transported in raffia bags to the Fish Technology Laboratory of the Federal University of Amazonas (UFAM), Manaus (AM), Brazil. In the laboratory, the material underwent mechanical fragmentation using an electric grinder (Multinox®, Betim, Minas Gerais, Brazil) and was subsequently divided into 15-L plastic containers, each receiving 10 kg of ground biomass for silage production.

For silage preparation, a mixture of propionic and formic acids (1:1 ratio) was added to the biomass at a proportion of 3% (*v/w*) of the acid solution relative to the residue weight, under constant homogenization, following the methodology proposed by Batalha et al. [5]. The acidification process aimed to promote pH reduction, inhibit undesirable microbial proliferation, and preserve the nutritional quality of the material during storage. The containers were maintained at room temperature for seven days.

To ensure complete homogenization and maximize acid contact with the residues, the ensiled material was manually stirred every 24 h throughout the seven-day storage period, contributing to silage stabilization and quality maintenance. During this period, samples were collected for pH determination using a digital pH meter, with results expressed to two decimal places. In addition, organoleptic characteristics, including color, aroma, and consistency, were monitored throughout the fermentation process to evaluate product stability and preservation.

At the end of the seven-day ensiling period, the material was spread onto aluminum trays and dried in a forced-air oven at 65 °C (149 °F) for 72 h to reduce moisture content and obtain a stable dried product. During drying, the material was periodically mixed to ensure uniform moisture removal and consistent product quality. The dried material was subsequently ground using a grain mill equipped with 4-mm mesh sieves to obtain ASP meal. Once dried, proximate composition analyses were conducted to determine moisture, mineral matter, lipid, fiber, and soluble carbohydrate contents of the ASP, following AOAC [25] methodologies. The results obtained are presented in Table 1. Microbiological analyses were also performed in accordance with the standards of the Brazilian Ministry of Agriculture, Livestock, and Supply [26] to confirm the safety of the ASP. After confirmation of physicochemical and microbiological quality, the processed silage was forwarded to the poultry research sector for use in the experimental trial with slow growing broilers.

2.2. Facilities, Animals, Diets and Experimental Design

The experiment was carried out at the Poultry Research Farm of the UFAM, Manaus, Amazonas, Brazil. The methodological procedures adopted in this study were adapted from those described by Santos [27]. Birds were housed in an open-sided poultry house measuring 25 m × 8 m, with a ceiling height of 3.25 m. Natural ventilation was favored by the surrounding vegetation, which contributed to maintaining adequate environmental conditions throughout the trial. Ambient temperature and relative humidity were monitored daily using a digital thermo-hygrometer. Mean temperature and relative humidity

during the experiment were 28.6 °C and 62.1%, respectively, values considered suitable for slow-growing broilers. No clinical signs of thermal stress were observed during the study.

Table 1. Composition of acid silage from pirarucu by-products.

Variables	Values
Dry matter, %	91.50
Crude protein, %	39.55
Fats, %	11.68
Minerals, %	27.15
Crude Fiber, %	0.62
Soluble carbohydrates, %	21.00
Gross energy, kcal/kg	3684.74
Metabolizable energy, kcal/kg	3270.36

A total of 240 one-day-old male Label Rouge broiler chicks, with an average initial body weight of 43.15 ± 0.21 g, were obtained from the UFAM hatchery. During the first week of life, chicks were reared inside brooding circles equipped with tray feeders, drinkers, wood-shaving litter, and electric heating to provide adequate brooding conditions. At eight days of age, birds were randomly distributed into experimental pens measuring 4 m², each equipped with tubular feeders and suspended drinkers over wood-shaving litter. Feed and water were supplied ad libitum throughout the experimental period.

The feeding trial was divided into three production phases: starter (8–28 days), grower (29–49 days), and finisher (50–56 days). A lighting schedule appropriate for slow-growing broilers was adopted, consisting of 23 h light during the first week, followed by 20 h light from 8 to 28 days and 18 h light from 29 to 56 days, according to the recommendations of Wu et al. [28]. A completely randomized experimental design was used, comprising four dietary treatments corresponding to 0, 1, 2, and 3% inclusion of acid silage from pirarucu by-products (ASP). Each treatment consisted of five replicates with twelve birds per replicate. Experimental diets (Table 2) were formulated using the nutritional recommendations of Rostagno et al. [29]. Nutrient composition of the ASP was based on the laboratory analyses performed in the present study, whereas the metabolizable energy value was obtained experimentally before diet formulation following the procedure described by Sakomura and Rostagno [30].

Table 2. Composition and guaranteed nutrient concentration (analyzed and calculated concentrations in dry matter) of experimental diets with different levels of acid silage from pirarucu by-products.

Ingredient	Pre-Ini (1–7 d)	Initial (8–28 d)				Grower (29–49 d)				Finisher (50–56 d)			
		0	1	2	3	0	1	2	3	0	1	2	3
Corn 7.88%	58.07	58.07	57.59	56.87	56.14	61.57	61.58	61.60	61.57	67.43	67.46	67.47	67.50
Soybean meal 46%	38.32	38.35	37.66	37.37	37.08	33.60	32.71	31.83	33.60	28.79	27.90	27.02	26.13
ASP	0.00	0.00	1.00	2.00	3.00	1.00	2.00	3.00	1.00	0.00	1.00	2.00	3.00
Limestone	0.93	0.82	0.82	0.82	0.82	0.88	0.89	0.89	0.88	0.69	0.69	0.69	0.69
Dical. phosp.	1.79	1.79	1.80	1.80	1.81	1.55	1.56	1.57	1.55	1.30	1.31	1.32	1.33
Common salt	0.30	0.37	0.52	0.52	0.52	0.27	0.28	0.28	0.27	0.28	0.28	0.28	0.28
Vit. Min. suppl.	0.50 ¹	0.50	0.50	0.50	0.50	0.50 ¹	0.50 ¹	0.50 ¹	0.50 ¹	0.50 ²	0.50 ²	0.50 ²	0.50 ²
DL-Met 99%	0.10	0.10	0.11	0.12	0.12	0.05	0.06	0.07	0.05	0.19	0.20	0.21	0.22
Soybean oil	0.00	0.00	0.00	0.00	0.00	0.58	0.42	0.26	0.58	0.82	0.66	0.51	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 2. *Cont.*

Ingredient	Pre-Ini (1–7 d)	Initial (8–28 d)				Grower (29–49 d)				Finisher (50–56 d)			
		0	1	2	3	0	1	2	3	0	1	2	3
Nutrients		Guarantee levels ³											
ME, kcal/kg	3000.00	3000.00	3000.00	3000.00	3000.00	3100.00	3100.00	3100.00	3100.00	3200.00	3200.00	3200.00	3200.00
Crude protein, %	22.50	22.50	22.50	22.50	22.50	21.00	21.00	21.00	21.00	19.00	19.00	19.00	19.00
Ca, %	0.94	0.90	0.90	0.90	0.90	0.85	0.85	0.85	0.85	0.70	0.70	0.70	0.70
Avail. P, %	0.46	0.45	0.45	0.45	0.45	0.40	0.40	0.40	0.40	0.35	0.35	0.35	0.35
Na, %	0.15	0.19	0.19	0.19	0.19	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Crude fiber, %	3.40	4.97	4.90	4.86	4.81	3.20	3.15	3.10	3.20	3.02	2.97	2.93	2.88
Lys, %	1.21	1.21	1.19	1.18	1.17	1.09	1.06	1.04	1.09	0.97	0.94	0.92	0.90
Met, %	0.45	0.45	0.45	0.45	0.46	0.37	0.37	0.38	0.37	0.49	0.49	0.50	0.50
Met + Cys, %	0.80	0.80	0.80	0.80	0.80	0.70	0.70	0.70	0.70	0.80	0.80	0.80	0.80
Total Thr, %	0.87	0.87	0.86	0.85	0.85	0.80	0.79	0.77	0.80	0.74	0.72	0.70	0.69
Total Trp, %	0.28	0.28	0.28	0.28	0.27	0.26	0.25	0.24	0.26	0.23	0.22	0.22	0.21

¹ Vitamin/mineral supplement (starter): Content per 1 kg of diet = Folic acid 800 mg, Pantothenic acid 12,500 mg, Antioxidant 0.5 g, Biotin 40 mg, Niacin 33,600 mg, Selenium 300 mg, Vitamin A 6,700,000 IU, Vitamin B1 1750 mg, Vitamin B12 9600 mcg, Vitamin B2 4800 mg, Vitamin B6 2500 mg, Vitamin D3 1,600,000 IU, Vitamin E 14,000 mg, Vitamin K3 1440 mg. Mineral supplement—content per 0.5 kg = Manganese 150,000 mg, Zinc 100,000 mg, Iron 100,000 mg, Copper 16,000 mg, Iodine 1500 mg. ² Vitamin/mineral supplement (finisher): Content per 1 kg of diet = Folic acid 650 mg, Pantothenic acid 10,400 mg, Antioxidant 0.5 g, Niacin 28,000 mg, Selenium 300 mg, Vitamin A 5,600,000 IU, Vitamin B1 550 mg, Vitamin B12 8000 mcg, Vitamin B2 4000 mg, Vitamin B6 2080 mg, Vitamin D3 1,200,000 IU, Vitamin E 10,000 mg, Vitamin K3 1200 mg. Mineral supplement—content per 0.5 kg = Manganese 150,000 mg, Zinc 100,000 mg, Iron 100,000 mg, Copper 16,000 mg, Iodine 1500 mg. ³ Levels analyzed and calculated on a dry matter basis. The nutrient composition of the experimental diets was calculated from ingredients composition.

Briefly, the metabolizable energy assay was conducted with 28-day-old broilers maintained in metabolism cages. Birds were allowed five days of adaptation followed by five consecutive days of total excreta collection. Feed consumption was recorded during the collection period, and both feed and excreta samples were analyzed for gross energy in order to estimate the apparent metabolizable energy of ASP. The analyzed gross energy and metabolizable energy values obtained for ASP were 3684.74 and 3270.36 kcal/kg of dry matter, respectively, and the latter value was adopted for formulation of the experimental diets.

2.3. Growth Performance

Growth performance was assessed separately during the starter (8–28 days), grower (29–49 days), and finisher (50–56 days) phases, as well as for the entire experimental period. Each experimental pen was considered the experimental unit for performance evaluation. The variables analyzed included feed intake, body weight gain, and feed conversion ratio. Feed intake (kg/bird) was determined by dividing the total amount of feed consumed in each pen by the number of birds housed in that pen during each production phase. Body weight gain (kg/bird) was calculated from the difference between the final and initial body weights recorded for each pen, expressed on a per-bird basis. Feed conversion ratio (kg feed/kg gain) was obtained by dividing feed consumption by the corresponding body weight gain. Overall performance was calculated using the cumulative data collected throughout the entire experimental period.

2.4. Physiological Parameters

At the end of the feeding trial and immediately before slaughter, blood samples were collected from ten broilers per treatment, corresponding to two birds randomly selected from each replicate. Approximately 1 mL of blood was obtained from the ulnar vein using disposable syringes containing heparin (5000 IU) as anticoagulant. After collection, samples were properly identified and maintained under refrigeration until laboratory processing.

For hematological evaluation, erythrocyte counts (M/mm³) were determined using a Neubauer hemocytometer following dilution in formaldehyde-citrate solution. Cell count-

ing was performed under an optical microscope (Nikon Eclipse E-50i, DM3000, Tokyo, Japan) equipped with a 40× objective lens. Hemoglobin concentration (g/dL) was measured by the cyanmethemoglobin (Drabkin) method using a UV–visible spectrophotometer (K37-UVVIS, Kasvi[®], São José dos Pinhais, Brazil) [31]. Hematocrit (%) was determined by the microhematocrit technique after centrifugation of heparinized capillary tubes at 10,000 rpm for 5 min [32]. The hematological indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were subsequently calculated according to the equations proposed by Tavares-Dias and Moraes [33]. Following hematological determinations, plasma was separated by centrifugation of whole blood at 5000 rpm for 5 min. Plasma aliquots were transferred to labeled microtubes and stored at −80 °C until biochemical analyses were performed [34].

Plasma concentrations of glucose, triglycerides, cholesterol, total protein, albumin, globulin, and uric acid were determined using commercial enzymatic-colorimetric kits supplied by InVitro Diagnóstica (Itabira, MG, Brazil). All analyses were conducted according to the manufacturer's recommendations, and absorbance was measured with a UV–visible spectrophotometer (K37-UVVIS, Kasvi[®], São José dos Pinhais, Brazil).

2.5. Meat Quality

At 56 days of age, twelve birds from each dietary treatment were randomly selected after a 12-h feed withdrawal period for carcass and meat quality evaluations. The birds were individually weighed, electrically stunned (40 V, 50 Hz), and slaughtered by severing the jugular vein. After bleeding, carcasses were scalded in water maintained at 60 °C for 62 s, mechanically defeathered, and manually eviscerated according to the procedures described by Mendes and Patrício [35].

Carcass yield was calculated, and the liver, gizzard, heart, and feet were individually weighed. Subsequently, commercial cuts, including breast, wings, back, thighs, drumsticks, and neck, were separated and weighed using a precision analytical balance (0.01 g). The yield of each cut was expressed as a percentage of carcass weight. Breast samples were collected immediately after processing and transported to the laboratory for chemical analyses. Moisture, crude protein, ether extract, and mineral matter were determined following the analytical procedures recommended by AOAC [25].

2.6. Statistical Analyses

The adopted statistical model (1) was as follows:

$$Y_{ik} = \mu + \alpha_i + \epsilon_{ik} \quad (1)$$

where

Y_{ik} = Observed value for the variable under study;

μ = Overall mean of the experiment;

α_i = Effect of the ASP levels;

ϵ_{ik} = Experimental error.

Prior to statistical analyses, all variables were tested for normality using the Shapiro–Wilk test. Data meeting the assumptions of normality were subjected to one-way analysis of variance (ANOVA) using R software (version 4.1.3), following the procedures described by Logan [36]. Whenever a significant treatment effect was detected ($p \leq 0.05$), polynomial regression analyses were performed to evaluate the response to increasing dietary levels of ASP [36,37]. Linear or quadratic regression models were selected according to the equation that provided the best fit to the observed data [37,38].

3. Results

3.1. Physical-Chemical Characteristics of ASP

During silage production, the color of the ensiled mass changed from brown to reddish-pink, reaching a dark brown coloration around days 6 to 7. The strong fishy odor gradually diminished, being replaced by a slightly acidic or fruity aroma. Liquefaction was observed within the first 72 h, resulting in a semi-pasty consistency that persisted throughout the entire process. The pH decreased from 6.49 on day 0 to 5.98 on day 3, stabilizing at approximately 5.99 by day 7. Titratable acidity increased from 2.27% to 10.04% (expressed as lactic acid equivalents), reflecting effective fermentation. Microbiological analysis showed very low levels of total coliforms, molds, and yeasts (<0.001 CFU/g), with no detection of *Escherichia coli*, *Salmonella* spp., or *Staphylococcus aureus*, confirming the microbiological safety of ASP for poultry diets. Proximate composition analysis revealed an excellent nutrient concentration in the dried product, with high contents of dry matter, protein, fat, and minerals.

3.2. Growth Performance

In the performance results (Table 3), during the starter stage, a significant effect ($p \leq 0.05$) was observed only for weight gain, with broilers fed diets containing 1% ASP inclusion showing higher values compared to the control group. Inclusion levels of 2% and 3% ASP did not exhibit the same pattern observed for the 1% ASP. During the grower stage, feed intake increased linearly ($p \leq 0.05$) with increasing silage inclusion in the diet. Weight gain showed higher ($p \leq 0.05$) values in treatments containing 1% and 2% inclusion levels. Feed conversion ratio showed lower ($p \leq 0.05$) values in broilers fed diets containing 2% ASP. During the finisher stage, birds fed diets with 2% inclusion showed higher ($p \leq 0.05$) weight gain and improved ($p \leq 0.05$) feed conversion results. The treatment containing 3% inclusion showed reduced performance for these variables compared to the other treatments. Considering the entire production period, birds fed diets containing between 1% and 2% ASP showed greater weight gain and improved feed conversion results compared to the control group.

Table 3. Growth performance of slow-growing broilers fed diets with increasing levels of acid silage from pirarucu by-products ¹.

Stage	Var. ²	Levels of ASP (%)				<i>p</i> -Value ³	CV ⁴	MM ⁵	R ²
		0.00	1.00	2.00	3.00				
Initial	FI	1.01 ± 0.07	0.88 ± 0.06	0.98 ± 0.20	1.21 ± 0.30	0.14	10.74	-	-
	WG	0.41 ± 0.03	0.45 ± 0.02	0.41 ± 0.03	0.39 ± 0.03	0.05	8.68	Y = 0.415 + 0.035x − 0.015x ²	0.73
	FC	2.46 ± 0.12	1.96 ± 0.15	2.39 ± 0.18	3.10 ± 0.19	0.10	13.36	-	-
Grower	FI	2.08 ± 0.11	2.13 ± 0.09	2.15 ± 0.10	2.19 ± 0.08	0.05	4.65	Y = 2.085 + 0.035x	0.97
	WG	0.78 ± 0.10	0.82 ± 0.10	0.85 ± 0.10	0.80 ± 0.03	0.05	10.83	Y = 0.7765 + 0.0765x − 0.0225x ²	0.90
	FC	2.67 ± 0.15	2.60 ± 0.11	2.53 ± 0.12	2.74 ± 0.14	0.05	9.35	Y = 2.684 − 0.196x + 0.07x ²	0.84
Finisher	FI	0.88 ± 0.09	0.88 ± 0.13	0.86 ± 0.03	0.95 ± 0.13	0.05	11.41	Y = 0.8865 − 0.0485x + 0.0225x ²	0.81
	WG	0.35 ± 0.06	0.37 ± 0.06	0.42 ± 0.02	0.33 ± 0.02	0.05	14.80	Y = 0.3415 + 0.0815x − 0.0275x ²	0.67
	FC	2.51 ± 0.17	2.38 ± 0.10	2.05 ± 0.13	2.88 ± 0.11	0.05	10.13	Y = 2.578 − 0.642x + 0.24x ²	0.73
Cumulative	FI	3.97 ± 0.06	3.89 ± 0.05	3.99 ± 0.0	4.35 ± 0.13	0.05	9.15	Y = 3.864 + 0.124x	0.71
	WG	1.54 ± 0.08	1.64 ± 0.07	1.68 ± 0.09	1.52 ± 0.03	0.05	8.66	Y = 1.533 + 0.193x − 0.065x ²	0.94
	FC	2.58 ± 0.12	2.37 ± 0.10	2.38 ± 0.13	2.86 ± 0.13	0.05	8.49	Y = 2.5925 − 0.4325x + 0.1725x ²	0.98

¹ All data represent the mean of 60 replicates (birds) per treatment. ² FI = Feed intake (kg/bird). WG = Weight gain (kg/bird). FC = Feed conversion ratio (kg/kg). ³ Statistical significance was established at $p \leq 0.05$, and significant treatment effects were further examined using polynomial regression analysis. ⁴ CV = Coefficient of variation. ⁵ Mathematical model fitted according to the influence of the independent variable on the dependent variable.

3.3. Physiological Parameters

In the hematological parameters (Table 4), significant effects ($p \leq 0.05$) were observed for hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin values showed a linear increase in response to increasing levels of ASP inclusion in the diet. Mean corpuscular hemoglobin concentration exhibited a quadratic response ($p \leq 0.05$) according to ASP inclusion levels, with the highest concentration observed in broilers fed diets containing 2% ASP. No significant differences ($p > 0.05$) were observed for erythrocyte count.

Table 4. Physiological parameters of slow-growing broilers fed diets with increasing levels of acid silage from pirarucu by-products ¹.

Par.	Var. ²	Levels of ASP (%)				<i>p</i> -Value ³	CV ⁴ , %	MM ⁵	R ²
		0.00	1.00	2.00	3.00				
Hematological	HEM	9.83 ± 2.12	10.79 ± 3.16	11.27 ± 2.85	11.69 ± 2.66	0.05	9.13	Y = 9.986 + 0.606x	0.95
	RBC	1.24 ± 0.32	1.19 ± 0.44	1.22 ± 0.37	1.19 ± 0.26	0.56	8.39	-	-
	HET	28.55 ± 3.26	29.37 ± 3.19	30.41 ± 3.65	31.69 ± 2.94	0.04	7.58	Y = 28.436 + 1.046x	0.99
	MCV	230.24 ± 10.12	246.81 ± 10.19	249.26 ± 10.98	266.30 ± 10.44	0.05	8.12	Y = 231.56 + 11.063x	0.93
	MCH	79.27 ± 5.15	90.67 ± 4.97	92.38 ± 4.99	98.24 ± 5.27	0.05	8.46	Y = 81.347 + 5.862x	0.90
	MCHC	34.43 ± 3.13	36.74 ± 3.29	37.06 ± 3.11	36.89 ± 3.44	0.03	8.69	Y = 34.505 + 2.63x - 0.62x ²	0.97
Plasma biochemical	GLI	50.80 ± 14.04	44.85 ± 11.64	44.32 ± 23.91	52.26 ± 11.84	0.68	12.82	-	-
	TRI	208.45 ± 56.46	232.12 ± 75.32	279.46 ± 69.86	202.53 ± 35.42	0.05	8.54	Y = 201.05 + 78.408x - 25.15x ²	0.70
	COL	72.67 ± 25.69	78.57 ± 31.95	65.79 ± 8.44	70.17 ± 9.55	0.68	9.16	-	-
	TOP	3.62 ± 0.63	3.73 ± 1.09	4.37 ± 1.21	4.57 ± 2.05	0.04	13.12	Y = 3.549 + 0.349x	0.92
	ALB	0.68 ± 0.51	0.67 ± 0.55	0.92 ± 0.52	1.20 ± 0.74	0.05	20.22	Y = 0.584 + 0.184x	0.87
	GLO	2.94 ± 0.51	3.06 ± 1.16	3.45 ± 1.29	3.37 ± 2.25	0.05	23.34	Y = 2.903 + 0.318x - 0.05x ²	0.84
	URE	1.27 ± 0.34	1.29 ± 0.18	1.23 ± 0.24	1.29 ± 0.15	0.96	18.24	-	-

¹ All data represent the average of 60 replicates (broilers) per treatment. ² HEM = Hemoglobin (g/dL). RBC = Erythrocytes (M/mm³). HET = Hematocrit (%). MCV = Mean Corpuscular Volume (um³). MCH = Mean Corpuscular Hemoglobin (pg/cel). MCHC = Mean Corpuscular Hemoglobin Concentration (g/dL). GLU = Glucose (mg/dL). TRI = Triglycerides (mg/dL). COL = Cholesterol (mg/dL). TOP = Total proteins (g/dL). ALB = Albumin (g/dL). GLO = Globulin (g/dL). URE = Urea (mg/dL). ³ Statistical significance was established at $p \leq 0.05$, and significant treatment effects were further examined using polynomial regression analysis. ⁴ CV = Coefficient of variation. ⁵ Mathematical model fitted according to the influence of the independent variable on the dependent variable.

In the plasma biochemical results (Table 4), no significant differences ($p > 0.05$) were observed for serum glucose, cholesterol, and urea levels among the evaluated treatments. Triglyceride levels showed a significant effect ($p \leq 0.05$), with a quadratic adjustment according to ASP inclusion levels, with the highest value observed in the treatment containing 2% inclusion. Total protein concentrations showed a linear increase ($p \leq 0.05$) with increasing ASP inclusion levels in broiler diets. Likewise, albumin values increased linearly ($p \leq 0.05$) in response to increasing inclusion levels. For globulin, a significant effect ($p \leq 0.05$) was observed, with a quadratic response according to ASP inclusion levels, with the highest concentration observed in broilers fed diets containing 2% ASP.

3.4. Meat Quality

The analysis of carcass traits (Table 5) demonstrated a significant effect on average slaughter weight, with birds fed diets containing ASP showing lower ($p \leq 0.05$) values compared to the control group. Carcass yield percentages did not show significant differences among the evaluated treatments. Relative liver and gizzard weights showed a significant reduction ($p \leq 0.05$) in broilers fed diets containing ASP compared to the control group.

Table 5. Slaughter weight, carcass yield, visceral organ weights and commercial cuts of slow-growing broilers fed diets with increasing levels of acid silage from pirarucu by-products ¹.

Var. ²	Levels of ASP (%)				<i>p</i> -Value ³	CV ⁴	MM ⁵	R ²
	0.00	1.00	2.00	3.00				
SW	1.99 ± 0.21	1.88 ± 0.22	1.88 ± 0.21	1.87 ± 0.13	0.05	10.60	Y = 1.984 – 0.111x + 0.025x ²	0.92
CY	75.13 ± 1.45	74.02 ± 3.78	73.17 ± 2.13	75.67 ± 3.04	0.28	3.75	-	-
FW	85.50 ± 12.86	75.50 ± 11.89	78.75 ± 14.84	79.75 ± 10.76	0.47	15.77	-	-
LW	31.87 ± 2.79	25.25 ± 6.20	24.62 ± 6.94	25.25 ± 5.87	0.05	13.12	Y = 29.821 – 2.049x	0.69
GW	41.37 ± 6.56	36.00 ± 8.45	37.12 ± 7.41	35.50 ± 5.92	0.05	9.18	Y = 40.908 – 4.4615x + 0.9375x ²	0.80
HW	8.75 ± 1.16	9.37 ± 3.06	8.00 ± 1.41	9.50 ± 1.41	0.39	11.59	-	-
BE	28.28 ± 2.52	28.87 ± 3.30	29.19 ± 3.70	28.99 ± 1.83	0.05	9.89	Y = 28.267 + 0.8375x – 0.1975x ²	0.99
DS	15.15 ± 0.89	15.44 ± 1.00	14.87 ± 1.40	15.18 ± 0.92	0.88	9.20	-	-
TH	16.14 ± 1.53	14.93 ± 0.95	14.72 ± 1.26	15.35 ± 1.28	0.14	8.74	-	-
BC	22.55 ± 3.70	22.05 ± 4.51	21.77 ± 1.98	22.08 ± 3.12	0.88	14.86	-	-
WI	12.21 ± 1.04	12.89 ± 0.44	13.96 ± 4.04	12.67 ± 0.33	0.05	16.28	Y = 12.073 + 1.7225x – 0.4925x ²	0.77
NE	5.67 ± 1.27	5.82 ± 1.34	5.49 ± 1.51	5.73 ± 1.06	0.96	11.97	-	-

¹ All data represent the average of 60 replicates (broilers) per treatment. ² SW = Slaughter weight (kg/bird). CY = Carcass yield (%). FP = Feet weight (g). LW = Liver weight (g). GW = Gizzard weight (g). HW = Heart weight (g). BE = Breast yield (%). DS = Drumstick yield (%). TH = Thigh yield (%). BC = Back yield (%). WI = Wing yield (%). NE = Neck yield (%). ³ Statistical significance was established at $p \leq 0.05$, and significant treatment effects were further examined using polynomial regression analysis. ⁴ CV = Coefficient of variation. ⁵ Mathematical model fitted according to the influence of the independent variable on the dependent variable.

Regarding commercial cuts (Table 5), breast yield showed a significant effect, with higher ($p \leq 0.05$) values observed in broilers fed diets containing 2% ASP. Relative wing yield also showed a significant difference, with better ($p \leq 0.05$) results likewise observed in broilers fed diets containing 2% ASP. Variables related to drumstick, thigh, back, and neck yields did not show significant differences among the evaluated treatments.

In the proximate composition analysis of the breast (Table 6), significant effects ($p \leq 0.05$) were observed for moisture, protein, fat, and mineral contents in broilers fed diets containing ASP. Breast moisture levels showed a linear decrease ($p \leq 0.05$) in response to increasing ASP inclusion levels in the diet. In contrast, protein content showed a linear increase ($p \leq 0.05$), with higher values observed in broilers fed increasing levels of ASP inclusion. Fat and mineral contents also showed a linear increase ($p \leq 0.05$) as ASP inclusion levels increased in the diets.

Table 6. Proximate composition of the breast of slow-growing broilers fed diets with increasing levels of acid silage from pirarucu by-products ¹.

Var. ²	Levels of ASP (%)				<i>p</i> -Value ³	CV ⁴	MM ⁵	R ²
	0.00	1.00	2.00	3.00				
MO	74.08 ± 0.47	73.68 ± 0.22	72.51 ± 0.58	71.01 ± 0.19	<0.01	3.94	Y = 74.377 – 1.038x	0.94
PR	18.97 ± 0.82	19.28 ± 0.48	19.36 ± 0.66	19.86 ± 0.91	0.05	3.98	Y = 18.955 + 0.275x	0.92
FT	4.02 ± 0.87	4.05 ± 0.44	5.07 ± 0.47	5.26 ± 0.92	0.05	6.71	Y = 3.889 + 0.474x	0.86
MI	2.93 ± 0.06	2.99 ± 0.07	3.06 ± 0.11	3.87 ± 0.20	0.05	2.15	Y = 2.779 + 0.289x	0.71

¹ All data represent the average of 60 replicates (broilers) per treatment. ² MO = Moisture (%). PR = Proteins (%). FT = Fats (%). MI = Minerals (%). ³ Statistical significance was established at $p \leq 0.05$, and significant treatment effects were further examined using polynomial regression analysis. ⁴ CV = Coefficient of variation. ⁵ Mathematical model fitted according to the influence of the independent variable on the dependent variable.

4. Discussion

The production of acid silage from pirarucu by-products proved to be an effective strategy for converting fish-processing residues into a microbiologically safe and nutritionally valuable feed ingredient suitable for poultry diets. The progressive reduction in pH, together with the increase in titratable acidity and the absence of pathogenic microorganisms, indicates that the preservation process successfully inhibited microbial deterioration while maintaining the chemical quality of the material [5,13]. Similar physicochemical changes have been described for fish silages produced from different species, in which acidification promotes stabilization of the biomass and extends its storage life by preventing microbial growth [11,23,24].

The modifications observed in color, odor, and consistency throughout the ensiling period are consistent with the biochemical events expected during acid preservation. Protein hydrolysis and structural changes in lipids contribute to the gradual transformation of the original fish residue into a homogeneous material with characteristics typically associated with stabilized silage [39,40]. These changes do not indicate deterioration but rather demonstrate the progression of the preservation process under controlled acidic conditions. Likewise, the combination of low pH and elevated acidity is recognized as one of the main factors responsible for maintaining microbiological safety and preserving nutrients in fish-derived silages destined for animal feeding [40,41].

Chemical analysis demonstrated that ASP contained high concentrations of crude protein, metabolizable energy, and minerals, confirming its potential as an alternative feed ingredient for poultry [5,13,41]. Fish by-products are naturally rich in highly digestible proteins, essential amino acids, and lipids containing biologically important fatty acids, making them attractive ingredients for non-ruminant nutrition [42–44]. Furthermore, the acid ensiling process partially hydrolyzes muscle proteins, generating peptides and free amino acids that may facilitate intestinal absorption and improve nutrient utilization [39,43,44]. Because all experimental diets were formulated to be isoenergetic and isoproteic, the productive responses observed in the present study are more likely related to qualitative characteristics of ASP than to differences in dietary nutrient concentrations. Therefore, improvements in bird performance may be associated with the biological value of fish-derived nutrients and bioactive compounds generated during the ensiling process rather than with increases in dietary energy or protein content.

The performance results indicate that low-to-moderate ASP inclusion levels promoted beneficial growth responses in the broilers evaluated, particularly between 1 and 2% inclusion levels. During the starter stage, the superior weight gain observed at 1% ASP suggests that moderate supplementation improved nutrient utilization without compromising diet acceptability, an important aspect to be considered when fish by-products are used in poultry diets [42]. Similar improvements in productive performance have been reported with fish-derived silage ingredients, although the mechanisms responsible for these responses may differ among studies depending on ingredient composition and dietary formulation [11,13,42,45].

During the grower and finisher stage, the increase in feed intake, accompanied by improved weight gain and enhanced feed conversion in broilers fed diets with moderate levels (1–2%) of ASP inclusion, was associated with improved productive performance under these conditions of the present experiment, suggesting more effective utilization of dietary nutrients. Furthermore, the absence of additional improvements at the highest inclusion level (3% ASP), together with the quadratic regression responses observed for weight gain and feed conversion ratio, suggests the existence of an optimal dietary inclusion threshold for ASP. Although the experimental diets were formulated to be isoenergetic and isoproteic, increasing ASP inclusion also increased the dietary supply of fish-derived lipids and minerals while reducing the proportions of soybean meal and vegetable oil. Fish silages are characterized by highly digestible proteins and lipids that can improve nutrient utilization and metabolic efficiency at moderate inclusion levels [20,45].

However, excessive inclusion may reduce productive efficiency due to decreased feed palatability associated with the characteristic odor and flavor of fish products, metabolic adjustments related to high lipid intake, or excessive mineral supply from bone ash, which may interfere with nutrient balance and digestive efficiency [13,15,40]. Therefore, fish-derived ingredients often have practical inclusion limits beyond which performance no longer improves or may even decline, reinforcing that moderate ASP inclusion favors

productive metabolism, whereas higher inclusion levels do not provide additional benefits [46,47].

Finally, the cumulative growth performance results confirmed that moderate ASP inclusion levels (1–2%) may favored broiler growth, promoting greater weight gain and improved feed conversion ratio. These responses may be associated with qualitative characteristics of ASP that are not fully reflected by conventional proximate composition analyses, including the presence of peptides, fish-derived lipids, and other bioactive compounds generated during the ensiling process [5,25,43,45]. Nevertheless, because nutrient digestibility, peptide availability, and other potential bioactive components were not determined, the mechanisms underlying the improved performance remain to be elucidated. Conversely, the absence of additional improvements at higher inclusion levels reinforces that fish by-product-derived ingredients may present optimal inclusion levels, as excessive concentrations could limit metabolic efficiency and productive responses [46,47].

The hematological findings indicate that dietary ASP inclusion did not adversely affect the physiological status of the broilers. Instead, progressive increases in hemoglobin concentration, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin suggest an improvement in blood oxygen-carrying capacity and erythrocyte functionality as dietary ASP levels increased. Because erythrocyte counts remained unchanged among treatments, these responses appear to reflect changes in erythrocyte quality rather than stimulation of erythropoiesis [48–53].

One possible explanation is that the highly digestible proteins and balanced amino acid profile supplied by fish-derived ingredients supported hemoglobin synthesis and red blood cell maturation. Fish proteins are recognized for their high biological value and may improve the availability of nutrients involved in erythrocyte metabolism [48,53]. Nevertheless, the present study did not evaluate mineral bioavailability or iron metabolism, preventing a direct association between ASP supplementation and hematopoietic activity. Consequently, the observed hematological improvements should be interpreted as evidence of an adequate physiological response rather than proof of a specific mechanism.

The plasma biochemical profile reinforces this interpretation. Increasing dietary ASP levels promoted higher concentrations of total protein, albumin, and globulin, indicating that birds maintained an improved protein metabolic status throughout the experimental period [54–56]. Albumin is synthesized by the liver and plays an essential role in maintaining osmotic balance and transporting numerous endogenous and exogenous compounds, whereas globulins are associated with immune function and several physiological regulatory processes [52,57,58]. Therefore, the increase observed in these proteins may reflect improved nutrient utilization and an adequate nutritional condition without suggesting pathological alterations.

Unlike the protein-related variables, glucose, cholesterol, and urea concentrations were not affected by dietary treatment. The stability of these biochemical indicators demonstrates that replacing conventional ingredients with ASP did not compromise carbohydrate metabolism, lipid homeostasis, or nitrogen excretion. This finding is particularly relevant because it suggests that moderate dietary inclusion of fish silage can be achieved without disturbing the birds' metabolic equilibrium.

Triglyceride concentration exhibited a quadratic response, with the highest values observed at the intermediate inclusion level. Considering that ASP partially replaced soybean meal and soybean oil in the diets, modest alterations in lipid metabolism were expected due to differences in fatty acid composition between vegetable and fish-derived lipids [59,60]. However, because no analyses of fatty acid profiles or lipid digestibility were performed, these changes should be interpreted cautiously and cannot be directly attributed to specific metabolic pathways.

On the other hand, carcass evaluation demonstrated that dietary ASP supplementation had little influence on overall carcass yield, despite the slight reduction observed in slaughter weight [61,62]. This result indicates that carcass deposition remained proportionally stable across treatments, suggesting that moderate inclusion of ASP did not negatively affect carcass development [20,45]. From a commercial standpoint, maintaining carcass yield is particularly important in slow-growing broilers, where product uniformity represents an important quality attribute [62,63].

The reductions observed in liver and gizzard weights may reflect physiological adaptations associated with the modified composition of the experimental diets rather than detrimental effects of ASP [64,65]. The liver is directly involved in nutrient metabolism, while gizzard development is influenced by physical characteristics of the diet, including ingredient particle size and fiber composition [50]. Because fish-derived ingredients contain relatively low concentrations of structural carbohydrates compared with plant feedstuffs, reduced mechanical activity of the gizzard may explain the smaller organ weight observed in birds receiving ASP [65–68].

Among the commercial cuts, breast yield showed the most pronounced positive response, reaching its highest value with the 2% inclusion level. This finding agrees with the improved productive performance observed during the experiment and suggests that moderate ASP supplementation favored lean tissue deposition. Since breast meat represents the most economically valuable portion of the carcass, even modest improvements in its yield may contribute positively to the economic efficiency of broiler production [69,70].

Changes in breast composition further support this interpretation. The progressive increase in protein, lipid, and mineral contents, accompanied by reduced moisture, indicates greater nutrient deposition within the muscle tissue. Similar responses have been reported for poultry fed diets containing fish-derived ingredients rich in highly digestible proteins and bioavailable minerals [20,21,52,53,67–74]. Nevertheless, because muscle amino acid composition and fatty acid profile were not determined, it is not possible to establish whether these compositional changes resulted solely from the nutritional characteristics of ASP or from broader metabolic adaptations induced by the experimental diets.

Finally, from a practical perspective, the use of fish by-product silages as ASP in animal feeding is consistent with circular bioeconomy principles and contributes to the sustainable utilization of residues generated by the aquaculture industry [9,41,75]. In Brazil, the use of processed fish by-products as feed ingredients is permitted provided that raw materials are obtained from inspected sources and processed in accordance with current sanitary and feed safety regulations [11,76,77], as used in this study.

Beyond the productive responses observed, the utilization of acid silage produced from pirarucu processing residues represents an alternative strategy for improving the sustainability of poultry production. The conversion of fish-processing by-products into feed ingredients contributes to the principles of the circular bioeconomy by reducing waste generation and increasing the value of materials that would otherwise require disposal [9,41,75].

The ASP used in the present study was produced from raw materials obtained from inspected fish-processing facilities and processed according to the sanitary standards established by Brazilian legislation [11,76,77]. Under these conditions, the ingredient demonstrated adequate physicochemical and microbiological quality, supporting its safe incorporation into poultry diets.

Taken together, the present findings indicate that moderate dietary inclusion of ASP provides productive and physiological benefits while simultaneously promoting a more efficient use of aquaculture by-products. Although additional studies are still necessary to evaluate long-term effects, nutrient digestibility, intestinal health, and economic feasibility

under commercial conditions, the present results support the use of ASP as a sustainable feed ingredient capable of contributing to both poultry production efficiency and environmental management.

5. Conclusions

The results of this study demonstrate that ASP can be used as a sustainable alternative ingredient in diets for slow-growing broilers, contributing to the valorization of aquaculture by-products. Diets containing moderate levels of ASP inclusion (1–2%) improved growth performance, particularly by increasing weight gain and improving feed conversion ratio, while also promoting favorable hematological and plasma biochemical responses, consistent with an improved physiological and nutritional status, without evidence of adverse effects on bird health. In addition, diets containing ASP increased breast yield (at the 2% inclusion level) and improved the proximate composition of breast meat by increasing its protein, lipid, and mineral contents. In contrast, increasing ASP inclusion to 3% did not provide additional productive benefits and resulted in reduced productive efficiency compared with the moderate inclusion levels, suggesting that excessive dietary ASP may exceed the optimal inclusion threshold under the conditions evaluated.

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Informed Consent Statement: This study did not involve human participants. Therefore, informed consent was not applicable and no informed consent statement is required. The study involved animals only and was conducted in accordance with the relevant institutional guidelines for animal care and use.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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