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PHYSIOLOGICAL AND PRODUCTIVE PARAMETERS OF COMMERCIAL LAYING HENS FED DIETS INCLUDING BIOLOGICAL SILAGE OF TAMBAQUI BY-PRODUCTS

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RESUMO

Esta tese de doutorado teve como objetivo avaliar o desempenho zootécnico e o perfil fisiológico de poedeiras comerciais alimentadas com silagem biológica elaborada a partir de subprodutos do processamento do tambaqui (*Colossoma macropomum*). O trabalho apresentou uma abordagem integrada, contemplando desempenho produtivo, respostas fisiológicas, qualidade dos ovos, análises metabolômicas e viabilidade econômica. O Capítulo 1 trata-se de uma introdução breve sobre a problemática da tese. No Capítulo II, discutiu-se o potencial da silagem de pescado como estratégia sustentável, fundamentando o estudo no contexto da bioeconomia circular. Os Capítulos III e IV contemplaram a avaliação de poedeiras em diferentes idades, demonstrando que a inclusão da silagem não comprometeu parâmetros zootécnicos nem as características físicas, centesimais e sensoriais dos ovos, além de manter estabilidade nos indicadores hematológicos e bioquímicos. O Capítulo V aplicou a espectroscopia de Ressonância Magnética Nuclear de prótons (RMN de ^{1}H) em baixo campo, associada a análises multivariadas, permitindo identificar modificações no perfil apolar da gema e ampliar a compreensão dos mecanismos bioquímicos e metabólicos modulados pela dieta. Por fim, o Capítulo VI integrou resultados de desempenho e custos, demonstrando maior eficiência alimentar e redução do custo de formulação, o que consolidou a viabilidade econômica. De forma geral, os resultados encontrados confirmaram o valor nutricional da silagem elaborada, e evidenciaram seu potencial de reduzir custos de produção, agregar valor à cadeia produtiva e mitigar impactos ambientais. Conclui-se que a integração entre avaliação zootécnica, análises fisiológicas e metabolômicas e estudo de viabilidade econômica posiciona a silagem biológica de tambaqui como alternativa inovadora e sustentável para a avicultura de postura, contribuindo para o fortalecimento de cadeias produtivas regionais e alinhando-se às demandas por sistemas alimentares resilientes e de baixo impacto ambiental.

Palavras-chave: Aproveitamento de resíduos; Biotecnologia aplicada; Qualidade do ovo; Peixe amazônico; Nutrição de aves

ABSTRACT

The objective of this thesis was to evaluate the productive performance and physiological profile of commercial laying hens fed biological silage produced from by-products of tambaqui processing (*Colossoma macropomum*). The study adopted an integrated approach, encompassing productive performance, physiological responses, egg quality, metabolomic analyses, and economic feasibility. Chapter 1 is a brief introduction to the thesis's problems. In Chapter II, the potential of fish silage as a sustainable strategy was discussed, framing the study within the context of a circular bioeconomy. Chapters III and IV addressed the evaluation of hens at different ages, demonstrating that the inclusion of silage did not compromise productive parameters or the physical, compositional, and sensory characteristics of eggs, while maintaining stable hematological and biochemical indicators. Chapter V applied low-field proton nuclear magnetic resonance spectroscopy (^{1}H NMR), coupled with multivariate analyses, enabling the identification of modifications in the apolar fraction of the yolk and expanding the understanding of biochemical and metabolic mechanisms modulated by the diet. Finally, Chapter VI integrated performance and cost results, demonstrating improved feed efficiency and reduced formulation costs, thereby confirming economic feasibility. Overall, the results validated the nutritional value of the silage and highlighted its potential to reduce production costs, add value to the production chain, and mitigate environmental impacts. In conclusion, the integration of productive evaluation, physiological and metabolomic analyses, and economic feasibility assessment positions tambaqui biological silage as an innovative and sustainable alternative for egg production, contributing to the strengthening of regional production chains and aligning with the demands for resilient, low-impact food systems.

Keywords: By-product utilization; Applied biotechnology; Egg quality; Amazonian fish and Poultry nutrition

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LIST OF ACRONYMS

A – Ash

ABPA – Brazilian Association of Animal Protein

AS – Acid Silage

B/C – Benefit-Cost Ratio

BE – Break-even Point

BS – Biological Silage

CAPES – Coordination for the Improvement of Higher Education Personnel

CHOL – Cholesterol

CNPq – National Council for Scientific and Technological Development

CP – Crude Protein

CV – Coefficient of Variation

DHA – Docosahexaenoic Acid

DM – Dry Matter

EE – Ether Extract

EM – Egg Mass

EP – Egg Production

EPA – Eicosapentaenoic Acid

EW – Egg Weight

FAPEAM – Amazonas Research Foundation

FC – Feed Cost

FCA/UFAM – Faculty of Agricultural Sciences, Federal University of Amazonas

FCR – Feed Conversion Ratio

FEDZ – Feed Efficiency per Dozen Eggs

FEKG – Feed Efficiency per Kilogram of Eggs

FI – Feed Intake

GC-MS – Gas Chromatography–Mass Spectrometry

GLU – Glucose

Hb – Hemoglobin

HDL – High-Density Lipoprotein

Ht – Hematocrit

IBGE – Brazilian Institute of Geography and Statistics

LAB – Lactic Acid Bacteria

LADETEC/UFRJ – Laboratory for Science and Technology in Chemistry, Federal University of Rio de Janeiro

LC-MS/MS – Liquid Chromatography–Tandem Mass Spectrometry

LDL – Low-Density Lipoprotein

MCH – Mean Corpuscular Hemoglobin

MCHC – Mean Corpuscular Hemoglobin Concentration

MCV – Mean Corpuscular Volume

OPM – Operational Profit Margin

PC – Phosphatidylcholine

PC1, PC2, PC3 – Principal Components

PCA – Principal Component Analysis

PE – Phosphatidylethanolamine

PI – Profitability Index

PO – Price of Dozen Eggs

ppm – Parts per Million

PR – Gross Revenue

PUFAs – Polyunsaturated Fatty Acids

RBC – Red Blood Cells

RE – Net Revenue

TAG – Triacylglycerols

TC – Total Cost

TG – Triglycerides

TP – Total Protein

UFAM – Federal University of Amazonas

VLDLy – Very Low-Density Lipoprotein (yolk fraction)

WBC – White Blood Cells

^{1}H NMR – Proton Nuclear Magnetic Resonance

ω-3 – Omega-3

SUMMARY

CHAPTER I – INTRODUCTION, OBJECTIVES AND LITERATURE REVIEW

1. INTRODUCTION

The search for alternative feeds in animal production aims to optimize diet formulation, reducing dependence on traditionally used ingredients and mitigating the financial impacts of commodity price fluctuations, notably protein and energy inputs, which represent the most costly portion of animal feed (CRUZ; RUFINO, 2017; SILVA *et al.*, 2023). In this context, the integration between production chains has stood out as a promising strategy, with the use of by-products from the fishing industry being a viable and sustainable option for poultry nutrition. Studies indicate that such by-products, due to their high nutritional quality, have great potential to be incorporated into balanced diets, promoting productive and economic gains (SCHADER *et al.*, 2015; VAN KERNEBEEK *et al.*, 2016; RÖÖS *et al.*, 2017; VAN HAL *et al.*, 2019; BRELAZ *et al.*, 2024).

The fishing industry generates a significant amount of by-products throughout its production chain, from capture to the final commercialization of products and derivatives. This process results in a substantial volume of organic waste, much of which is improperly discarded due to structural limitations, lack of investment, deficiencies in knowledge about its nutritional potential, and difficulties in accessing technologies for its utilization (GUIMARÃES *et al.*, 2021; SANTANA *et al.*, 2024). The improper disposal of these by-products can lead to severe environmental impacts, such as soil and water contamination, as well as contributing to greenhouse gas emissions (Rossetto; Signor, 2021).

Transforming this waste into feed ingredients represents a technically viable and economically attractive alternative, given that feed costs account for approximately 70% of the total expenses in animal production (CRUZ *et al.*, 2016; CRUZ; RUFINO, 2017; SILVA *et al.*, 2023). Thus, the utilization of fishing by-products can significantly reduce diet formulation costs, especially in regions where logistical challenges hinder access to conventional ingredients such as soybean meal and corn (CRUZ *et al.*, 2016).

Studies have demonstrated that fish meals, protein hydrolysates, and other derivatives obtained from fishing by-products are rich sources of essential amino acids, omega-3 fatty acids, and micronutrients, enhancing poultry performance and improving the quality of poultry products such as meat and eggs (RAMÍREZ *et al.*, 2013; SILVA *et al.*, 2017; GUIMARÃES *et al.*, 2019). The inclusion of these ingredients in broiler and layer diets can improve feed conversion rates, enhance digestibility, and strengthen the

birds' immune systems, reducing the need for antibiotics and synthetic additives (Batalha *et al.*, 2017, 2018; Brelaz *et al.*, 2019).

Given this scenario, it is imperative to develop systems and policies that encourage the efficient use of fish processing by-products, promoting a circular bioeconomy in animal production and minimizing environmental impacts. (RAMÍREZ *et al.*, 2013; BATALHA *et al.*, 2017; SILVA *et al.*, 2017; BATALHA *et al.*, 2018; BRELAZ *et al.*, 2019; GUIMARÃES *et al.*, 2019; SANTANA *et al.*, 2024). The implementation of processing technologies, such as drying and enzymatic hydrolysis, can enhance the feasibility of using these ingredients, ensuring their safe and effective inclusion in poultry diets. Furthermore, public policies and government incentives can play a crucial role in enabling this strategy by promoting research, funding, and the necessary infrastructure for the adoption of this practice.

Therefore, the use of by-products from the fishing industry in poultry nutrition represents a promising alternative for the sustainability of the poultry production chain. In addition to reducing costs and dependence on traditional inputs, this strategy enhances food security, mitigates environmental impacts, and contributes to the improvement of production and nutritional standards in poultry farming. Therefore, the integration between animal and fishing production chains should be encouraged through innovative strategies, investment in research, and the development of sustainable policies for the sector.

In parallel with nutritional strategies, the application of analytical biotechnologies is essential to understand how alternative ingredients affect egg quality, particularly the yolk lipid fraction, which is highly sensitive to dietary changes. In this regard, proton nuclear magnetic resonance (^{1}H NMR) (BISCHOF *et al.*, 2024), even at low field, combined with multivariate statistical analyses, has proven to be a robust, fast, and non-destructive tool for molecular characterization (TAHERI *et al.*, 2024). This approach allows the detection of subtle variations in the apolar profile of egg yolk lipids, including triglycerides, phospholipids, and unsaturated fatty acids, generating insights into the functional and nutritional value of eggs. The integration of low-field ^{1}H NMR with chemometric methods thus represents an innovative strategy for monitoring the effects of fish by-product silage inclusion in layer diets, linking sustainable feeding practices to molecular-level nutritional assessment.

2. OBJETIVES

2.1.General objective

To evaluate the productive performance and physiological profile of commercial laying hens fed biological silage produced from by-products of tambaqui processing.

2.2.Specific objectives

- To evaluate the potential of fish by-product silage as a sustainable alternative in poultry feed;
- To evaluate the effects of increasing levels of tambaqui biological silage in diets for older commercial laying hens on productive performance, hematological and plasma biochemical parameters, as well as egg quality (physical, centesimal composition and sensory characteristics);
- To evaluate the effects of increasing levels of tambaqui biological silage in diets for young commercial laying hens on productive performance, hematological and plasma biochemical parameters, as well as on the quality (physical, centesimal composition and sensory characteristics) of eggs;
- To evaluate the efficiency of metabolic data obtained by low field ^{1}H NMR, associated with multivariate statistical analyses, to generate molecular insights into the nonpolar profile of egg yolk from commercial laying hens fed diets with different levels levels of tambaqui biological silage;
- To evaluate the effects of increasing levels of tambaqui biological silage in the diets of commercial laying hens in the first and second production cycle, on productive performance and economic viability.

3. LITERATURE REVIEW

3.1. Sustainable Valorization of Tambaqui By-products: Integrated Approach

Fish farming production in Brazil has shown continuous growth, registering an increase of 2.3% between 2021 and 2022. This advance was also reflected in the cultivation of native species, whose production grew by 1.8% in the same period (PEIXE BR, 2023). Among these species, tambaqui (*Colossoma macropomum*, Cuvier, 1818) and its hybrids stand out, consolidating themselves as the main native species in national aquaculture (VALENTI *et al.*, 2021; LEAL *et al.*, 2020; LIMA *et al.*, 2021).

The prominence of tambaqui is related to its productive performance, characterized by hardiness, rapid growth, meat quality, and high sensory value (GUIMARÃES *et al.*, 2014; COSTA *et al.*, 2012). In addition to being an important source of animal protein, it represents a fish of great economic importance (BORGES *et al.*, 2013), widely accepted by consumers (LEAL *et al.*, 2020), and highly valued in the Amazon region, where it is the most marketed species in fairs, ports, and local markets (NÓBREGA *et al.*, 2020).

The processing of tambaqui for commercialization involves steps such as washing, cutting, evisceration, and removal of fins and scales, generating a significant amount of by-products (COSTA; SOUZA, 2012). The proportion of these residues varies according to factors such as production scale, slaughter weight, rearing period, equipment, and operator skill (MACHADO *et al.*, 2020; GARCIA; MACIEL, 2021). It is estimated that they may represent from 50% (OLSEN *et al.*, 2014) to 70% of fish biomass (Vaz *et al.*, 2020), including head (9–10%), skin (1–3%), bones (9–15%), scales (5%), and viscera (12–18%) (BORONAT *et al.*, 2023).

Inadequate disposal of these by-products constitutes an environmental problem, with potential impacts on aquatic and terrestrial ecosystems (LIN *et al.*, 2017; DECKER *et al.*, 2016). As an alternative, sustainable use has been proposed to minimize environmental damage and add value to the production chain, by transforming waste into higher value-added products (COPPOLA *et al.*, 2021; FAO, 2022). Among the possibilities, the production of silage (BATALHA *et al.*, 2017; GUIMARÃES *et al.*, 2021), fish oil and meal (LIMA, 2013), as well as the extraction of biopolymers such as collagen (CALDATO *et al.*, 2019) and gelatin (DA SILVA *et al.*, 2018; SHI *et al.*, 2022) stand out.

In the state of Amazonas, fish farming is distributed across all municipalities, with an estimated production of 20.77 thousand tons of fish (PEIXE BR, 2022). Tambaqui is

the predominant species, present in 92.36% of farms (LIMA, 2018). However, the production chain generates a large volume of solid waste (STEVENS *et al.*, 2018), whose inadequate disposal intensifies environmental impacts (COPPOLA *et al.*, 2021). Studies on tambaqui carcass yield demonstrate significant variation: Borghesi (2017) reported a yield of 93.46% eviscerated fish and 6.54% by-products; Fernandes *et al.* (2010) identified 83.3% yield and 16.7% residues; whereas Souza and Inhamuns (2011), working with individuals weighing 1,071 g, observed approximately 45% by-products. These discrepancies highlight the need for efficient reuse systems, considering limitations such as infrastructure, investment, and access to appropriate technologies (GUIMARÃES *et al.*, 2021).

Proper utilization of these residues could reduce animal production costs (BATALHA *et al.*, 2017). Considering that feed accounts for about 70% of total production costs, with corn and soybean as the main energy and protein ingredients (BATALHA *et al.*, 2017), the search for local and affordable alternatives becomes highly relevant.

The production of silage from fish by-products emerges as a low-cost technological solution, based on biomass hydrolysis. The product presents a high protein content, essential fatty acids, and good biological value for animal nutrition (ROSSETTO; SIGNOR, 2021). In addition to reducing the amount of waste discarded into the environment, it constitutes an economically viable alternative for small producers, using rejects from fish farming, fishing, and industrial processing (ARRUDA *et al.*, 2006; CYRINO *et al.*, 2010; OLIVEIRA *et al.*, 2012; GUIMARÃES *et al.*, 2021).

3.2.Fish silage: an alternative for by-product utilization

In the preparation of fish silage, two methodologies are usually employed: the addition (combined or not) of propionic, sulfuric, acetic, formic, and hydrochloric acids, known as “Acid Silage” (ARRUDA, 2004), or the addition of lactic acid-producing microorganisms as inoculants, resulting in “Biological Silage” (VIDOTTI; GONÇALVES, 2006). With the addition of these components, preservation occurs through pH reduction in the medium via a controlled fermentation process, either by the addition of acids or by microbial lactic fermentation, which conserves the ensiled mass for a longer period (BORGHESI, 2004). Both techniques are characterized by the hydrolysis of the original protein matter in the residue to the state of oligopeptides, amino acids, and peptides (VIDOTTI, 2001).

Studies on silage preparation emphasize that the process represents a viable alternative for the utilization of fish residues. Its production technology is simple, does not require specific machinery or specialized labor, and stands out because it does not release unpleasant or polluting odors, nor does it pose problems related to pathogens (VIDOTTI *et al.*, 2003). According to Santos and Salles (2011), biological silage is an easily stored, stable product, and constitutes a protein source of high biological value. Lactic acid bacteria produce substances favorable to the ensiling process, such as lantibiotics and hydrogen peroxide, which, when reducing the pH below 4.2, drastically diminish pathogenic microorganisms, and diacetyl, a substance responsible for the pleasant fruity aroma of silage, with antifungal and antibacterial properties, except against lactic acid bacteria. This is an important factor during the process, as the preservation of fish residues depends on lactic acid formation (CÂNDIDO *et al.*, 2017).

According to Vidotti and Gonçalves (2006), success in the process requires favoring the predominance of homofermentative bacteria, best achieved through the addition of a starter inoculant such as lactic acid bacteria (Streptococcus lactis, Saccharomyces platensis, Pediococcus, and Lactobacillus plantarum), as well as other lactic acid-producing microorganisms. Residues contain only a small amount of carbohydrates, insufficient to ensure fermentation with adequate changes in pH and acidity to preserve the ensiled mass (BORGHESI, 2004). Thus, a carbohydrate source should also be added to the biomass, as carbohydrates are crucial to accelerate the fermentation process: the microbial inoculum will utilize the sugars to produce lactic acid, small amounts of CO_2, and other organic acids (VIDOTTI *et al.*, 2003). Consequently, pH decreases, triggering protein hydrolysis into amino acids and peptides (OLIVEIRA *et al.*, 2014). High-quality biological silage will be the final product of the interactions in the medium, where microorganisms promote process stability through lactic acid (VIDOTTI, 2001; VIDOTTI; GONÇALVES, 2006).

During the silage preparation process, alterations occur in the texture and appearance of the ensiled residual mass; however, its chemical and nutritional characteristics are preserved, depending on the raw material, including species, head size, rearing system, fat, and other components (VIDOTTI; GONÇALVES, 2006). One factor that may influence the nutritional quality of fish silage is prolonged storage, due to the high number of free amino acids present, endogenous enzyme activity, and the lipid content of the product (BORGHESI, 2004). According to Arruda (2004), lipid oxidation can lead to peroxide formation, which binds to proteins through covalent bonds, resulting

in amino acid destruction. The same author argues that centrifugation of residues is the best method for lipid extraction, making it possible to increase storage time.

With respect to the evaluation of the chemical and nutritional composition of fish silage with microbial inoculum, Cândido *et al.* (2017) obtained the following results: 39.01% crude protein (CP); 29.78% ether extract (EE); 14.45% ash (A); and 30.69% dry matter (DM). The crude protein content was similar to the values reported by Guimarães *et al.* (2021) when evaluating the physicochemical composition of biological silage from tambaqui residues with different amounts of microbial inoculum, showing crude protein contents of 32.49%, 33.65%, and 37.15%; lipid contents of 30.44%, 36.44%, and 33.92%; and ash contents of 15.44%, 16.46%, and 15.67%, respectively, for treatments T1, T2, and T3. In the study by Ge Fei *et al.* (2010) evaluating the chemical composition, amino acids, trace elements, hydrolysis degree, and protein in prepared silages, the authors indicated that fermentation temperature and time contribute to the degree of hydrolysis and protein content in silages prepared with fish residues. The complete presence of amino acids constituting the fish favors the nutritional quality of silage and directly influences its high digestibility (ARRUDA *et al.*, 2007). Therefore, fish residues can be directed toward silage production, standing out for their excellent protein value and potential inclusion in animal diets (JUNIOR; SALES, 2013).

3.3. Alternative nutrition in layer poultry farming

In recent years, poultry farming has achieved excellent results in its production due to advances in all areas, nutrition, genetics, management, health, and environment, making it a highly competitive activity in the meat and egg market (SCHIAVONE *et al.*, 2022). This activity is of great importance since one of the main goals of poultry production is to efficiently and economically convert raw materials into nutritious food (SILVA *et al.*, 2023). Changes in diet and the search for low-cost proteins have shaped the consumer market toward concentrated, healthier, and cheaper food options, opening new opportunities for poultry and egg production enterprises (RODRIGUES *et al.*, 2020).

In Brazil, egg production has proven to be significant. In 2020, it remained stable, with around 3.976 billion dozen chicken eggs produced (IBGE, 2020). According to ABPA (2024), most of this production is consumed domestically. *Per capita* egg consumption increased from 148 units/year in 2010 to 242 units/year in 2023. In 2022, it rose by 2.5%, reaching the remarkable figure of 262 units/year per inhabitant, with only a small percentage exported. In the Brazilian poultry sector, especially in egg production,

the use of alternative feeds is strategic, since the central goal is to convert low-attractiveness raw materials into high-value nutritional products (SILVA *et al.*, 2023). In the state of Amazonas, poultry farming became self-sufficient in egg production for consumption in 2001, and since then, production has been steadily growing due to technological advances and the increasing number of producers interested in the activity (CRUZ *et al.*, 2016). However, many systems are based on family farming, meaning that poultry farming, as with other agricultural activities, is deeply rooted in riverine communities. The high costs of acquiring raw materials for feed formulation remain an obstacle for production (SCHOR, 2015).

Despite these challenges, the state meets about 90% of its egg demand, with an annual production in 2018 of 42.152 million dozen eggs in the municipality of Manaus alone, ranking as the 12th largest egg-producing municipality in Brazil (IBGE, 2019). The use of alternative feeds in production systems has been encouraged to promote the utilization of materials otherwise considered unusable but with nutritional properties (SILVA *et al.*, 2023), thereby increasing productivity and reducing production costs without harming the animal's physiology (ASSUNÇÃO *et al.*, 2019). In recent years, the search for alternative feed has contributed to reducing expenses in animal nutrition (AMÂNCIO *et al.*, 2010).

Studies have reported relevant results on productive and economic performance using fish by-products as alternative feed. Silva *et al.* (2017), using fish residue meal in laying hen diets, observed improvements in egg production, egg weight, and reductions in feed cost and production cost, corroborating Brelaz *et al.* (2021a) who found better economic viability at a 1.50% inclusion level of fish residue oil in egg production. Evaluating the physicochemical and nutritional properties of acid silage meal from *Arapaima gigas* residues in diets for commercial light layers, Batalha *et al.* (2017), reported significant differences in crude protein digestibility, nitrogen-free extract (soluble carbohydrates), ether extract, ash, metabolizable energy, and metabolizable energy utilization coefficient. Their results indicated that silage can be included in the form of meal at up to 3% in diets for light laying hens. Guimarães *et al.* (2019) reported that biological silage meal made from tambaqui residual biomass, when included in commercial laying diets, showed high CP digestibility compared with control diets. The authors further highlighted that hydrolyzed proteins resulting from lactic fermentation during ensiling yielded autolyzed proteins of high biological value.

Brelaz *et al.* (2019), including fish residual oil in layer diets, observed increased feed intake up to a 2.50% inclusion level. Above this, feed intake, egg mass, and flavor were negatively affected. Besides fish residues, other agro-industrial by-products have also been used as alternative feeds for layers. Rufino *et al.* (2017) showed that buriti residue meal used as a feed additive did not increase costs or cause revenue losses. However, inclusion of up to 25% of this residue led to higher feed intake, production costs, and reduced profitability. In another study, layers fed diets containing 25% açaí bran showed low metabolization and utilization of energy content (ether extract), dry matter, and fibrous carbohydrates, but improved utilization of crude protein, non-fibrous carbohydrates, and ash (RUFINO *et al.*, 2020). In this sense, the use of by-products in poultry farming represents an alternative to prevent environmental damage, optimize production through biotechnical reuse strategies, and improve productive performance of birds (RUFINO *et al.*, 2015).

According to Pessôa *et al.* (2012), the pursuit of maximum feed efficiency and cost reduction in poultry farming is a critical point to consider. A properly balanced feed for animal nutrition is nutritionally complete when it reduces stress, minimizes deficiencies, enhances immune competence, and improves production performance. Specifically for laying hens, achieving good productive performance and egg quality indices is key to the success of the commercial egg sector. An important factor in diet formulation is knowledge of the chemical composition of alternative feeds, as well as their nutrient availability, which contributes to reduced costs, good quality, and high digestibility (FERNANDES *et al.*, 2012). Hematological parameters also allow the evaluation of the bioefficacy of an alternative feed, reflecting its effect on animal nutrition by indicating physiological status (VILA, 2013). Nutritional stress or environmental conditions are reflected in quantitative and morphological changes in blood cells, with variations in hematocrit, circulating leukocyte count, erythrocyte content, and hemoglobin concentration (MACARI *et al.*, 1994).

Blood values can be influenced by nutritional status (feed), sex, age, habitat, season, reproductive state, trauma, rearing conditions, and environmental stress (CAMPBELL, 2004). Poultry nutrition studies show considerable interest in monitoring serum lipid, triacylglycerol, and cholesterol levels, in addition to fatty acids, as a way to estimate certain physiological conditions in birds (HILL, 1983). The inclusion of alternative feeds in the diet influences the blood metabolism of birds and should be carefully observed. A study by Brelaz *et al.* (2021b), analyzing the blood biochemistry of

layers fed diets with increasing levels (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5%) of fish residue oil, found significant differences in chloride, triglycerides, glucose, and uric acid parameters, with higher total cholesterol levels (226.18 mg/dL) and a marked decrease in plasma triglyceride levels in the group that received diets with the highest levels of fish oil. Feijó *et al.*, (2016) adding increasing levels (0, 5, 10, 15, 20, and 25%) of yam (*Dioscorea trifida* L.) meal to the diets of commercial light layers, observed significant differences in blood triglyceride concentrations (479.68 mg/dL) at 4.05% yam meal inclusion, while blood pH reached its highest value (7.09) at 6.15% inclusion, indicating sensitivity of avian blood metabolism to high levels of free carbohydrates in the diet. These studies demonstrate that the interaction between nutrition and health is essential in animal production systems, since, as a consequence, the productive performance of individuals and flocks is influenced by environmental and management factors (GONÇALVES *et al.*, 2009)

3.4.Economic and sustainable aspect of by-product use in poultry farming

Poultry farming is one of the main pillars of global food production, playing a fundamental role in food security and in the affordable supply of high-quality animal protein (PICCOLO *et al.*, 2024). However, the continuous increase in input costs, especially those related to feed, compromises the economic and environmental sustainability of the sector, since feed expenses can represent 60% to 80% of total production costs (BOWLES *et al.*, 2019; CRUZ *et al.*, 2016). This challenge is even more critical in the Amazon region, where logistical limitations significantly increase the cost of formulating balanced diets (CRUZ *et al.*, 2016). In this scenario, the search for nutritional alternatives that are technically and economically viable, reduce dependence on traditional ingredients such as corn and soybean meal, and strengthen the sustainability of the production chain becomes indispensable (RUFINO *et al.*, 2015; CRUZ *et al.*, 2016).

In this context, the use of agro-industrial by-products emerges as a promising strategy, as it reduces costs, enables waste recovery, and integrates circular economy principles into poultry farming (CRUZ; RUFINO, 2017). Among these alternatives, residues from fruit and fish processing, often discarded, have relevant nutritional value and, when processed into meals or silages, can be incorporated into diets as supplements, generating direct economic benefits (by reducing feed costs) and indirect benefits, such

as increased productivity and improved system profitability (GUIMARÃES *et al.*, 2021; SILVA *et al.*, 2023).

The Amazon region has great potential for the use of alternative ingredients, given its biodiversity and availability of raw materials with recognized nutritional, technological, and economic value (SANTOS *et al.*, 2024). In this context, the fishing industry represents a strategic opportunity: in addition to its growth in recent decades, driven by global demand for fish (COPPOLA *et al.*, 2021), it generates large volumes of biological residues that, if poorly managed, entail high environmental and economic costs (DECKER *et al.*, 2016; COPPOLA *et al.*, 2021). Transforming these residues into feed inputs, therefore, not only helps mitigate environmental impacts but also reduces disposal costs and creates value within the production chain itself.

Fish biological silage emerges as a low-cost, practical technological alternative with great economic potential. Obtained through biomass hydrolysis, it results in a product rich in proteins and fatty acids, with high nutritional value, suitable for use in feed formulations (ROSSETTO; SIGNOR, 2021), especially for poultry (GUIMARÃES *et al.*, 2019; GUIMARÃES *et al.*, 2025). In addition to reducing dependence on conventional inputs, its use can improve production economic indicators, such as the average feed cost per kilogram of eggs produced, operational profit margin, and the benefit/cost ratio of the activity (ARRUDA *et al.*, 2006; OZYURT *et al.*, 2017).

Among the species of greatest relevance for this use is tambaqui (C. macropomum), considered the most cultivated native fish in Brazil due to its high productivity, adaptability to tropical conditions, and consequent large-scale residue generation (ARIDE *et al.*, 2018; LIMA *et al.*, 2021; VALENTI *et al.*, 2021). Processing these residues into tambaqui biological silage can reduce feed formulation costs and increase poultry sector profitability, while simultaneously strengthening local production chains and promoting sustainable regional development.

3.5.Analytical biotechnologies applied to egg evaluation: emphasis on Proton Nuclear Magnetic Resonance (^{1}H NMR) and metabolomics

Sustainable and economically viable technological innovations in animal production have driven the adoption of feeding strategies based on the use of agro-industrial by-products and organic residues (ABBAS, 2023). In poultry systems, the partial replacement of conventional ingredients with alternative sources can reduce feeding costs (DIARRA; DEVI, 2015; CRUZ; RUFINO, 2017), contribute to

environmental sustainability, and enhance the nutritional functionality of meat and eggs (GAUTRON *et al.*, 2022; YOGESWARI *et al.*, 2024). The nutritional evaluation of alternative feeds for laying hens involves the analysis of productive parameters, with emphasis on the physical quality and composition of eggs, particularly the yolk lipid fraction, which is highly sensitive to dietary changes (XIAO *et al.*, 2020; XIA *et al.*, 2022).

Egg yolk is a remarkably complex nutritional matrix (XIA *et al.*, 2022), composed of a wide variety of macronutrients and micronutrients (YOGESWARI *et al.*, 2024) that not only provide high biological value (XIA *et al.*, 2022), but also confer relevant functional and technological properties (KASSIS *et al.*, 2010). A fresh chicken egg consists of approximately 48–52% water, 30–33% lipids, and 16–18% proteins (GAUTRON *et al.*, 2022). The lipid fraction includes triglycerides, phospholipids, and cholesterol (XIAO *et al.*, 2020), while proteins are mostly organized into low-density (LDL) and high-density lipoproteins (HDL), livetins, and phosvitin (XU *et al.*, 2012; YU *et al.*, 2019).

The lipid composition of egg yolk is strongly influenced by the laying hen's diet (GAUTRON *et al.*, 2022), and may include long-chain polyunsaturated fatty acids, such as ω-3 polyunsaturated fatty acids and triglycerides (XIAO *et al.*, 2020), which are recognized for their nutritional value to human health (MIRANDA *et al.*, 2015). Moreover, the yolk contains carotenoids, minerals such as iron and zinc, and fat-soluble vitamins (including vitamins A and E) (XIA *et al.*, 2022), reinforcing its role as a functional food (MIRANDA *et al.*, 2015)

Beyond its nutritional profile, egg yolk stands out for its technological properties (WANG *et al.*, 2023), widely used in the food industry as an emulsifier (HOHMANN *et al.*, 2015), and gelling agent (AU *et al.*, 2016). Emulsification, in particular, is enhanced by the structure of LDL and phospholipids such as phosphatidylcholine, which are essential for the stability of emulsions in food and pharmaceutical formulations (CHEN *et al.*, 2014). Detailed characterization of these effects requires robust and comprehensive analytical approaches (TAHERI *et al.*, 2024).

Metabolomics, particularly when combined with proton nuclear magnetic resonance spectroscopy (^{1}H NMR), offers a powerful, rapid, and simple methodology for studying metabolic profiles in animal-derived foods (BISCHOF *et al.*, 2024). ^{1}H NMR enables the simultaneous detection of multiple lipidic and water-soluble compounds, allowing comparative analyses across different dietary treatments and rearing systems

(CARDOSO *et al.*, 2022; BISCHOF *et al.*, 2024). Studies have demonstrated its effectiveness in differentiating eggs produced in conventional, free-range, and organic systems, as well as in authenticating origin and detecting adulteration (ACKERMANN *et al.*, 2019; XIA *et al.*, 2022; BISCHOF *et al.*, 2024).

Furthermore, recent investigations employing multivariate methods and machine learning have demonstrated the ability of ^{1}H NMR to distinguish egg profiles based on diet, bird age, and housing conditions with high accuracy (CARDOSO *et al.*, 2022; BISCHOF *et al.*, 2024). The observed variations include changes in signals attributed to unsaturated fatty acids (δ ~2.8–2.9 ppm), glycerol (δ ~4.1 ppm), phospholipids (phosphatidylcholine-PC, phosphatidylethanolamine-PE), and choline-dependent compounds, often used as biomarkers in the nutritional evaluation of eggs (XIA *et al.*, 2022). Such analyses allow not only for product quality assessment but also for inferences about dietary physiological effects and the metabolic efficiency of birds in different production stages (GU *et al.*, 2023; YOGESWARI *et al.*, 2024). The integration of sustainable nutritional strategies with advanced analytical approaches such as metabolomics represents a significant step forward for the valorization of regional ingredients and for the qualitative improvement of poultry products.

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CHAPTER II – USING FISH BY-PRODUCTS AS SILAGE FOR ALTERNATIVE FOOD IN POULTRY DIETS

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5. MATERIALS AND METHODS

For the preparation of this chapter, a bibliographic survey was conducted using indexed literature references, through consultation of the main portals and databases of scientific journals: Google Scholar (www.scholar.google.com.br), SciELO (www.scielo.org), CAPES Journal Portal (www.periodicos.capes.gov.br), as well as international databases such as Scopus and Web of Science.

This research is classified as exploratory and descriptive, as it sought specific information and characteristics related to the subject under study. Searches were carried out in Portuguese and English without temporal limitation, using combinations of the following terms as Keywords:

Portuguese and English: poultry nutrition, alternative feeding, fish by-products, fish silage, fish processing waste, poultry sustainability, circular bioeconomy, animal performance, laying hens. To simplify the reading and discussion of the findings, the chapter was structured into thematic topics, according to the main axes addressed in the publications surveyed.

6. RESULTS

6.1.A general overview of fish by-products

One of the major concerns of the fish production chain, both in Brazil and worldwide, is the large amount of solid waste generated (STEVENS *et al.*, 2018), most of which is improperly disposed of, consequently creating a serious environmental problem (COPPOLA *et al.*, 2021). According to Borghesi *et al.* (2007), the more diversified the production line or the greater the processing the fish undergoes, the more waste-generating stages there are. From slaughter to fish processing, such as the removal of the head, viscera, fins, tail, spine, scales, and meat scraps, and considering the species and the form of processing, the amount of waste can vary (ARRUDA *et al.*, 2007).

According to Vidal *et al.* (2011), to determine the yield of the edible portion of the fish, and consequently the volume of waste generated, the filleting equipment, the worker's manual efficiency, and the shape and size of the fish must also be considered. Technological or market limitations, regardless of the species, tend to generate a waste volume ranging from 30 to 70% of the initial weight of the fish (SOUZA; INHAMUNS, 2011). In the past, studies have already pointed out that such waste has the potential to be treated by biological processes, employing innovative and emerging technologies for use in the development of new products for both human consumption and animal feed (GUIMARÃES *et al.*, 2021).

Miranda and Lenz (2021) highlighted the biological quality of fish, with products that can reach a protein content of up to 90%, as well as minerals and vitamins with functional properties of proteins, with potential for use as nutritional supplements. Feltes *et al.* (2010), in research aimed at finding value-added alternatives for fish processing waste, mention that it has a composition rich in organic and inorganic compounds, raising concerns about the potential negative environmental impacts of disposing of this material directly into the environment.

In this regard, the fishing sector has been seeking a significant reduction in pollution from waste for decades by implementing circularity in its production chains, which can become a distinguishing factor for animal production, consequently diversifying its production and ensuring sustainable development (FELTES *et al.*, 2010; GUIMARÃES *et al.*, 2021). It is clear that this waste needs to be treated appropriately, complying with environmental standards (CONAMA, 2005) and the national waste management policy (BRASIL, 2010), making it possible to convert complex organic

matter (carbohydrates, proteins, and lipids) into simpler forms that can be assimilated by other compartments of the ecosystem (CHERNICHARO, 2007).

According to ABNT NBR 10.004 (2004), fish viscera are classified as solid or semi-solid waste resulting from industrial, domestic, agricultural activities, among others, including sludge from effluent treatment plants and waste generated in pollution control equipment and facilities, which cannot be directly discharged into public sewage systems or the environment. Thus, the use of by-products from fish processing becomes of utmost importance in reducing this improper disposal, consequently mitigating this pollution (TRAN *et al.*, 2015; DECKER *et al.*, 2016; COPPOLA *et al.*, 2021).

In this context, there is a wide range of technological possibilities available for the utilization of fish by-products, which can be applied in various sectors. In the food industry, for example, these by-products can be used for human consumption, provided they undergo rigorous hygienic and sanitary controls (STEVANATO *et al.*, 2007), offering a sustainable and profitable alternative for the food industry (ANBE *et al.*, 2015).

Additionally, they can be transformed into silage for use as ingredients in high-quality nutritional diets for production animals (BATALHA *et al.*, 2017; BATALHA *et al.*, 2018; GUIMARÃES *et al.*, 2019; GUIMARÃES *et al.*, 2025), used in the production of fertilizers (PAES *et al.*, 2016), leather goods, bioproducts, as well as in the extraction of enzymes, collagen, carotenoids, gelatin, and oils (AGUIAR *et al.*, 2014; NÓBREGA *et al.*, 2024; BARAI *et al.*, 2025). These processes not only add value to the waste but also contribute to the sustainability and diversification of industries that work with fish.

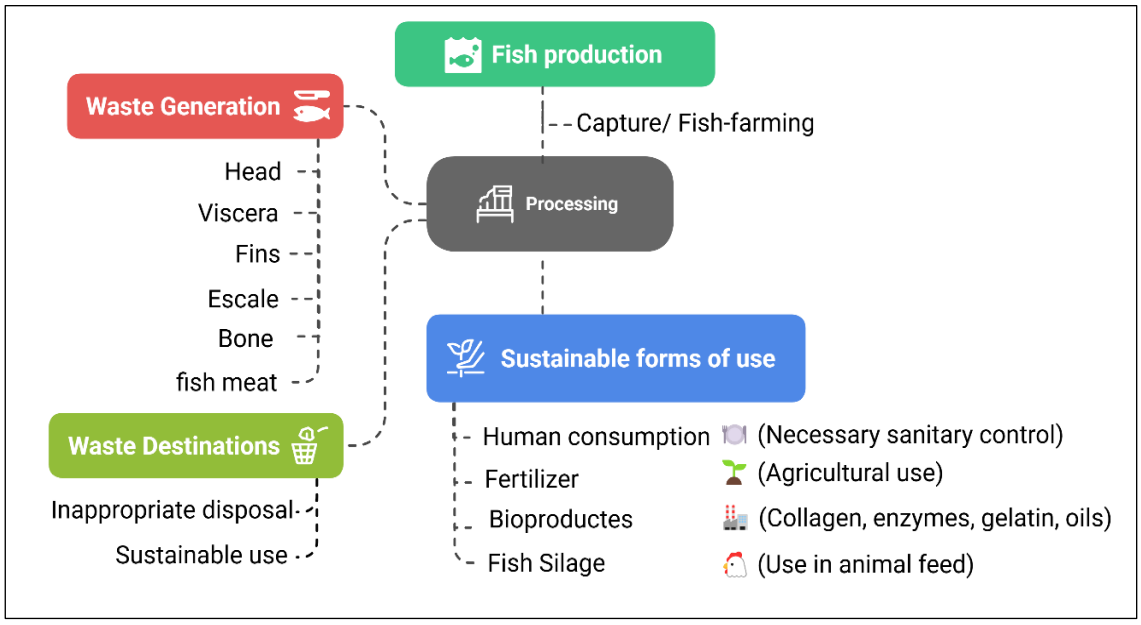

Figure 1. Sustainable use of fish by- products.

6.2.Fish silage as an alternative for the utilization of fish by-products

Many studies have highlighted the production of silage as a viable alternative for utilizing the residues from the fish production chain. This is particularly because it is a relatively simple processing technology that does not require specific machinery or highly specialized labor (VIDOTTI *et al.*, 2003). According to Machado (2010), fish residue silage is a microbiologically stable product, easy to store, and based on its nutritional composition, it constitutes a protein source of high biological value. Historically, fish residue silage emerged in the 1920s in Finland, aiming to utilize these residues. The method used for its production was adapted from those used in forage preservation, consisting of a combination of sulfuric and hydrochloric acids. In the 1930s, Sweden began more technically advanced production of this silage, where, in addition to the acids mentioned above, formic acid and molasses were introduced during the process (HISANO *et al.*, 2012). In the 1940s, other countries like Canada, Australia, Norway, and Germany started producing fish residue silage in small quantities (SANTOS; SALES, 2011; HISANO *et al.*, 2012). Later, from the 1960s onward, Denmark, Poland, and Norway began commercial-scale silage production using the acid combination method, with the product being used in animal feed as a protein supplement (ARRUDA *et al.*, 2007).

However, despite the numerous studies conducted on silage production techniques over the decades, fish silage is still not produced on a large scale in many countries with

a strong primary sector, such as Brazil. This is due to various factors, most notably the inconsistent supply of quality material for the silage process, the need for minimal infrastructure and technology for larger-scale production beyond artisanal methods, among others (BATALHA *et al.*, 2017; BATALHA *et al.*, 2018).

Technologically, two basic methodologies are used for producing fish silage: acid silage, which is produced by adding organic or inorganic acids such as formic, sulfuric, hydrochloric, propionic, and acetic acids; and biological silage, which is produced using lactic acid-producing microorganisms along with a carbohydrate source (ARRUDA *et al.*, 2007). Both of these methods are based on a controlled fermentation process that makes this residue suitable for longer preservation. The preservation of the ensiled material is achieved by lowering the pH in the medium, either through acid addition or microbial lactic fermentation (BORGHESI *et al.*, 2007).

Chemically, the autolyzed material during silage production is characterized by the degradation of the original protein matter from the fish residue into peptides, oligopeptides, and amino acids (ARRUDA *et al.*, 2007; BORGHESI *et al.*, 2007). Despite the alterations that occur during the production process, fish residue silages retain chemical and nutritional characteristics similar to the original material, meaning they depend on species, head size, farming system, fat content, and other factors that make up the silage (CÂNDIDO *et al.*, 2017).

In terms of composition, Oliveira *et al.* (2014) evaluated the chemical composition of biological fish silage in wet and semi-dry forms and obtained crude protein values of 13.30% for wet silage and 38.94% for dry silage. For the wet silage, they also observed the following contents: 61.80% moisture; 3.45% lipids; 6.85% ash; 14.60% carbohydrates; and a caloric value of 1,015 Kcal/100g. For semi-dry silage, the moisture was 14.34%; lipids 4.77%; ash 31.98%; carbohydrates 9.97%; and caloric value 1,479.70 Kcal. Nascimento *et al.* (2014) conducted a study to evaluate the physicochemical and nutritional characteristics of acidic, biological, and enzymatic silages produced from fish residues (fins, head, viscera, and scales). These authors found that the silages had: 46.07; 29.19 and 38.6 of crude protein; 41.58; 29.83 and 42.87 of ash; 8.29; 23 and 46 of lipids; and 4.50; 2.50 and 0.43 of moisture, respectively. The crude protein levels obtained in acidic silage were higher than those observed in biological silage, which can be explained by the production process that allowed better protein utilization and is also related to the variety of residues used in the preparation.

In evaluating the chemical and nutritional composition of fish silage with microbiological inoculum, Cândido *et al.* (2017) obtained the following results: 30.69% dry matter; 14.45% mineral matter; 29.78% ether extract; 39.01% crude protein; and 5143 kcal of gross energy/kg. The crude protein content found was similar to the values obtained by Guimarães *et al.* (2019) when evaluating the physicochemical composition of biological silage from tambaqui residue with different amounts of microbiological inoculum.

It is important to highlight that one of the factors that can influence the nutritional quality of fish silage is prolonged storage, due to the high number of free amino acids present in the silage, the activity of endogenous enzymes, and the lipid content in the product (BORGHESI *et al.*, 2007). According to Arruda *et al.* (2007), lipid oxidation can lead to the formation of peroxides, which complex with proteins through covalent bonds, consequently destroying amino acids. This same author argues that centrifuging the residues is the best method for lipid extraction, making it possible to increase the storage time.

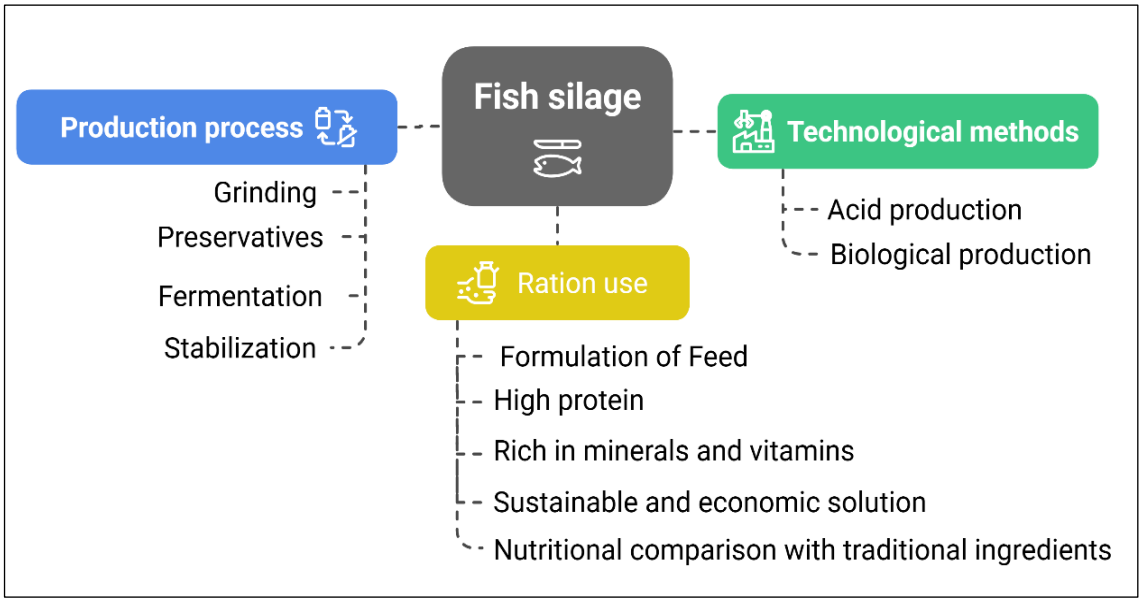

Figure 2. Production process and use of fish silage.

6.3.Fish silage as an alternative food in poultry diets

The use of alternative feeds in poultry production has been employed to encourage the reuse of by-products that were previously considered waste, but which possess nutritional properties to enhance productivity and reduce production costs without harming the birds' physiology (AMÂNCIO *et al.*, 2010; GIROTTO; SANTOS, 2012).

This becomes even more crucial for regions facing logistical challenges in sourcing inputs, such as the Amazon region states (CRUZ *et al.*, 2016).

When evaluating the use of fish silage as an alternative feed in poultry diets, Ramírez *et al.* (2013) assessed the effect of biological silage mixed with soybean meal (1:1) at inclusion levels (0, 10, 20, and 30%) on the performance and meat quality of Japanese quails (*Coturnix coturnix japonica*). The authors observed improvements in the fatty acid composition of the breast meat, but no effect on growth rate and feed conversion, and no significant impact on carcass yield in the treatments. Similar results were obtained in a study by Enke *et al.* (2010), which evaluated the use of fish silage meal combined with defatted rice bran in Japanese quail diets, and also found no effect on weight gain, feed conversion, and carcass yield. However, positive results were found by Collazos and Guio (2007) in an experiment with inclusion levels of up to 6.0% of biological fish silage for Japanese quails, with the authors recommending its use in diets to improve performance and egg quality.

Al-Marzooqi *et al.* (2010) evaluated the effect of four levels (0, 10, 20, and 30%) of sardine silage on the performance and meat quality characteristics of broilers raised in both closed and open housing systems. They found that, in both systems, broilers fed diets containing 10 and 20% silage gained more weight than those fed 30%. On the other hand, Shabani *et al.* (2018), replacing soybean meal in broiler diets with biological fish silage, reported improvements in performance and meat quality, classifying silage as an adequate protein source in diets.

In another study, Kjos *et al.* (2001) using acid silage in diets for light laying hens reported that its inclusion did not cause negative effects on performance and egg quality. The same authors also commented that fish silage is an excellent protein source for these diets. Evaluating the physicochemical and nutritional characteristics of acid silage meal from arapaima residues in diets for commercial layer hens, Batalha *et al.* (2017, 2018, and 2019) found significant differences in the digestibility of crude protein, non-nitrogen extract (soluble carbohydrates), ether extract, mineral matter, metabolizable energy, and the coefficient of metabolizable energy, indicating that silage can be included in meal form up to 3% in diets for light layers. The same authors found that this reduces egg production costs, being a product with low operational cost and an alternative to minimize the environmental impact generated during the disposal of fish processing waste. Guimarães *et al.* (2019), testing biological silage meal made from tambaqui residual biomass in commercial layer diets, observed high digestibility of crude protein in these

diets compared to the control diet, attributed by the authors to the proteins resulting from lactic fermentation by microorganisms during the ensiling process, yielding high biological value autolyzed proteins, with the alternative feed being classified as a potential source of dietary energy and protein.

Guimarães *et al.* (2025) investigated the inclusion of biological silage in the diet of older laying hens and found that levels between 1% and 2% improved egg weight, eggshell quality, and feed efficiency without compromising bird health. However, higher concentrations reduced feed intake, egg production, and egg mineral content, possibly due to sensory rejection. Additionally, there was an increase in MCV and MCH values, elevated triglycerides, and reduced plasma cholesterol, suggesting potential benefits for lipid metabolism at moderate levels. Sensory analysis indicated greater acceptance of eggs with 3% to 4% silage inclusion due to enhanced yolk coloration, although these levels were not ideal for productivity. The study highlights the potential of biological silage for by-product valorization and circular economy practices, suggesting that processing improvements could optimize sensory acceptance without compromising.

7. CONCLUSIONS AND OUTLOOK

In conclusion, the study highlights the significant potential of utilizing fish by-products as alternative feedstuffs in animal production, particularly in poultry diets. The transformation of these by-products into fish silage not only addresses the environmental concerns associated with improper waste disposal but also offers a cost-effective solution to enhance the nutritional quality of animal feed. The research demonstrates that fish silage can be successfully incorporated into poultry diets, improving performance and reducing feed costs, especially in regions with limited access to traditional feed resources. This approach not only supports sustainable animal production but also contributes to the overall circular economy by adding value to what would otherwise be waste.

Moreover, the findings underscore the importance of integrating technological innovations in the fish production chain to maximize the utilization of by-products. By adopting methods such as acid and biological silage production, industries can effectively manage waste while providing high-quality, nutritionally rich feed for poultry. The study also emphasizes the need for further research to optimize silage production techniques and to explore the broader application of fish by-products in various animal production systems. Ultimately, this approach aligns with the goals of sustainability, resource efficiency, and environmental stewardship, offering a viable alternative for the animal feed industry while mitigating the ecological impact of fish processing activities.

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CHAPTER III – BIOLOGICAL SILAGE FROM TAMBAQUI BY-PRODUCTS ON THE PRODUCTIVE PERFORMANCE, HEMATOLOGICAL PARAMETERS AND EGG QUALITY OF OLDER COMMERCIAL HENS

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9. MATERIALS AND METHODS

The current study was conducted at the Research Poultry Farm of the Federal University of Amazonas (UFAM), on the University campus located in Manaus City, Amazonas, Brazil. This study was conducted between the months of July and September, which are characterized as the beginning of summer in the Amazon Rainforest. All experimental procedures were performed in accordance with the Local Experimental Animal Care Committee and were approved (protocol number 005/2022) by the institutional ethics committee of the UFAM, Brazil.

9.1.Processing of tambaqui by-products and silage production

The tambaqui by-products used in the production of biological silage, consisting of viscera, gills, scales, and fins, which are materials discarded during the processing of tambaqui (fish weighing between 800 to 1,300 g), were obtained from an industrial fish processing plant in Manaus city, Amazonas. These were transported in thermal boxes to the laboratory and stored in a freezer until processing. During the processing, the by-products were removed and left at room temperature to thaw, after which they underwent a thermal treatment at 65°C for 5 minutes. Subsequently, they were filtered through a sieve, and the residual mass after filtration was pressed using a hydraulic press. The final content was weighed and distributed into 40-liter plastic buckets.

For silage production, the residual fish mass was mixed with the following ingredients in the specified proportions: (10% w/w) cassava trimmings (*Manihot*

esculenta Crantz), acquired from municipal markets in Manaus. These were cleaned and ground to be used as a carbohydrate source in the silage; (2.5% v/w) inoculum, consisting of pure cultures of the bacterium *Lactobacillus plantarum* as proteolytic microorganisms; 0.1% (w/w) benzoic acid as a fungicidal and bactericidal preservative; and 0.1% (w/w) ascorbic acid as an antioxidant, following the methodology proposed by Vidotti *et al.* (2003) and Vidotti *et al.* (2011).

All ingredients were stirred in the buckets until complete uniformity of the silage components was achieved. Subsequently, they were hermetically sealed to maintain the material under anaerobic conditions. For this purpose, a sterilized plastic bag was placed in contact with the surface of the ensiled mass before sealing the buckets. The ensiled mass was then stored and stirred every 24 hours to facilitate a more efficient fermentation process. It remained stored for 14 days at room temperature under anaerobic conditions.

During this period, the silage was evaluated for its organoleptic characteristics using subjective criteria (GUIMARÃES *et al.* 2019), including odor, color, and texture. Specifically, observations were made every three days to assess these attributes. The mass hydrogen ionic potential (pH) was measured using a bench pH meter, while the titratable acidity was determined through titration with 0.1 N NaOH, utilizing 0.5 mL of phenolphthalein (1.5%) as an indicator. These assessments allowed for a comprehensive evaluation of the silage's quality and stability over time. At the end of the product processing, the silage was placed in aluminum trays and placed in a forced ventilation oven at 65°C (149°F) for 72 hours to reduce moisture. During this period, the biomass was stirred to ensure a uniform drying process (Figure 3).

After drying, proximate composition analyses were conducted to evaluate moisture, crude protein, fats, fiber, minerals, and soluble carbohydrate contents, following the methodologies recommended by AOAC (2019). Microbiological analyses were carried out in accordance with the standards of the Brazilian Ministry of Agriculture, Livestock, and Supply (2018), which specify the analysis of total and thermotolerant coliforms, *Salmonella* sp., *Staphylococcus* sp., molds, and yeasts. After completing the analyses, the product was sent to the poultry sector to proceed with the experiment involving commercial hens.

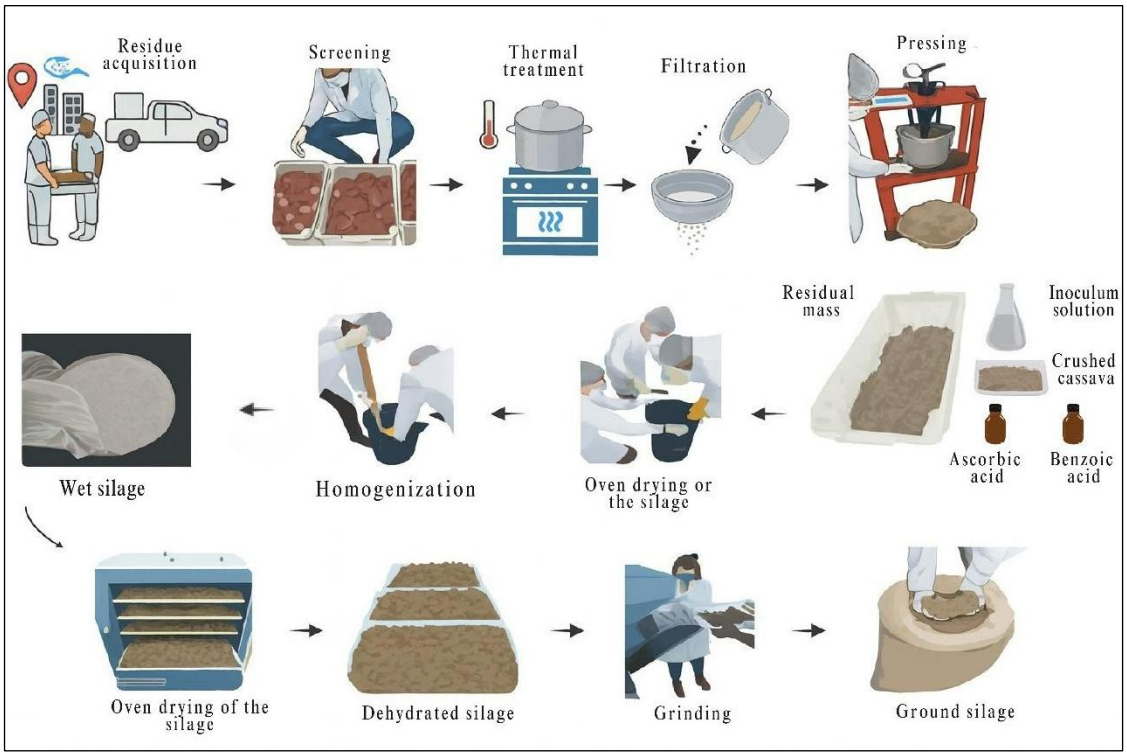

Figure 3. Processing of biological silage from tambaqui by-products.

9.2.Facilities, animals, diets and experimental design

The aviary used had 17 meters in length, 3.5 meters in width, and a ceiling height of 3.25 m, with structural adaptations to improve bird welfare. The temperature and relative humidity were monitored using a digital thermohygrometer, registering averages of 28.6 °C (83.48 °F) and 54.15%, respectively. Throughout the entire experimental period, the hens were monitored for signs of thermal stress caused by the environment, a situation that was not observed during all this time.

The experimental period lasted 63 days, divided into three subperiods of 21 days each. One-hundred twenty commercial hens of the Hisex Brown strain, 83 weeks old, previously subjected to a seven-day adaptation period to the diets and facilities, were used. The birds were weighed at the beginning of the experimental period to standardize the plots, with an average weight of 1.83 ± 0.158kg. Hens were housed in galvanized wire cages (0.45 meters in height, 0.40 meters in width, and 1.00 meters in length), accommodating 6 birds each suspended in a single line. It also had trough feeders and nipple drinkers. The birds were provided with 16 hours of light per day (12 hours of natural light + 4 hours of artificial light) throughout the experimental period. The collection of eggs was carried out twice a day (9 a.m. and 3 p.m.), with a record of each daily occurrence (mortality, number of eggs, among others).

The hens were distributed in a completely randomized design consisting of the control treatment (without inclusion) and four inclusion levels of tambaqui biological silage (1, 2, 3, and 4%) in the diets, with four repetitions of six birds each, totaling twenty-four birds per treatment and, consequently, a total of one-hundred twenty birds. The experimental diets (Table 1) were formulated to meet the nutritional requirements of the commercial hens using the reference values described by Rostagno *et al.* (2017) and the values obtained for the tambaqui biological silage composition previously. For comparison purposes, a proximate analysis of the diets was performed to evaluate the accuracy of the calculated values.

Table 1: Experimental diet composition.

Feedstuffs	Biological silage from tambaqui by-products levels, %				
	0	1	2	3	4
Corn (7.88%)[1]	65.80	65.30	64.42	64.29	63.79
Soybean meal (46%)[1]	21.41	20.76	20.11	19.46	18.81
Tambaqui biol. silage	0.00	1.00	2.00	3.00	4.00
Limestone	9.78	9.91	10.42	10.17	10.30
Dicalcium phosphate	1.93	1.94	1.95	1.95	1.96
Vit. min. supplement[2]	0.60	0.60	0.60	0.60	0.60
Salt	0.29	0.29	0.30	0.30	0.30
DL-methionine (99%)[3]	0.19	0.20	0.21	0.22	0.23
Total	100.00	100.00	100.00	100.00	100.00
Nutrient	Nutritional levels				
M.E., kcal.kg^{-1}	2,900.00	2,900.00	2,900.00	2,900.00	2,900.00
Crude protein, %	15.50	15.50	15.50	15.50	15.50
Calcium, %	4.32	4.37	4.42	4.47	4.52
Available phosphorus, %	0.45	0.45	0.45	0.45	0.45
Crude fiber, %	2.55	2.52	2.48	2.45	2.41
NDF, %	10.54	10.39	10.24	10.09	9.94
ADF, %	3.92	3.85	3.78	3.71	3.64
Methionine + Cystine, %	0.70	0.70	0.70	0.70	0.70
Methionine, %	0.43	0.44	0.45	0.45	0.46
Lysine, %	0.76	0.74	0.72	0.70	0.68
Threonine, %	0.60	0.58	0.57	0.56	0.54
Tryptophan, %	0.18	0.17	0.17	0.16	0.16
Sodium, %	0.15	0.15	0.15	0.15	0.15

[1] Values in parentheses indicate the protein content of these feedstuffs.

[2] Guaranteed levels per kilogram of the product: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2

760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, Calcium Pantothenate 2,000 mg, Folic acid 50 mg, Coccidiostat 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg. Selenium 40 mg. Vehicle q.s.p. 1,000 g.

[3] The value in parentheses indicates the purity of the amino acid.

9.3. Performance and physical quality of the eggs

The performance of the hens was evaluated according to the methodology described by Rufino *et al.* (2021). At every 7 days, the following parameters were measured: feed intake (g/bird/day), egg production (%), feed conversion ratio (kg of feed per kg of egg produced – kg/kg), feed conversion ratio (kg of feed per dozen eggs produced – kg/dz), and egg mass (g) for each repetition. In the last two days of each 21-day subperiod, four eggs from each replicate were randomly selected for quality analysis.

For physical quality of the eggs analyses, this study used the same methodology described by Rufino *et al.* (2021). The eggs were stored for one hour at room temperature and weighed using an electronic balance (0.01 g). The eggs were placed in wire baskets and immersed in buckets containing different levels of sodium chloride (NaCl) with density variations from 1,075 to 1,100 g/cm^3 (interval of 0.005) to evaluate the specific gravity. Then, the eggs were placed on a flat glass plate to determine albumen and yolk height, and yolk diameter using an electronic caliper. To separate albumen and yolk, a manual separator was used. Each one was placed in a plastic cup and weighed in analytical balance. Eggshells were washed, dried in an oven (50 ºC / 122 ºF) for 48 hours, and weighed. Dry eggshells were used to determine the eggshell thickness using a digital micrometer. Average eggshell thickness was analyzed considering three regions: basal, meridional, and apical. The yolk color was evaluated using a ROCHE© colorimetric fan with a scale of 1 to 15. Haugh unit was calculated using the following formula (HAUGH, 1937):

$$H_{unit} = 100 * log\ log\ (H + 7.57 - 1.7 * W^{0.37}) \qquad (1)$$

Where:

H = albumen height (mm)

W = egg weight (g).

9.4.Hematological and plasma biochemical parameters

It was randomly selected eight hens per treatment for blood collection and analysis of hematological and plasma biochemical parameters. One milliliter of blood was collected from these hens directly from the ulnar vein, using disposable syringes containing heparin anticoagulant (5000 IU per sample). These samples were immediately centrifuged at 6,848 G for 10 minutes to separate the red blood cells for evaluation of hematological parameters and the plasma was used for biochemical parameters analysis. These samples were identified and preserved at -4 °C (24.8 °F) throughout the process to be sent to the laboratory. In the analysis of hematological parameters, the collected blood was used for the count of circulating erythrocytes (M/mm^3) using a Neubauer chamber after dilution in formaldehyde-citrate and toluidine blue and visualized using an optical microscope (Nikon Eclipse E-50i, DM3000, Tokyo, Japan) with a 40x objective lens. The hemoglobin concentration (g/dL) was determined by the cyanomethemoglobin method, while the hematocrit (%) by the microhematocrit method (GOLDENFARB *et al.*, 1971), with centrifugation of heparinized microcapillary tubes at 12,000 rpm for 5 minutes (ARIDE *et al.*, 2018). Through these analyses, the mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin (MCH, pg/cell), and mean corpuscular hemoglobin concentration (MCHC, g/dL) were calculated according to Tavares-Dias and Moraes (2004).

In the analysis of plasma biochemical parameters, the remaining plasma samples after centrifugation were subjected to commercial enzymatic-colorimetric assay kits according to the manufacturer's specific recommendations, and the readings were taken on a mass spectrophotometer (model K37-UVVIS, Kasvi©, São José dos Pinhais, Brazil) at a specific wavelength for each assay. The biochemical parameters analyzed were the concentrations of total proteins, triglycerides, glucose, cholesterol, and albumin.

9.5.Proximate composition of the eggs

Eight eggs per treatment were collected and immediately sent to the Fish Technology Laboratory of UFAM. These samples had their proximate composition, focused in the percentages of moisture, minerals, fats and proteins, also determined according to the methods described by AOAC (2019).

9.6. Sensory characteristics of the eggs

For each treatment, we used one egg from each replicate per treatment, with a fifth egg being randomly chosen among the replicates to complete a total of five per treatment for sensory analysis. 20 untrained judges of both genders were randomly selected to evaluate the appearance, acidity, aroma, color and taste of the eggs. A 9-point hedonic test was applied to measure each of these variables using scales from "liked extremely" (9) to "disliked extremely" (1) following the methodology described by Dutcosky (1996). Each of these eggs was cut into four parts after cooked in hot water for 10 minutes, with only a sample per treatment being served to the judges.

9.7.Statistical Analyses

The adopted statistical model was as follows:

$$Y_{ik} = \mu + \alpha_i + \epsilon_{ik}$$

where:

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

μ = Overall mean of the experiment;

α_i = Effect of the tambaqui biological silage levels;

ϵ_{ik} = Experimental error.

All data were analyzed by one-way ANOVA using the R *software* (2021), following the guidelines outlined by Logan (2010). Significant variables ($p<0.05$) were subjected to polynomial regression to analyze the influence of the independent variable (tambaqui biological silage levels) on dependent variables evaluated (CHATTERJEE, 2006; LOGAN, 2010). The mathematical model, linear ($Y = a + bx$) or quadratic ($Y = c + bx + ax^2$), was chosen based on the influence of each independent variable on the dependent variable analyzed (DORMANN *et al.*, 2013). Values of R squared were also considered to indicate the best model (CHATTERJEE, 2006; DORMANN *et al.*, 2013).

10. RESULTS

10.1. Physical-chemical characteristics of tambaqui biological silage

During the production process, upon visual analysis, a brown color was initially observed in the ensiled mass. Over the subsequent days, the color transitioned to reddish-pink, eventually reaching a dark brown shade by the fourteenth day of processing. The characteristic fish smell gradually diminished during the process, being replaced by a slightly acidic or fruity aroma. In the initial 72 hours, liquefaction of the homogeneous ensiled mass was observed, resulting in a semi-pasty appearance that persisted throughout the process. Overall, the tambaqui biological silage exhibited a natural color and aroma characteristic of this product.

The pH and titratable acidity of the tambaqui biological silage were monitored for periods of 72 hours over 14 days, and the results are presented in Table 2. The initial pH of the silage on day 0 was 6.47 ± 0.02, which dropped to 5.93 ± 0.02 on day 3 and then slightly increased to 5.94 ± 0.01 on day 6. However, on days 9 and 12, it dropped again to 5.87 ± 0.01 and 5.85 ± 0.01, respectively. By day 14, the pH was recorded at 5.95 ± 0.01. The results for titratable acidity, to estimate the % of acid in the ensiled mass, showed an increasing trend on the evaluation days. It started at 2.13% on day 0, and increased progressively to 5.50%, 5.70%, and 5.90% on days 3, 6, and 9, respectively. However, it slightly rose to 7.13% on day 12, reaching 10.13% on the 14th day of silage processing.

Table 2. pH value and titratable acidity during the processing of biological silage from tambaqui by-products.

Inoculation days	pH*	Titratable acidity (%)*
0	6.47 ± 0.02	2.13± 0.06
3	5.93 ± 0.02	5.5± 0.44
6	5.94 ± 0.01	5.7± 0.10
9	5.87 ± 0.01	5.9± 0.17
12	5.85 ± 0.01	7.13± 0.55
14	5.95 ± 0.01	10.13± 0.40

* Mean ± standard deviation of three replicates.

10.2. Microbiological quality and proximate composition of tambaqui biological silage

In the results of the microbiological quality of tambaqui biological silage (Table 3), it was observed a very low count of total and thermotolerant coliforms, and fungi and molds in the samples analyzed, in addition to the absence of *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. This indicates that tambaqui biological silage can be used as a feedstuff in animal diets, in the case of this study for older commercial hens, without causing potential negative effects or pathologies due to contamination.

Table 3. Microbiological quality of biological silage from tambaqui by-products.

Microorganisms	Dry biological silage
Total coliforms, CFU/g	$<3.0\times10^{-1}$
Thermotolerant coliforms, CFU/g	$<3.0\times10^{-1}$
Escherichia coli	Non-detected
Salmonella spp.	Non-detected
Staphylococcus spp.	Non-detected
Molds and yeasts, CFU/g	1.0×10^{-1}

In the proximate composition (Table 4) of the residual mass composed of tambaqui by-products before the ensilage process, and tambaqui biological silage after the drying process, it was observed an improved nutrient availability in the tambaqui biological silage compared to the residual mass, especially exposed in the percentage values of dry matter. Additionally, the tambaqui biological silage showed an increase in protein, fat and mineral contents, which may be attributed to the concentration effect resulting from moisture reduction during the drying process. As water is removed, the remaining nutrients become more concentrated, thus enhancing their apparent levels in the final product. This increase in nutrient density not only improves the overall quality of the tambaqui biological silage but may also enhance its potential as a nutritional supplement, particularly in animal feed formulations where higher protein and fat contents are desirable for growth and development. The enhanced bioavailability of these nutrients also suggests that tambaqui biological silage could serve as a more efficient source of energy and essential amino acids compared to the untreated residual mass.

Table 4. Proximate composition of fresh residual mass and biological silage from tambaqui by-products.

Proximate composition	Fresh waste*	Biological silage*
Dry matter, %	44.61±2.87	87.69±0.17
Crude protein, %	22.67±1.81	44.98±0.59
Fats, %	7.38±0.35	17.89±0.47
Minerals, %	11.70±1.15	21.87±0.69
Crude fiber, %	1.62±0.28	2.44±1.24
Soluble carbohydrates, %	1.23±0.32	0.50±1.06

*All nutrient values were calculated on a dry matter basis. Mean ± standard deviation of three replicates.

10.3. Performance and egg quality

The use of tambaqui biological silage significantly affected ($p<0.05$) all performance variables evaluated in older commercial hens (Table 5). All these variables displayed linear behavior, with the increasing inclusion of tambaqui biological silage in the diets causing a linear reduction in feed intake, egg production, and egg mass. In contrast, feed efficiency values (kg/kg and kg/dozen) increased linearly as tambaqui biological silage levels in the diets rose.

Regarding egg quality (Table 6), the variables egg weight, eggshell percentage, eggshell thickness, Haugh unit, yolk pH, and albumen pH were significantly affected ($p<0.05$) by the inclusion of tambaqui biological silage in the diets, with all these variables exhibiting quadratic equations as the mathematical model that best expressed the behavior of their data. By deriving the equations to determine the tambaqui biological silage levels that would yield the highest results in these variables, it was found that including 1.50% tambaqui biological silage in the diets would achieve the highest values for egg weight, eggshell percentage, Haugh unit, yolk pH, and albumen pH, while a 2% level would result in greater eggshell thickness.

Table 5. Performance of older commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables[1]	Tambaqui biological silage levels, %					p-value[2]	CV[3] %	Model[4]	R^2
	0	1	2	3	4				
Feed intake, g/bird/day	97.63	111.48	89.05	71.28	57.29	<0.01	2.30	Y = 109.52 - 12.088x	0.79
Egg production, %	67.25	69.00	66.50	40.75	36.25	0.04	3.87	Y = 74.01 - 9.025x	0.79
Feed efficiency, kg/kg	2.08	2.17	2.43	3.36	3.95	0.03	5.35	Y = 1.812 + 0.493x	0.90
Feed efficiency, kg/dz	1.36	1.59	1.99	2.70	2.95	0.05	5.25	Y = 1.26 + 0.429x	0.96
Egg mass, g	35.57	37.49	39.40	29.86	17.83	0.02	4.41	Y = 40.652 - 4.311x	0.61

[1] Feed efficiency, kg/kg = Quantity of feed (kg) to produce one kilogram (kg) of egg. Feed efficiency, kg/dz = Quantity of feed (kg) to produce one dozen (dz) of eggs.

[2] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[3] CV - Coefficient of variation.

[4] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

Table 6. Quality of eggs produced by older commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Egg weight, g	48.63	58.40	54.59	52.72	49.26	<0.01	7.97	$Y = 54.101 + 6.5637x - 2.1814x^2$	0.69
Yolk, %	24.99	24.81	24.85	23.41	26.33	0.21	6.89	-	-
Albumen, %	58.99	60.08	59.84	63.44	60.00	0.12	4.21	-	-
Eggshell, %	8.89	10.18	9.50	9.39	8.94	0.02	7.12	$Y = 9.8523 + 0.7624x - 0.253x^2$	0.59
Yolk height, mm	18.49	18.67	19.22	18.76	17.97	0.12	3.70	-	-
Albumen height, mm	7.33	7.44	8.20	7.85	8.04	0.44	9.77	-	-
Yolk diameter, mm	40.27	41.22	41.22	41.71	41.45	0.46	2.71	-	-
Yolk color	5.43	6.62	6.00	6.37	6.06	0.27	10.85	-	-
Specific gravity, g/ml³	1082.18	1085.62	1081.56	1084.37	1083.43	0.26	0.26	-	-
Eggshell thickness, µm	0.26	0.29	0.32	0.28	0.22	<0.01	5.33	$Y = 0.2663 + 0.0624x - 0.0156x^2$	0.95
Haugh unit	88.84	92.35	91.85	88.66	88.24	0.05	4.77	$Y = 91.459 + 2.5253x - 0.8416x^2$	0.68
Yolk pH	5.90	6.50	6.34	6.32	6.16	<0.01	3.33	$Y = 6.2389 + 0.4283x - 0.1426x^2$	0.71
Albumen pH	8.45	8.71	8.66	8.68	8.53	<0.01	1.18	$Y = 8.5729 + 0.2273x - 0.0759x^2$	0.84

[1] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[2] CV - Coefficient of variation.

[3] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

10.4. Hematological and plasma biochemical parameters

In the results for hematological parameters (Table 7), MCV and MCH values were significantly influenced ($p<0.05$) by the inclusion of tambaqui biological silage in the diets, showing a positive linear trend; that is, MCV and MCH values gradually increased as tambaqui biological silage levels in the diets rose. Erythrocytes (RBC) and Hematocrit values were also significantly affected ($p<0.05$) by the inclusion of tambaqui biological silage in the diets. However, for these variables, the quadratic mathematical model best expressed the data behavior. By deriving these equations, it was found that the highest RBC levels would be achieved at a 1.00% tambaqui biological silage level in the diets, while the highest Hematocrit values would be obtained at a 3.00% tambaqui biological silage level in the diets. The results for plasma biochemical parameters (Table 8) showed a significant linear increase ($p<0.05$) in triglyceride levels in hens as the dietary tambaqui biological silage levels rose. Conversely, cholesterol levels displayed a significant linear decrease ($p<0.05$) with increasing tambaqui biological silage levels in the diets.

10.5. Proximate composition of the eggs

In the analysis of the proximate composition of the eggs (Table 9), the percentage of minerals were significantly influenced ($p<0.05$) by the inclusion of tambaqui biological silage in the diets, showing a negative linear trend. The percentage values of moisture and fats were also significantly affected ($p<0.05$) by the inclusion of tambaqui biological silage in the diets. However, for these variables, the quadratic mathematical model best expressed the data behavior. By deriving these equations, it was found that the highest moisture percentages would be achieved at a 2.00% tambaqui biological silage level in the diets, while the highest fat percentages would be obtained at a 3.90% tambaqui biological silage level.

10.6. Sensory characteristics of the eggs

The results of the sensory characteristics (Table 10) showed a significant effect ($p<0.05$) of tambaqui biological silage inclusion in the diets of the older commercial hens on the appearance and color of the eggs, where the quadratic mathematical model best expressed the data behavior. By deriving these equations, it was found that the highest appearance evaluation values would be achieved at a 3.82% tambaqui biological silage level in the diets, while the highest color evaluation values would be achieved at a 3.67% tambaqui biological silage level in the diets.

Table 7. Hematological parameters of older commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables[1]	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R²
	0	1	2	3	4				
Hemoglobin, g/dL	13.02	11.46	12.16	12.89	13.31	0.19	19.56	-	-
Erythrocytes RBC, M/mm^3	3.49	2.49	2.76	2.86	3.14	<0.01	18.84	$Y = 3.9554 - 0.7159x + 0.3707x^2$	0.71
Hematocrit, %	36.50	31.12	30.87	38.81	37.87	<0.01	16.16	$Y = 45.001 - 3.8341x + 0.6387x^2$	0.55
MCV, um³	106.24	110.23	121.31	127.02	141.06	<0.01	18.26	$Y = 103.89 + 8.643x$	0.96
MCH, pg/cel	38.05	41.17	42.83	48.34	50.05	0.02	17.71	$Y = 37.854 + 3.117x$	0.96
MCHC, g/dL	36.09	37.49	40.06	34.33	35.28	0.41	14.41	-	-

[1] MCV = Mean Corpuscular Volume. MCH = Mean Corpuscular Hemoglobin. MCHC = Mean Corpuscular Hemoglobin Concentration.

[2] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[3] CV - Coefficient of variation.

[4] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

Table 8. Plasma biochemical parameters of older commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Total proteins, g/dL	4.96	4.98	5.24	4.47	4.35	0.71	9.96	-	-
Triglycerides, mg/dL	385.34	531.28	639.24	670.23	685.45	<0.01	7.06	Y = 434.47 + 73.917x	0.86
Glucose, mg/dL	165.83	194.11	185.86	195.48	185.59	0.42	6.03	-	-
Cholesterol, mg/dL	89.61	85.31	79.53	62.52	56.05	0.05	3.99	Y = 92.586 - 8.991x	0.84
Albumin, mg/dL	4.83	4.89	4.79	4.99	4,63	0.95	8.77	-	-

[1] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[2] CV - Coefficient of variation.

[3] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

Table 9. Proximate composition of eggs produced by older commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Moisture, %	78.71	79.02	80.59	79.66	77.37	0.01	1.78	$Y = 78.678 + 1.996x - 0.499x^2$	0.81
Minerals, %	0.73	0.72	0.71	0.64	0.68	0.03	8.03	$Y = 0.732 - 0,018x$	0.61
Fats, %	8.13	7.69	6.89	7.85	9.05	<0.01	9.83	$Y = 12.306 - 1.272x + 0.162x^2$	0.85
Proteins, %	12.43	12.41	11.81	12.01	12.90	0.34	5.46	-	-

[1] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[2] CV - Coefficient of variation.

[3] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

Table 10. Sensory characteristics of eggs produced by older commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Appearance	6.82	5.95	5.75	5.20	7.37	<0.01	3.33	$Y = 10.9666 - 1.6021x + 0.2093x^2$	0.78
Acidity	6.80	6.57	6.52	6.20	6.77	0.40	13.14	-	-
Aroma	6.42	6.35	6.30	6.50	6.70	0.41	7.56	-	-
Color	7.02	6.27	5.97	5.77	7.60	<0.01	9.20	$Y = 10.1454 - 1.4369x + 0.1957x^2$	0.86
Taste	7.07	6.77	6.42	6.35	6.97	0.27	16.81	-	-

[1] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[2] CV - Coefficient of variation.

[3] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

11. DISCUSSION

Firstly, focusing on silage production, liquefaction of the homogeneous mass began around the third day after inoculation, gradually increasing until a liquid-pasty product formed. This aligns with Mousavi *et al.* (2013), who attributed the process to continuous protein hydrolysis by proteolytic enzymes naturally found in fish viscera. Silva *et al.* (2014) elaborated that these enzymes help solubilize residual muscle proteins, separating the insoluble fraction (bones and undigested components) from the soluble fraction containing hydrolyzed proteins suitable for animal feed. Observed changes in color and odor, including the disappearance of the characteristic fish smell, corroborate findings by Suarez *et al.* (2018), Guimarães *et al.* (2021), and Mahale *et al.* (2024), who noted these as normal indicators in fish silage processing.

Mahale *et al.* (2024) further emphasized the role of pH values as indicators of hydrolysis and silage quality. This study recorded an initial pH decrease with fluctuations, stabilizing at 5.95 ± 0.01, consistent with Bezerra and Fonseca's (2023) findings on preservation via lactic fermentation from various inoculum sources. As noted by Arruda *et al.* (2006) and Santana *et al.* (2023), pH reduction not only curtails undesirable microbial growth but also activates enzymes for protein hydrolysis. Özyurt *et al.* (2016) highlighted that pH reduction in silages is driven by carbohydrate sources, producing organic acids, while pH fluctuations result from component dissolution, such as fish bones and scales. Higher titratable acidity, linked to lactic acid from carbohydrate fermentation, further enhanced biological preservation (VIDOTTI *et al.*, 2011; NASCIMENTO *et al.*, 2014), improving microbiological quality with low levels of *E. coli*, *Salmonella spp.*, *Staphylococcus spp.*, coliforms, molds, and yeasts, consistent with Ozyurt *et al.* (2020) and Mayta-Apaza *et al.* (2021).

Considering these factors during production, some studies suggest that the final composition of silage is strongly influenced by its processing method, whether acidic or biological, as it determines the nutrient profile (BATALHA *et al.*, 2017; GUIMARÃES *et al.*, 2019; GUIMARÃES *et al.*, 2021). The use of lactic acid bacteria in fish silage fermentation, a biological approach as used in this study, can improve quality, flavor, and increase protein and vitamin levels (OZYURT *et al.*, 2020). However, as noted by Bezerra and Fonseca (2023), silage composition also depends on factors such as fish species, feed, and biological variables, leading to variability in nutritional value. The protein levels observed in the tambaqui biological silage produced in this study were consistent with other freshwater fish silage studies (VIDOTTI *et al.*, 2003; BEZERRA; FONSECA,

2023). Overall, the tambaqui biological silage showed high protein content and low-fat content due to pre-silage defatting, aligning with findings from Ramírez *et al.* (2013), Gaviria G *et al.* (2020), and Yhoan *et al.* (2020), who reported similar fat reductions. Thus, the favorable protein-to-fat ratio and nutritional profile of the tambaqui biological silage suggest it is a nutritionally valuable feedstuff for use in poultry diets, as proposed in this study.

However, despite these positive findings regarding the nutritional quality of tambaqui biological silage, its increasing inclusion in the diets did not yield favorable performance results for the older commercial hens. It became evident that only the 1% tambaqui biological silage inclusion level in the diets provided good performance results. Beyond this level, there was a considerable decline in the birds' productive efficiency, as indicated by gradual decreases in feed intake, egg production, and egg mass, along with a loss in feed efficiency due to increased feed requirements to produce a kilogram or dozen eggs. This aligns with other studies that also used fish by-products in hens' diets (BATALHA *et al.*, 2017; GUIMARÃES *et al.*, 2019; BRELAZ *et al.*, 2024), where the authors reported that high levels of these feedstuffs, above 3% inclusion, tend to lead to significant sensory rejection by the birds, which gradually decreases feed intake over time and, consequently, productivity, especially in older birds (AYED *et al.*, 2015; BRELAZ *et al.*, 2019). These same authors further explain that the primary factor behind this rejection of fish by-products when included at high levels (above 3%) is the presence of polyunsaturated fatty acid chains derived from fish, which significantly alter the sensory properties of the diets. Although literature generally notes these effects at inclusion levels above 3%, in this study, they were observed at just over 1%, possibly indicating that tambaqui biological silage, even after processing, retains substantial chemical and sensory characteristics of the tambaqui residues used.

Similarly, the best results for egg quality and proximate composition were achieved with 1% to 2% tambaqui biological silage inclusion in diets, with levels above this leading to a gradual decline in significant variables. This drop in the physical and chemical quality of eggs due to high levels of fish by-products, such as tambaqui biological silage, in layer diets has also been reported by other studies (BERTIPAGLIA *et al.*, 2016; SILVA *et al.*, 2017; BATALHA *et al.*, 2018; BRELAZ *et al.*, 2019; GAVIRIA G *et al.*, 2020), with authors attributing this effect to decreased production efficiency. In other words, the increased dietary rejection by birds due to elevated levels of fish by-products results in reduced feed intake, thus providing fewer nutrients available

for egg formation, leading to poorer physical structure and chemical composition in eggs (BERTIPAGLIA *et al.*, 2016; FAITARONE *et al.*, 2016; SILVA *et al.*, 2017; CARIOCA *et al.*, 2010).

Once the increasing the inclusion of fish by-products in diets for older commercial hens can lead to lower feed intake, this phenomenon reduces the overall availability of essential nutrients for basal metabolism and egg production. Physiologically, the unique lipids in these by-products in the duodenum stimulate the release of cholecystokinin, a hormone that regulates digestion. This hormone slows gastric emptying, extending food transit time through the digestive system, which may hinder the absorption of critical nutrients for egg production. Consequently, with limited availability of these nutrients, the nutritional profile of the eggs may be altered, affecting their quality, composition, and sensory characteristics, such as flavor and texture (PINHEIRO *et al.*, 2012; HEYING *et al.*, 2014; TORRES; DREHE, 2015; TOOMER *et al.*, 2019).

In this context, hematological and plasma biochemical parameters are important indicators for understanding how the inclusion of tambaqui biological silage in diets is affecting the metabolism of birds (CAMPBELL, 2004), especially at higher inclusion levels, which previous results have shown to be detrimental to the productivity of older commercial hens. The linear increase in MCV and MCH values may suggest an improvement in red blood cell quality, possibly due to specific nutrients in tambaqui biological silage that can stimulate hemoglobin synthesis (CAMPBELL, 2004; GUTIÉRREZ-CASTRO; CORREDOR-MATUS, 2017), even though fewer nutrients are available when birds consume diets with higher levels of tambaqui biological silage due to reduced feed intake. The RBC and Hematocrit results, which showed optimal levels at 1.00% and 3.00% tambaqui biological silage, respectively, suggest that moderate levels of tambaqui biological silage in diets optimize the production of erythrocytes and the volume of circulating red blood cells, indicating improved metabolic activity for nutrient transport, while higher levels may overload the birds' metabolism, or this may again be related to reduced nutrient availability under these conditions (CAMPBELL, 2004; ETIM *et al.*, 2014; GUTIÉRREZ-CASTRO; CORREDOR-MATUS, 2017).

In plasma biochemistry, the increase in triglycerides and reduction in plasma cholesterol with higher levels of tambaqui biological silage indicate that lipid and antioxidant compounds present in tambaqui residues impact lipid metabolism, possibly promoting greater lipid availability for energy and reducing cholesterol synthesis (CHERIAN, 2015; GUTIÉRREZ-CASTRO; CORREDOR-MATUS, 2017; MOON,

2018; BRELAZ *et al.*, 2021). These results suggest that tambaqui biological silage may benefit the health and performance of birds when included in a balanced way (between 1 and 2%) in diets but is detrimental when included in excess.

On the other hand, despite the above results showing a decline starting from higher inclusion levels of tambaqui biological silage in the diets, the sensory analysis of the eggs indicated better acceptance by the judges when the eggs were from hens that consumed diets with higher levels (between 3 and 4%) of tambaqui biological silage. As indicated in other studies with layers that also used fish by-products in the diets, this may have occurred due to the presence of lipid compounds in tambaqui residues, which, when transferred to the egg yolk, provide a vibrant color that is more aesthetically pleasing to consumers (TORRES; DREHER, 2015; BRELAZ *et al.*, 2019). However, at very low levels, the benefits may be limited, while excessive concentrations can negatively impact the health of the birds or the quality of the egg.

Finally, another important point to consider in this study was the evaluation of tambaqui biological silage inclusion in the diets of older commercial hens, specifically those over 80 weeks of age, which naturally presents a challenge for the poultry industry in maintaining both productivity and egg quality. The literature indicates that advancing hen age typically leads to a decline in egg quality, particularly after 60 weeks, with reductions in eggshell quality and yolk color observed (GUO *et al.*, 2021; MOLNÁR *et al.*, 2017). However, the improvement in several parameters assessed in this study following the inclusion of 1% tambaqui biological silage in the diets, such as egg weight, eggshell percentage, shell thickness, yolk color, Haugh Unit, as well as sensory evaluation of appearance and color, highlights the potential of tambaqui biological silage to mitigate age-related declines, aiding older hens, specifically those at 83 weeks of age in this study, in producing eggs of better quality.

This enhancement in egg quality among older commercial hens consuming tambaqui biological silage -enriched diets can be attributed to the excellent nutritional profile of tambaqui biological silage, similar to other fish by-products. According to Özyurt *et al.* (2016), fish waste silages often exhibit better amino acid content and protein quality compared to conventional fish meal while providing higher concentrations of essential free fatty acids, such as omega-3 (VIDOTTI *et al.*, 2011). This nutritional richness can support the metabolic functions of aging hens, emphasizing the potential of tambaqui biological silage to improve egg quality in hens during production cycles beyond conventional standards.

12. CONCLUSION

In conclusion, the use of tambaqui biological silage in the diets of older commercial hens has demonstrated its potential to serve as an alternative feed ingredient with notable nutritional benefits. This study revealed that tambaqui biological silage inclusion at levels between 1% and 2% is optimal, improving egg quality, feed efficiency, and some hematological parameters without compromising bird health or productivity. However, higher levels of tambaqui biological silage negatively impacted feed intake, egg production, and some quality indicators, possibly due to sensory rejection by the hens. These findings suggest that controlled inclusion of tambaqui biological silage can contribute to sustainable poultry nutrition by valorizing fish by-products, aligning with the goals of circular economy practices in the agro-industry. Further research may explore refining tambaqui biological silage formulations to balance nutritional advantages with sensory acceptance in older hens.

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CAPTER IV – EFFECTS OF BIOLOGICAL SILAGE FROM TAMBAQUI BY-PRODUCTS ON THE DIETS OF YOUNGER COMMERCIAL HENS

14. MATERIALS AND METHODS

The current experiment was conducted at the Research Poultry Farm of the Federal University of Amazonas (UFAM), on the University campus located in Manaus City, Amazonas, Brazil. All experimental procedures were performed in accordance with the Local Experimental Animal Care Committee and were approved (protocol number 005/2022) by the institutional ethics committee of the UFAM, Brazil.

14.1. Processing of tambaqui residues and silage production

The residues comprising viscera, gills, scales, and fins discarded during the processing of tambaqui (fish weighing between 800 and 1,300 g) were obtained in an industrial fish processing facility in Manaus, Amazonas. These residues were transported to the laboratory in insulated containers and stored in a freezer until further processing. During this initial screening, the residues were thawed at room temperature and subjected to thermal treatment at 65°C for 5 minutes. Afterward, they were filtered through a sieve, and the remaining mass was pressed using a hydraulic press. The processed material was then weighed and transferred into 40-liter plastic buckets for subsequent use.

For the production of silage, the residual fish mass was combined with the following ingredients in specified proportions: 10% (w/w) cassava trimmings (*Manihot esculenta* Crantz), obtained from local markets in Manaus. The trimmings were cleaned and ground to serve as a carbohydrate source for the silage. Additionally, 2.5% (v/w) inoculum, consisting of pure cultures of the bacterium *Lactobacillus plantarum* as proteolytic microorganisms, was added, along with 0.1% (w/w) benzoic acid as a fungicidal and bactericidal preservative, and 0.1% (w/w) ascorbic acid as an antioxidant. This process followed the methodology described by Vidotti *et al.* (2003) and Vidotti *et al.* (2011).

All ingredients were thoroughly mixed in the buckets until the silage components were completely uniform. The buckets were then hermetically sealed to ensure anaerobic conditions. To achieve this, a sterilized plastic bag was placed directly on the surface of the ensiled mass before sealing the buckets. The silage was stored and stirred every 24 hours to enhance the fermentation process. It was kept for 14 days at room temperature under anaerobic conditions. During this period, the silage was assessed for its

organoleptic properties, including odor, color changes, texture, pH, and titratable acidity, which was determined through titration with 0.1 N NaOH.

After processing, the silage was transferred to aluminum trays and placed in a forced-ventilation oven at 65°C (149°F) for 72 hours to reduce moisture content. During this time, the biomass was periodically stirred to ensure uniform drying. Once dried, proximate composition analyses were performed to determine the levels of moisture, minerals, fats, fiber, and soluble carbohydrates of tambaqui biological silage, following the methodologies outlined by AOAC (2019). Microbiological analyses were also conducted in compliance with the standards set by the Brazilian Ministry of Agriculture, Livestock, and Supply (2018), including tests for total and thermotolerant coliforms, *Salmonella* sp., *Staphylococcus* sp., molds, and yeasts. Following the completion of these analyses, the product was sent to the poultry sector for use in an experimental trial involving commercial hens.

14.2. Facilities, animals, diets and experimental design

The aviary measured 17 meters in length, 3.5 meters in width, and had a ceiling height of 3.25 meters, featuring structural adaptations designed to enhance bird welfare. Temperature and relative humidity were monitored using a digital thermohygrometer, which recorded average values of 29.4°C (84.92°F) and 64.22%, respectively. Throughout the experimental period, the hens were observed for any signs of thermal stress related to environmental conditions; however, no such signs were detected during the entire study.

The experimental period lasted 63 days, divided into three sub-periods of 21 days each. A total of 120 commercial hens of the Hisex Brown strain, aged 23 weeks, were used, following a seven-day adaptation period to the diets and housing conditions. At the start of the experimental period, the birds were weighed to standardize the groups, with an average weight of 1.87±0.181 kg. The hens were housed in galvanized wire cages measuring 0.45 meters in height, 0.40 meters in width, and 1.00 meter in length, with each cage accommodating six birds in a single line. The cages were equipped with trough feeders and nipple drinkers. The hens were provided with 16 hours of light per day (12 hours of natural light supplemented with 4 hours of artificial light) throughout the experimental period. Eggs were collected twice daily (at 9 a.m. and 3 p.m.), and all daily occurrences, including mortality and egg count, were recorded.

The hens were allocated in a completely randomized design consisting of a control treatment (without tambaqui biological silage inclusion) and four levels of tambaqui biological silage inclusion (1%, 2%, 3%, and 4%) in the diets. Each treatment had four replicates, with six birds per replicate, totaling 24 birds per treatment and 120 birds overall. The experimental diets (Table 11) were formulated to meet the nutritional requirements of commercial hens based on the reference values provided by Rostagno *et al.* (2024) and the previously determined composition of tambaqui biological silage. To ensure accuracy, a proximate analysis of the diets was conducted to verify the calculated nutritional values.

Table 11. Experimental diet composition.

Feedstuffs	Biological silage from tambaqui by-products levels, %				
	0	1	2	3	4
Corn (7.88%)[1]	65.80	65.30	64.42	64.29	63.79
Soybean meal (46%)[1]	21.41	20.76	20.11	19.46	18.81
Tambaqui biol. silage	0.00	1.00	2.00	3.00	4.00
Limestone	9.78	9.91	10.42	10.17	10.30
Dicalcium phosphate	1.93	1.94	1.95	1.95	1.96
Vit. min. supplement[2]	0.60	0.60	0.60	0.60	0.60
Salt	0.29	0.29	0.30	0.30	0.30
DL-methionine (99%)[3]	0.19	0.20	0.21	0.22	0.23
Total	100.00	100.00	100.00	100.00	100.00
Nutrient	Nutritional levels				
M.E., kcal.kg^{-1}	2,900.00	2,900.00	2,900.00	2,900.00	2,900.00
Crude protein, %	15.50	15.50	15.50	15.50	15.50
Calcium, %	4.32	4.37	4.42	4.47	4.52
Available phosphorus, %	0.45	0.45	0.45	0.45	0.45
Crude fiber, %	2.55	2.52	2.48	2.45	2.41
NDF, %	10.54	10.39	10.24	10.09	9.94
ADF, %	3.92	3.85	3.78	3.71	3.64
Methionine + Cystine, %	0.70	0.70	0.70	0.70	0.70
Methionine, %	0.43	0.44	0.45	0.45	0.46
Lysine, %	0.76	0.74	0.72	0.70	0.68
Threonine, %	0.60	0.58	0.57	0.56	0.54
Tryptophan, %	0.18	0.17	0.17	0.16	0.16
Sodium, %	0.15	0.15	0.15	0.15	0.15

[1] Values in parentheses indicate the protein content of these feedstuffs.

[2] Guaranteed levels per kilogram of the product: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2

760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, Calcium Pantothenate 2,000 mg, Folic acid 50 mg, Coccidiostat 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg. Selenium 40 mg. Vehicle q.s.p. 1,000 g.

[3] The value in parentheses indicates the purity of the amino acid.

14.3. Performance and physical quality of the eggs

The performance of the hens was assessed following the methodology outlined by Rufino *et al.* (2021). Every seven days, the following parameters were recorded: feed intake (g/bird/day), egg production (%), feed conversion ratio (kg of feed per kg of eggs produced – kg/kg), feed conversion ratio (kg of feed per dozen eggs produced – kg/dz), and egg mass (g) for each replicate. During the final two days of each 21-day subperiod, four eggs from each replicate were randomly selected for quality analysis.

For physical egg quality analysis, the study adhered to the methodology described by Rufino *et al.* (2021). Eggs were stored for one hour at room temperature and then weighed using an electronic balance with a precision of 0.01 g. They were placed in wire baskets and immersed in buckets containing sodium chloride (NaCl) solutions with densities ranging from 1.075 to 1.100 g/cm³, in intervals of 0.005, to determine specific gravity. Subsequently, eggs were placed on a flat glass plate to measure albumen height, yolk height, and yolk diameter using an electronic caliper. Albumen and yolk were separated using a manual separator, then transferred to individual plastic cups and weighed on an analytical balance. Eggshells were washed, dried in an oven at 50 °C (122 °F) for 48 hours, and weighed. Dried eggshells were used to measure thickness with a digital micrometer, considering three regions: basal, meridional, and apical. Yolk color was evaluated using a ROCHE© colorimetric fan, with a scale ranging from 1 to 15. The Haugh unit was calculated using the following formula (HAUGH, 1937):

$$H_{unit} = 100 * log\ log\ (H + 7.57 - 1.7 * W^{0.37}) \qquad (1)$$

Where:

H = albumen height (mm)

W = egg weight (g).

14.4. Hematological and plasma biochemical parameters

It was randomly selected eight hens per treatment for blood collection and analysis of hematological and plasma biochemical parameters. One milliliter of blood was

collected from these hens directly from the ulnar vein, using disposable syringes containing heparin anticoagulant (5000 IU per sample). These samples were immediately centrifuged at 6,848 G for 10 minutes to separate the red blood cells for evaluation of hematological parameters and the plasma was used for biochemical parameters analysis. These samples were identified and preserved at -4 °C (24.8 °F) throughout the process to be sent to the laboratory. In the analysis of hematological parameters, the collected blood was used for the count of circulating erythrocytes (M/mm^3) using a Neubauer chamber after dilution in formaldehyde-citrate and toluidine blue and visualized using an optical microscope (Nikon Eclipse E-50i, DM3000, Tokyo, Japan) with a 40x objective lens. The hemoglobin concentration (g/dL) was determined by the cyanomethemoglobin method, while the hematocrit (%) by the microhematocrit method (GOLDENFARB *et al.*, 1971), with centrifugation of heparinized microcapillary tubes at 12,000 rpm for 5 minutes (ARIDE *et al.*, 2018). Through these analyses, the mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin (MCH, pg/cell), and mean corpuscular hemoglobin concentration (MCHC, g/dL) were calculated according to Tavares-Dias and Moraes (2004).

In the analysis of plasma biochemical parameters, the remaining plasma samples after centrifugation were subjected to commercial enzymatic-colorimetric assay kits according to the manufacturer's specific recommendations, and the readings were taken on a mass spectrophotometer (model K37-UVVIS, Kasvi©, São José dos Pinhais, Brazil) at a specific wavelength for each assay. The biochemical parameters analyzed were the concentrations of total proteins, triglycerides, glucose, cholesterol, and albumin.

14.5. Chemical composition

Eight eggs from each treatment were subjected to chemical composition analysis, which included the evaluation of moisture (%), minerals (%), fats (%), and proteins (%). These analyses were performed following the methods described by the Association of Official Analytical Chemists (AOAC, 2019).

14.6. Sensory characteristics of the eggs

For sensory analysis, one egg from each replicate per treatment was selected, along with a fifth egg randomly chosen among the replicates, totaling five eggs per treatment. Twenty untrained judges of both genders were randomly selected to evaluate the eggs' appearance, acidity, aroma, color, and taste. A 9-point hedonic scale was used

to assess these variables, ranging from "liked extremely" (9) to "disliked extremely" (1), following the methodology described by Dutcosky (1996). Each egg was cooked in boiling water for 10 minutes, then cut into four pieces, with only one sample per treatment served to the judges.

14.7. Statistical Analyses

The adopted statistical model was as follows:

$$Y_{ik} = \mu + \alpha_i + \epsilon_{ik} \qquad (2)$$

where:

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

μ = Overall mean of the experiment;

α_i = Effect of the tambaqui biological silage levels;

ϵ_{ik} = Experimental error.

All data were analyzed by one-way ANOVA using the R *software* (2021), following the guidelines outlined by Logan (2010). Significant variables ($p<0.05$) were subjected to polynomial regression to analyze the influence of the independent variable (tambaqui biological silage levels) on dependent variables evaluated (CHATTERJEE; HADI, 2006; LOGAN, 2010). The mathematical model, linear ($Y = a + bx$) or quadratic ($Y = c + bx + ax^2$), was chosen based on the influence of each independent variable on the dependent variable analyzed (DORMANN *et al.*, 2013). Values of R squared were also considered to indicate the best model (CHATTERJEE; HADI, 2006; DORMANN *et al.*, 2013).

15. RESULTS

15.1. Physical-chemical and microbiological quality of tambaqui biological silage

During the silage production, the color of the ensiled mass evolved from brown to reddish-pink, reaching dark brown by the 14th day. The strong fish odor diminished, replaced by a mildly acidic or fruity aroma. Liquefaction occurred within the first 72 hours, resulting in a semi-pasty consistency that persisted throughout the process (Table 12). The pH dropped from 6.47 on day 0 to 5.93 on day 3, stabilizing around 5.95 by day 14. Titratable acidity increased from 2.13% to 10.13%, reflecting effective fermentation.

Table 12. pH value and titratable acidity during the processing of biological silage from tambaqui by-products.

Inoculation days	pH*	Titratable acidity (%)*
0	6.47 ± 0.02	2.13± 0.06
3	5.93 ± 0.02	5.5± 0.44
6	5.94 ± 0.01	5.7± 0.10
9	5.87 ± 0.01	5.9± 0.17
12	5.85 ± 0.01	7.13± 0.55
14	5.95 ± 0.01	10.13± 0.40

* Mean ± standard deviation of three replicates.

Microbiological analysis (Table 13) showed low levels of coliforms, molds, and yeasts, with no detection of *E. coli*, *Salmonella spp.*, or *Staphylococcus aureus*, confirming the safety of tambaqui biological silage for poultry diets. Proximate composition analysis (Table 14) revealed enhanced nutrient concentration after drying, with increased dry matter, protein, fat, and mineral content compared to the raw residues. This nutrient concentration, due to moisture reduction, improves the nutritional value and potential energy efficiency of tambaqui biological silage in feed formulations, offering a sustainable and cost-effective feed alternative.

Table 13. Microbiological quality of biological silage from tambaqui by-products.

Microorganisms	Dry biological silage
Total coliforms, CFU/g	$<3.0\times10^{-1}$
Thermotolerant coliforms, CFU/g	$<3.0\times10^{-1}$
Escherichia coli	Non-detected
Salmonella spp.	Non-detected
Staphylococcus spp.	Non-detected
Molds and yeasts, CFU/g	1.0×10^{-1}

Table 14. Proximate composition of fresh residual mass and biological silage from tambaqui by-products.

Proximate composition	Fresh waste*	Biological silage*
Dry matter, %	44.61±2.87	87.69±0.17
Crude protein, %	22.67±1.81	44.98±0.59
Fats, %	7.38±0.35	17.89±0.47
Minerals, %	11.70±1.15	21.87±0.69
Crude fiber, %	1.62±0.28	2.44±1.24
Soluble carbohydrates, %	1.23±0.32	0.50±1.06

*All nutrient values were calculated on a dry matter basis. Mean ± standard deviation of three replicates.

15.2. Performance and physical quality of the eggs

The use of tambaqui biological silage significantly affected ($p<0.05$) all performance variables evaluated in younger commercial hens (Table 15). Feed intake showed a quadratic response, with levels of tambaqui biological silage inclusion up to 2% increasing intake, but higher levels resulted in a decrease, reaching the lowest value at 4% inclusion. Egg production also decreased linearly with increasing tambaqui biological silage levels, indicating a negative impact on performance as inclusion increased. Feed efficiency, measured both per kilogram of eggs produced and per dozen eggs, exhibited distinct patterns: efficiency per kilogram followed a quadratic model, increasing with moderate tambaqui biological silage inclusion, while efficiency per dozen showed a linear increase with higher tambaqui biological silage levels, suggesting a worst feed efficiency according to increase the tambaqui biological silage levels. Egg mass

decreased significantly with increased tambaqui biological silage inclusion, indicating reduced egg size at higher tambaqui biological silage levels.

Regarding egg quality (Table 16), the inclusion of tambaqui biological silage had significant effects ($p<0.05$) on specific gravity, albumen height, and Haugh unit, while other parameters, were not significantly influenced ($p>0.05$). Specific gravity showed a quadratic response, with values initially increasing up to 2% tambaqui biological silage inclusion, followed by a decline at higher levels. Albumen height exhibited a similar quadratic pattern, peaking at 2% inclusion before decreasing, indicating a potential reduction in albumen quality with higher silage levels. Haugh unit, a key indicator of egg freshness, also followed a quadratic model, reaching the highest value at 2% tambaqui biological silage inclusion, suggesting an optimal point for maintaining egg quality.

Table 15. Performance of younger commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables[1]	Tambaqui biological silage levels, %					p-value[2]	CV[3] %	Model[4]	R^2
	0	1	2	3	4				
Feed intake, g/bird/day	118.69	121.79	123.61	108.94	105.90	<0.01	7.51	$Y = 119.36 + 4.377x - 2.055x^2$	0.82
Egg production, %	88.24	90.02	80.05	70.38	68.00	0.03	6.71	$Y = 91.362 - 6.012x$	0.90
Feed efficiency, kg/kg	2.61	2.81	2.80	2.54	2.31	<0.01	8.76	$Y = 2.6294 + 0.2301x - 0.0793x^2$	0.95
Feed efficiency, kg/dz	1.60	1.61	1.84	1.91	1.91	0.05	5.77	$Y = 1.59 + 0.092x$	0.85
Egg mass, g	39.98	38.94	35.30	30.10	31.27	0.04	7.22	$Y = 40.37 - 2.626x$	0.88

[1] Feed efficiency, kg/kg = Quantity of feed (kg) to produce one kilogram (kg) of egg. Feed efficiency, kg/dz = Quantity of feed (kg) to produce one dozen (dz) of eggs.

[2] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[3] CV - Coefficient of variation.

[4] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

Table 16. Physical quality of eggs produced by younger commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Egg weight, g	50.97	49.67	50.38	51.48	48.39	0.95	15.60	-	-
Yolk, %	29.48	29.98	29.59	30.23	31.73	0.30	8.97	-	-
Albumen, %	60.64	60.10	60.70	60.96	59.69	0.98	15.04	-	-
Eggshell, %	9.88	9.92	9.71	8.81	8.58	0.26	13.83	-	-
Yolk height, mm	16.82	16.97	17.82	16.95	16.79	0.50	7.58	-	-
Albumen height, mm	7.82	7.97	8.55	7.60	7.35	0.03	9.99	$Y = 7.7871 + 0.5347x - 0.1664x^2$	0.68
Yolk diameter, mm	40.95	41.33	42.28	40.65	41.85	0.27	3.97	-	-
Yolk color	4.59	4.43	4.50	4.37	4.50	0.76	7.40	-	-
Specific gravity, g/ml^3	1084.68	1085.31	1085.62	1083.12	1080.41	<0.01	0.22	$Y = 1084.6 + 1.6384x - 0.6779x^2$	0.97
Eggshell thickness, µm	0.42	0.43	0.42	0.42	0.40	0.10	5.05	-	-
Haugh unit	89.98	91.46	92.86	89.19	88.13	0.05	11.11	$Y = 90.068 + 2.303x - 0.725x^2$	0.78

[1] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[2] CV - Coefficient of variation.

[3] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

15.3. Hematological and plasma biochemical parameters

The inclusion of biological silage from tambaqui by-products in the diets of younger commercial hens significantly influenced several hematological and plasma biochemical parameters (Tables 17 and 18). Regarding hematological parameters, hemoglobin concentration exhibited a negative linear response ($p<0.01$), decreasing as tambaqui biological silage levels increased. Hens fed with the control diet (0% tambaqui biological silage) presented the highest hemoglobin values (17.24 g/dL), while those receiving 4% tambaqui biological silage had the lowest values (11.22 g/dL). Similarly, the erythrocyte count (RBC) followed a decreasing linear trend ($p<0.05$), with a reduction from 2.29 M/mm³ in the control group to 1.70 M/mm³ at the highest tambaqui biological silage level. Conversely, the hematocrit percentage did not show a significant difference ($p>0.05$), despite a slight reduction at 3% and 4% tambaqui biological silage levels. The mean corpuscular hemoglobin concentration (MCHC) exhibited a linear decrease ($p<0.05$), dropping from 55.27 g/dL in the control group to 35.71 g/dL at 4% tambaqui biological silage inclusion. However, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were not significantly affected by tambaqui biological silage inclusion ($p>0.05$).

For plasma biochemical parameters, total protein levels followed a quadratic pattern ($p<0.05$), peaking at 2% tambaqui biological silage inclusion (4.57 g/dL) before declining at higher levels. Triglyceride concentrations decreased significantly ($p<0.05$) with increasing tambaqui biological silage levels, with the control group presenting the highest values (734.04 mg/dL) and hens fed 3% and 4% tambaqui biological silage exhibiting the lowest levels (447.55 and 463.47 mg/dL, respectively). Similarly, plasma glucose levels exhibited a decreasing linear trend ($p=0.03$), dropping from 269.56 mg/dL in the control to 222.54 mg/dL in the 4% tambaqui biological silage group. Cholesterol concentrations also declined linearly ($p<0.05$), with the highest value recorded in the control diet (67.06 mg/dL) and the lowest at 4% tambaqui biological silage inclusion (33.23 mg/dL). Albumin levels, on the other hand, followed a positive linear trend ($p<0.05$), increasing with tambaqui biological silage inclusion, reaching the highest value at 4% tambaqui biological silage (3.50 mg/dL). A similar pattern was observed for uric acid levels ($p<0.05$), with values increasing progressively from 2.06 mg/dL (control) to 3.08 mg/dL (4% tambaqui biological silage).

Table 17. Hematological parameters of younger commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables[1]	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Hemoglobin, g/dL	17.24	12.88	11.51	11.23	11.22	<0.01	11.80	Y = 15.554 – 1.369x	0.71
Erythrocytes RBC, M/mm^3	2.29	1.75	1.74	1.73	1.70	<0.01	17.41	Y = 2.082 – 0.12x	0.77
Hematocrit, %	31.62	33.25	32.87	27.87	27.37	0.08	18.24	-	-
MCV, um³	157.04	194.86	191.56	164.08	158.07	0.27	16.73	-	-
MCH, pg/cel	81.28	75.11	66.62	66.17	65.05	0.51	10.85	-	-
MCHC, g/dL	55.27	41.35	40.51	39.44	35.71	0.03	12.14	Y = 50.662 – 4.103x	0.75

[1] MCV = Mean Corpuscular Volume. MCH = Mean Corpuscular Hemoglobin. MCHC = Mean Corpuscular Hemoglobin Concentration.

[2] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[3] CV - Coefficient of variation.

[4] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

Table 18. Plasma biochemical parameters of older commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Total proteins, g/dL	3.57	4.55	4.57	3.65	3.07	0.01	12.83	$Y = 3.682 + 0.97x - 0.29x^2$	0.89
Triglycerides, mg/dL	734.04	789.64	716.99	447.55	463.47	0.05	14.39	$Y = 806.98 - 88.323x$	0.74
Glucose, mg/dL	269.56	255.18	232.05	228.42	222.54	0.03	15.44	$Y = 265.71 - 12.08x$	0.91
Cholesterol, mg/dL	67.06	63.56	36.55	38.49	33.23	0.05	17.41	$Y = 66.324 - 9.273x$	0.82
Albumin, mg/dL	2.71	3.05	3.27	3.36	3,50	0.05	13.38	$Y = 2.8 + 0.189x$	0.94
Uric acid, mg/dL	2.06	2.00	2.82	2.88	3.08	0.05	16.91	$Y = 1.984 + 0.292x$	0.85

[1] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[2] CV - Coefficient of variation.

[3] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

15.4. Chemical composition of the eggs

In the analysis of the proximate composition of the eggs (Table 19), the inclusion of tambaqui biological silage significantly influenced ($p<0.05$) the protein content, while moisture, mineral, and fat contents were not significantly affected ($p>0.05$). Protein content exhibited a quadratic response, with values increasing up to 2% tambaqui biological silage inclusion, followed by a decline at higher levels, indicating an optimal point for protein enrichment at moderate inclusion rates. Moisture, minerals, and fats remained relatively stable across all treatments, suggesting that tambaqui biological silage inclusion did not compromise the overall balance of these nutrients.

15.5. Sensory characteristics of the eggs

The results of the sensory characteristics (Table 20) showed that tambaqui biological silage inclusion significantly affected ($p<0.05$) only the taste of the eggs, while appearance, texture, aroma, and color were not significantly influenced ($p>0.05$). Taste followed a negative linear trend, with increasing levels of tambaqui biological silage reducing the overall acceptability of flavor. Despite this, the scores remained within the acceptable range across all treatments. The absence of significant effects on appearance, texture, aroma, and color indicates that tambaqui biological silage inclusion did not compromise these sensory attributes, suggesting its potential for use in poultry diets with minimal impact on the eggs' overall sensory appeal, provided that inclusion levels are carefully managed to balance taste acceptability.

Table 19. Proximate composition of eggs produced by younger commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Moisture, %	79.43	77.85	77.72	78.48	78.51	0.13	1.65	-	-
Minerals, %	0.74	0.81	0.80	0.78	0.79	0.37	5.56	-	-
Fats, %	7.71	8.28	8.26	8.65	8.68	0.23	13.39	-	-
Proteins, %	12.10	13.06	13.22	12.09	12.02	0.05	8.13	$Y = 12.245 + 0.8441x - 0.2393x^2$	0.66

[1] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[2] CV - Coefficient of variation.

[3] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated

Table 20. Sensory characteristics of eggs produced by younger commercial hens fed diets containing increasing levels of biological silage from tambaqui by-products.

Variables	Tambaqui biological silage levels, %					p-value[1]	CV[2] %	Model[3]	R^2
	0	1	2	3	4				
Appearance	7.25	7.10	6.70	6.94	6.81	0.49	5.31	-	-
Texture	7.45	7.63	7.29	7.03	7.07	0.29	13.14	-	-
Aroma	6.78	6.54	6.63	6.61	6.58	0.97	17.38	-	-
Color	7.41	7.30	6.70	7.29	6.85	0.13	14.57	-	-
Taste	7.76	7.58	7.20	7.16	7.18	0.05	11.67	Y = 7.692 – 0.158x	0.81

[1] Treatments with means on the same line show significant differences when $p \leq 0.05$.

[2] CV - Coefficient of variation.

[3] Mathematical model adjusted according to the influence of the independent variable (tambaqui biological silage levels) on the dependent variable evaluated.

16. DISCUSSION

The color changes in tambaqui biological silage during the fermentation process reflect the biochemical and microbiological transformations occurring in the organic matrix, as described by Guimarães *et al.* (2025). Ozyurt *et al.* (2017) indicate that protein degradation and metabolite production influence the coloration of biological silages. The transition from a brown color to a reddish-pink hue, culminating in a dark brown shade on the 14th day, may be related to the oxidative degradation of carotenoid pigments and the formation of melanoidin compounds via the Maillard reaction (STAROWICZ; ZIELIŃSKI, 2019). This reaction is characteristic of fermentative processes in which proteins and carbohydrates react under controlled temperature and pH conditions, leading to organoleptic changes in the final product (ABBASILIASI *et al.*, 2017).

The reduction of the strong fish odor, replaced by a slightly acidic or fruity aroma, is attributed to the action of fermentative microorganisms in the degradation of lipids and proteins (YAFETTO *et al.*, 2022). According to Abbasiliasi *et al.* (2017), during fermentation, lactic acid bacteria metabolize carbohydrates and soluble proteins, producing organic acids such as lactic acid and acetic acid, which contribute to the characteristic aroma and microbiological stability of the silage.

The initial pH of 6.51 and its stabilization around 5.47 by the 14th day reflect the progressive acidification of the silage. This process is essential for inhibiting pathogens and preserving the silage's nutrients (YANG *et al.*, 2020). The increase in titratable acidity from 1.66% to 7.51% indicates efficient lactic fermentation, which is crucial for maintaining the stability and safety of the final product (LIU *et al.*, 2022). The absence of pathogenic microorganisms suggests that the fermentation process (RASHID *et al.*, 2022) was effective in reducing microbiological contamination, ensuring the biosafety of the ingredient for commercial poultry diets (GUIMARÃES *et al.*, 2025). As a high-quality protein source, rich in essential amino acids, particularly lysine and methionine, crucial for protein synthesis and energy metabolism in commercial hens (GUIMARÃES *et al.*, 2019; JIAN *et al.*, 2021; BEZERRA; FONSECA, 2023), tambaqui biological silage contributes to increased feed intake and improved feed conversion, due to its high biological value (ADEDOKUN *et al.*, 2016).

This increase is attributed to protein hydrolysis promoted by enzymatic and microbial activity during fermentation, releasing bioactive peptides and free amino acids (BEZERRA; FONSECA, 2023), which enhance digestibility and absorption in the birds' gastrointestinal tract (GUIMARÃES *et al.*, 2019). Similarly, the lipid concentration,

combined with mineral balance and microbiological stability, reinforces its potential as an economically viable (BATALHA *et al.*, 2019) and nutritionally efficient alternative for feed formulation (GUIMARÃES *et al.*, 2025).

The availability and balance of nutrients are essential for maintaining the productive performance and egg quality of younger commercial hens, as they regulate physiological processes and the bird's biochemical metabolism (SAKOMURA; ROSTAGNO, 2016). In the present study, the inclusion of tambaqui biological silage in the diet showed a quadratic response pattern in feed intake, where moderate levels (up to 2%) improved feed conversion per kilogram of eggs produced, while higher levels resulted in reduced feed intake and a decline in laying rate, corroborating Guimarães *et al.* (2025). This response is associated with the regulation of energy metabolism via hepatic activity, given that vitellogenin, a precursor of yolk lipoproteins, is highly dependent on the bird's energy balance (BRYDEN *et al.*, 2021).

Changes in feed intake are also related to feed palatability and the presence of compounds derived from biological production (GUIMARÃES *et al.*, 2025), which may influence gut microbiota and, consequently, digestive efficiency (CLASSEN, 2017). The digestibility of nutrients presents in tambaqui biological silage, particularly proteins and lipids, influenced essential biochemical processes in the synthesis of egg components such as albumen, yolk, and eggshell. The egg yolk is mainly composed of lipoproteins (Alvarenga *et al.*, 2021) synthesized in the liver, where vitellogenin and apolipoprotein VLDL-II play critical roles in lipid deposition (CHERIAN; QUEZADA, 2016). Hepatic lipid metabolism is regulated by hormones such as estrogen, which stimulates the synthesis of very low-density lipoproteins (VLDL), essential for yolk formation (VAN ECK *et al.*, 2023).

The reduction in egg mass with increasing inclusion of tambaqui biological silage suggests that the bioavailability of lipids from the silage may have been insufficient to provide adequate support of essential fatty acids for vitellogenin properties. According to Cherian and Quezada (2016), the incorporation of omega-3 fatty acids into the diet of laying hens modifies the lipid composition of the yolk, influencing the structure and functionality of cell membranes. Similarly, the presence of polyunsaturated fatty acids derived from tambaqui residues may have altered yolk membrane fluidity (BAIN *et al.*, 2016), impacting its structural stability. This effect may be associated with reduced feed intake in diets with high levels of tambaqui biological silage, leading to an energy deficit and, consequently, lower mobilization of hepatic lipids for yolk enrichment and

deposition. Thus, the decrease in egg mass in diets with high tambaqui biological silage levels may be related to the prioritization of hepatic metabolism for the bird's homeostatic maintenance and a decline in egg production.

The moderate inclusion of tambaqui biological silage (up to 2%) improved albumen height and Haugh unit, reflecting higher protein quality in the albumen. This effect may be related to the greater availability of essential amino acids (BEZERRA; FONSECA, 2023), particularly sulfur-containing ones (methionine and cysteine), which are crucial for the stabilization of egg proteins (VAN ECK *et al.*, 2023). The fermentation process in silage generates proteins and bioactive peptides that can optimize the absorption and utilization of these amino acids by commercial hens (SAKOMURA; ROSTAGNO, 2016; GUIMARÃES *et al.*, 2019).

In the hematological and serum biochemical parameters of the commercial hens, physiological changes were observed, related to protein and lipid metabolism and the availability of essential nutrients for hematopoiesis and metabolic homeostasis (JIAN *et al.*, 2021). A linear reduction in hemoglobin concentration and red blood cell (RBC) count was observed with increasing levels of tambaqui biological silage in the diet. According to Tactacan *et al.* (2012), the availability of iron and B-complex vitamins, particularly vitamin B12 and folic acid (BUNCHASAK; KACHANA, 2009), is essential for hemoglobin synthesis and efficient erythropoiesis. Since tambaqui by-products may have a distinct nutritional profile in terms of mineral bioavailability, the reduction in hemoglobin may reflect lower efficiency in oxygen uptake by tissues (BRELAZ *et al.*, 2021), potentially impacting the birds' energy metabolism.

Despite the reduction in hemoglobin and RBC count, the absence of significant differences in hematocrit suggests that blood viscosity and globular volume were not severely compromised, possibly due to compensatory hematopoietic homeostasis mechanisms (CAMPBELL, 2004). The linear decrease in mean corpuscular hemoglobin concentration (MCHC) reinforces the hypothesis of lower efficiency in iron incorporation into hemoglobin, while the unchanged values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) indicate that erythrocyte morphology and volume were not affected.

The quadratic response of total protein levels, peaking at 2% tambaqui biological silage inclusion, has a moderately positive effect on protein metabolism, associated with the increased availability of bioactive peptides and amino acids derived from silage fermentation (SAKOMURA; ROSTAGNO, 2016; GUIMARÃES *et al.*, 2019).

Conversely, high levels of tambaqui biological silage may indicate limitations in protein digestibility or increased excretion of nitrogenous metabolites.

The significant reduction in triglyceride and cholesterol levels with increased tambaqui biological silage inclusion can be attributed to the presence of polyunsaturated fatty acids (PUFAs) (VAN ECK *et al.*, 2023) in tambaqui by-products, which modulate hepatic lipid metabolism and reduce the synthesis of very low-density lipoproteins (VLDL), essential for lipid transport to the yolk (BAIN *et al.*, 2016). Since vitellogenin and apolipoprotein VLDL-II depend on an adequate lipid supply for yolk formation, the decline in triglycerides may partially explain the reduction in egg mass observed at high tambaqui biological silage inclusion levels.

The linear reduction in plasma glucose suggests modulation of carbohydrate metabolism due to the higher presence of bioactive compounds in the silage, such as peptides and organic acids, which may influence insulin sensitivity and glucose uptake by tissues (LEESON; SUMMERS, 2009). On the other hand, the progressive increase in albumin levels with tambaqui biological silage inclusion indicates a positive plasma amino acid balance and increased hepatic protein synthesis. The rise in uric acid levels with higher dietary tambaqui biological silage reflects intensified protein metabolism and greater purine catabolism, indicating that silage protein may be used as an energy source at high inclusion levels.

The reduction in egg protein content observed with increasing levels of tambaqui biological silage can be attributed to an imbalance between the supply of essential amino acids and energy availability, as discussed by Blanco *et al.* (2014). This adverse situation may significantly impact vitellogenin synthesis, a crucial protein for yolk formation, as well as the production of other plasma proteins in the birds' liver (JIAN *et al.*, 2021). Meanwhile, up to 2% tambaqui biological silage inclusion in the diet resulted in higher egg protein content, associated with the bioavailability of essential amino acids and bioactive peptides (BEZERRA; FONSECA, 2023) derived from silage fermentation. This favored protein synthesis, particularly albumen formation, which depends on sulfur-containing amino acids for structural stabilization. The stability of moisture, lipid, and mineral contents indicates the maintenance of osmotic homeostasis (BLANCO *et al.*, 2014), lipid metabolism, and mineral deposition, preserving yolk and eggshell integrity.

The sensory analysis of eggs from hens fed with tambaqui biological silage revealed that the inclusion of this ingredient significantly affected only flavor, while appearance, texture, aroma, and color remained unchanged. The observed negative linear

trend in flavor indicates that increasing tambaqui biological silage levels reduced the overall acceptance of egg taste, although the values remained within an acceptable range. This alteration in flavor may be attributed to the lipid and protein composition of tambaqui biological silage, which influences the formation of volatile compounds during hepatic metabolism and yolk deposition. Fish by-products, even after fermentation processes, contain polyunsaturated fatty acids susceptible to lipid oxidation, leading to the generation of compounds such as aldehydes and ketones (VIDOTTI *et al.*, 2011; NASCIMENTO, 2023), which impart undesirable flavor notes to the eggs. Additionally, the presence of bioactive peptides may alter the sensory profile of the yolk, affecting consumer taste perception (BRELAZ *et al.*, 2021).

On the other hand, the stability of visual, textural, and aromatic attributes suggests that tambaqui biological silage did not compromise the structural integrity of the eggs. Yolk appearance and color are largely determined by the presence of carotenoids and the uniformity of the lipoprotein emulsion (BAIN *et al.*, 2016), factors that appear to have been preserved, indicating that pigment bioavailability was not negatively affected by tambaqui biological silage. Similarly, the absence of changes in texture (LEESON; SUMMERS, 2009) suggests that the protein composition of both albumen and yolk was maintained, preserving its viscosity and cohesion.

17. CONCLUSION

The present study demonstrated that the inclusion of tambaqui biological silage in the diet of younger commercial hens influences productive performance, physiological parameters, and egg quality. The inclusion of up to 2% tambaqui biological silage showed positive effects on feed conversion and albumen quality, indicating an optimal inclusion level to maximize nutritional benefits without compromising bird performance. However, levels above 2% resulted in reduced egg production, lower feed intake, and alterations in blood biochemical composition, suggesting possible limitations in digestibility and metabolic utilization of the ingredient at high concentrations. Despite the stability of the physical and sensory characteristics of the eggs, flavor acceptability decreased with increasing tambaqui biological silage inclusion, highlighting the need for strategies to mitigate potential organoleptic impacts. Thus, tambaqui biological silage proves to be a viable and sustainable alternative for commercial hen nutrition, provided it is used at controlled levels, contributing to the reuse of aquaculture waste and the reduction of environmental impacts in the production chain.

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CHAPTER V – METABOLIC INSIGHTS INTO EGG YOLK LIPIDS: A ^{1}H NMR STUDY OF TAMBAQUI SILAGE INCLUSION IN FIRST- AND SECOND-CYCLE LAYERS

19. MATERIALS AND METHODS

This study was conducted in partnership with the Fish Technology Laboratory and the Poultry Sector, both belonging to the Faculty of Agricultural Sciences at the Federal University of Amazonas (UFAM), and the Food Analysis Center at the Laboratory for Technological Development Support (LADETEC) of the Institute of Chemistry at the Federal University of Rio de Janeiro (UFRJ). The study is registered under protocol N°. 005/2022 with the Ethics Committee on the Use of Animals (CEUA) at the Federal University of Amazonas.

19.1. Processing of biological silage from tambaqui processing residues

The by-products used in the production of tambaqui (C. macropomum) biological silage, consisting of viscera, gills, few scales, and fins (fish weighing 800 to 1300 g), underwent heat treatment at 65°C for 5 minutes. The resulting residual mass was then filtered and pressed using a hydraulic press. The final content was weighed, placed in buckets, and supplemented with 10% (weight/weight) cassava (*Manihot esculenta* Crantz) peels as a carbohydrate source for the silage, 2.5% (volume/weight) inoculum of pure cultures of the bacterium Lactobacillus plantarum as proteolytic microorganisms, 0.1% (weight/weight) benzoic acid as a fungicidal and bactericidal preservative, and 0.1% (weight/weight) ascorbic acid as an antioxidant. All ingredients were mixed, and then the mixture was kept under anaerobic conditions for 14 days at room temperature, as shown in Figure 3.

At the end of processing, the silage was placed in aluminum trays and transferred to a forced-ventilation oven for 72 hours at 65°C to reduce moisture. Once dried, proximate composition analyses were performed following the methodologies described by Van Soest *et al.* (1991) and AOAC (2019), with the results presented in Table 21.

Table 21. Proximate composition of fresh residual mass and biological silage from tambaqui by-products.

Proximate composition	Fresh waste*	Biological silage*
Dry matter, %	44.61±2.87	87.69±0.17
Crude protein, %	22.67±1.81	44.98±0.59
Fats, %	7.38±0.35	17.89±0.47
Minerals, %	11.70±1.15	21.87±0.69
Crude fiber, %	1.62±0.28	2.44±1.24
Soluble carbohydrates, %	1.23±0.32	0.50±1.06

* All nutrient values were calculated on a dry matter basis. Mean ± standard deviation of three replicates.

19.2. Animals, Facilities, and Experimental Design

Commercial laying hens of the Hisex Brown line were used at different production cycles. Experiment 1 evaluated the effects of including biological silage from tambaqui by-products in older birds (83 weeks) during the second production cycle, while Experiment 2 analyzed these effects in young birds (23 weeks) during the first production cycle.

In each experiment, 120 commercial laying hens were used. The experimental period lasted 63 days, divided into three phases of 21 days each, preceded by a seven-day adaptation period to the diets and facilities. Birds were weighed at the beginning of the experimental period to standardize the groups, with an average weight of 1.83 ± 0.158 kg in Experiment 1 and 1.87 ± 0.181 kg in Experiment 2. A poultry house with a ceiling height of 3.25 m was used. Birds were housed in galvanized wire cages (0.45 m high, 0.40 m wide, and 1.00 m long), each containing six birds. The cages were equipped with linear feeders and nipple drinkers. Throughout the experimental period, birds were exposed to 16 hours of light per day (12 hours of natural light and 4 hours of artificial light).

Air temperature and relative humidity were monitored using a portable digital weather station, which recorded average values of 28.6 °C and 54.15%, respectively. Eggs were collected twice daily (at 9 a.m. and 3 p.m.), with all occurrences (mortality, egg count, among others) recorded daily. The experiments were conducted in a completely randomized design, with treatments consisting of a control diet (without

tambaqui biological silage inclusion) and four levels of tambaqui biological silage inclusion (1, 2, 3, and 4%) in the diets, with four replicates of six birds each. The experimental diets were formulated based on the reference values of Rostagno *et al.* (2017; 2024), except for tambaqui biological silage, whose composition was based on previous bromatological analyses. Metabolizable energy was determined based on the values described by Guimarães *et al.* (2019). tambaqui biological silage was considered a fixed component in the formulations, and the other ingredients were adjusted according to the proposed inclusion levels in the treatments. Diets were formulated using SuperCrac *software* (TD *Software*©, Viçosa, Brazil), and their compositions were analyzed for proximate composition following the methods described by Van Soest *et al.* (1991) and AOAC (2019).

19.3. Egg Collection

During the last two days of the experimental period, eggs were collected to obtain samples for ^{1}H NMR spectroscopy of the yolk. In Experiment 1, 16 eggs were collected per treatment, while in Experiment 2, 32 eggs were collected per treatment. All eggs were properly labeled and sent to the Poultry Sector Laboratory at the Federal University of Amazonas (UFAM). In the laboratory, the eggs were broken, and the yolk was separated from the albumen using a manual separator and then transferred to a beaker.

19.4. Low-field NMR instrumentation and spectral acquisition

^{1}H spectra were acquired using a Spinsolve Multi X benchtop spectrometer (Magritek GmbH) equipped with permanent magnets, capable of generating a homogeneous magnetic field of 1.5 T (corresponding to 60 MHz for protons and 15 MHz for the carbon Larmor frequency). The system was also fitted with a Spinsolve autosampler with a capacity for 20 tubes. Egg yolk samples were obtained from two experiments as illustrated in Figure 4: (i) laying hens in the second production cycle and (ii) laying hens in the first production cycle. In both trials, the birds received either control diets (without tambaqui biological silage) or diets supplemented with four inclusion levels of tambaqui biological silage (1, 2, 3, and 4%).

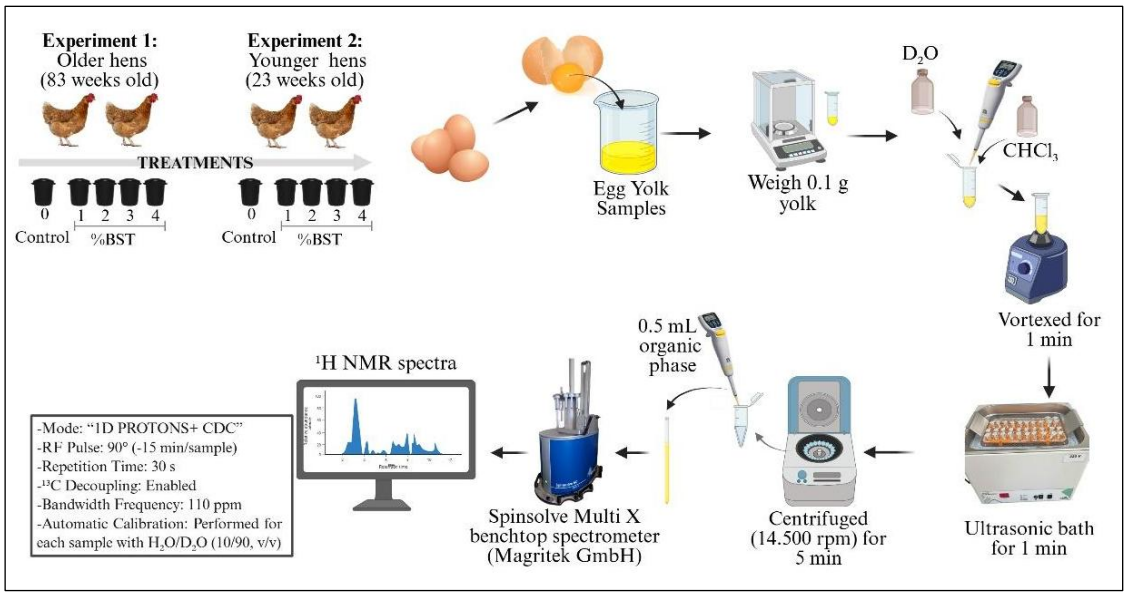

Figure 4. Low-field NMR instrumentation and acquisition of yolk spectra.

The yolks were previously thawed at room temperature and subsequently prepared for apolar metabolic profile analysis, according to the methodology described by Cardoso *et al.* (2022), with adaptations. Briefly, 0.1 g of yolk was weighed and transferred into 2 mL plastic microtubes, to which 0.7 mL of chloroform ($CHCl_3$) and 0.2 mL of deuterated water (D_2O) were added. The samples were vortexed for 1 min, sonicated in an ultrasonic bath for 1 min, and then centrifuged at approximately 9,400 × g (14,500 rpm) for 5 min. After phase separation, 0.5 mL of the organic phase was carefully transferred into 5 mm NMR tubes for spectroscopic analysis.

^1H spectra were acquired in "1D PROTON+ CDC" mode using a 90° radiofrequency pulse. A total of 180 scans were collected, corresponding to an acquisition time of approximately 15 min per sample, with a repetition delay of 30 s between acquisitions. ^{13}C decoupling was enabled, the bandwidth was set at 328 ppm, and the central frequency was adjusted to 110 ppm. The instrument was automatically calibrated every 30 min of analysis using a standard H_2O/D_2O solution (10/90, v/v

19.5. Multivariate Analysis

All spectral preprocessing, multivariate analysis, and post-processing procedures were performed using Matlab 2021a® (The MathWorks Inc., Natick, USA) in conjunction with a user-friendly graphical interface called the Multivariate Analysis Group in Food Matrices (GAMMA-GUI), which is freely available for download on GitHub at https://github.com/appGAMMA. Prior to initiating any preprocessing protocol

or multivariate analysis, the spectral data were converted into numerical matrices within the Matlab environment.

19.6. Spectral Preprocessing

Free induction decay (FID) signals were initially processed with zero-order phase correction and baseline correction to ensure higher quality and reproducibility of the spectra. Subsequently, spectral alignment was performed using the Icoshift algorithm, with the characteristic chloroform signal ($CHCl_3$, δ 7.26 ppm) as a reference. After alignment, this reference peak was removed from the dataset to avoid interference in subsequent multivariate analyses. For the chemometric modeling steps, a spectral range from δ 0.00 to δ 6.50 ppm was defined, excluding the water signal and regions of low analytical relevance. In addition, a 0.5 Hz filter was applied to smooth high-frequency noise and optimize the quality of the processed spectra.

19.7. Principal Component Analysis (PCA)

PCA was used to examine the data's natural behavior. This method reduces the dimensionality of the dataset by using linear combinations of the original variables to maximize variance in the multivariate space. The new variables resulting from PCA are called principal components (PCs), which are orthogonal projections of each other. They can be obtained through the Singular Value Decomposition (SVD) of the matrix X, producing the score (T) and loading (L) matrices, as well as the residual matrix (E) for a given set of *A* PCs, Equation 1.

(Eq. 1)

$$X = \sum_{a=1}^{A} t_a l_a^T + e = T_A L_A^T + E$$

PCA aims to identify patterns and simplify data visualization while preserving as much of the total variance of the original dataset as possible. Principal components represent directions in the data space that explain the most significant variance, allowing for a more precise and focused analysis of the relationships between samples and their characteristics in a reduced-dimensional space (KURITA, 2019).

20. RESULTS

20.1. ^{1}H NMR spectra for laying hens in the second production cycle

Figure 5 shows the average low-field ^{1}H NMR spectra of the apolar metabolic profile of egg yolks from laying hens in the second production cycle, subjected to control diets (CT) and diets containing different levels of tambaqui biological silage inclusion: 1% (1S), 2% (2S), 3% (3S), and 4% (4S). To facilitate interpretation, the overall spectrum (δ 0.00–δ 6.50 ppm) was segmented into six distinct regions (Reg A–F), each corresponding to specific classes of lipid metabolites. This division allowed a targeted assessment of the dietary effects on different constituents of the yolk's apolar fraction.

In general, an increase in lipid signal intensities was observed in tambaqui biological silage supplemented diets compared with the control. Among treatments, the 3% inclusion (3S) stood out, showing the highest intensities in most spectral regions, indicating greater deposition or mobilization of apolar lipids. The exception was found in the vinyl PUFAs (Region F), where the 1S treatment exhibited the highest signals, suggesting that lower inclusion levels selectively favor this group of compounds.

In Region A (δ 0.60–δ 1.10 ppm), characteristic signals of saturated fatty acids (δ 0.70–0.87 ppm), ω-3 fatty acids (δ 0.95 ppm), and cholesterol (δ 1.02–1.07 ppm) were detected. Region B (δ 1.70–δ 2.60 ppm) showed signals related to the α-CH_2 group of fatty acids (δ 2.27–2.38 ppm), allylic CH_2 (δ 2.03–2.12 ppm), phosphatidylethanolamine-PE (δ ~2.50 ppm), and ketoacids (δ 1.66–1.79 ppm). In Region C (δ 2.70–δ 3.40 ppm), signals were observed corresponding to phosphatidylcholine-PC (δ ~3.30 ppm) and bis-allylic polyunsaturated fatty acids (δ 2.70–2.90 ppm). Region D (δ 3.50–δ 4.00 ppm) was characterized by the predominance of phospholipids, with variations in intensity among treatments. Region E (δ 4.10–δ 4.80 ppm) showed signals from triacylglycerols (δ ~4.28 ppm), PC (δ ~4.21 ppm), phospholipids (δ ~4.70 ppm), and glycerol (δ 4.10–4.54 ppm). Finally, Region F (δ 5.10–δ 5.80 ppm) presented signals of vinyl PUFAs (δ 5.16–5.23 ppm and δ 5.65 ppm) and triacylglycerols (δ 5.34–5.42 ppm). Unlike the other regions, the highest intensity of vinyl PUFAs was observed in the 1S treatment, suggesting that lower inclusion levels of silage may selectively favor the deposition of this group of unsaturated fatty acids.

Spectral analysis demonstrated that tambaqui silage inclusion promoted relevant modifications in the yolk lipid profile, with particular emphasis on the 3% level (3S), which intensified most lipid signals, reflecting an enrichment of the nutritional profile,

whereas the 1% level (1S) had a more selective impact on vinyl PUFAs. Visual inspection of the average spectra allowed qualitative and intensity variations among treatments to be identified; however, a deeper understanding of these differences requires multivariate statistical analyses. In this regard, Principal Component Analysis (PCA) was conducted as a subsequent step and will be discussed below, enabling the identification of clustering patterns and providing more consistent insights into the metabolic changes induced by silage inclusion.

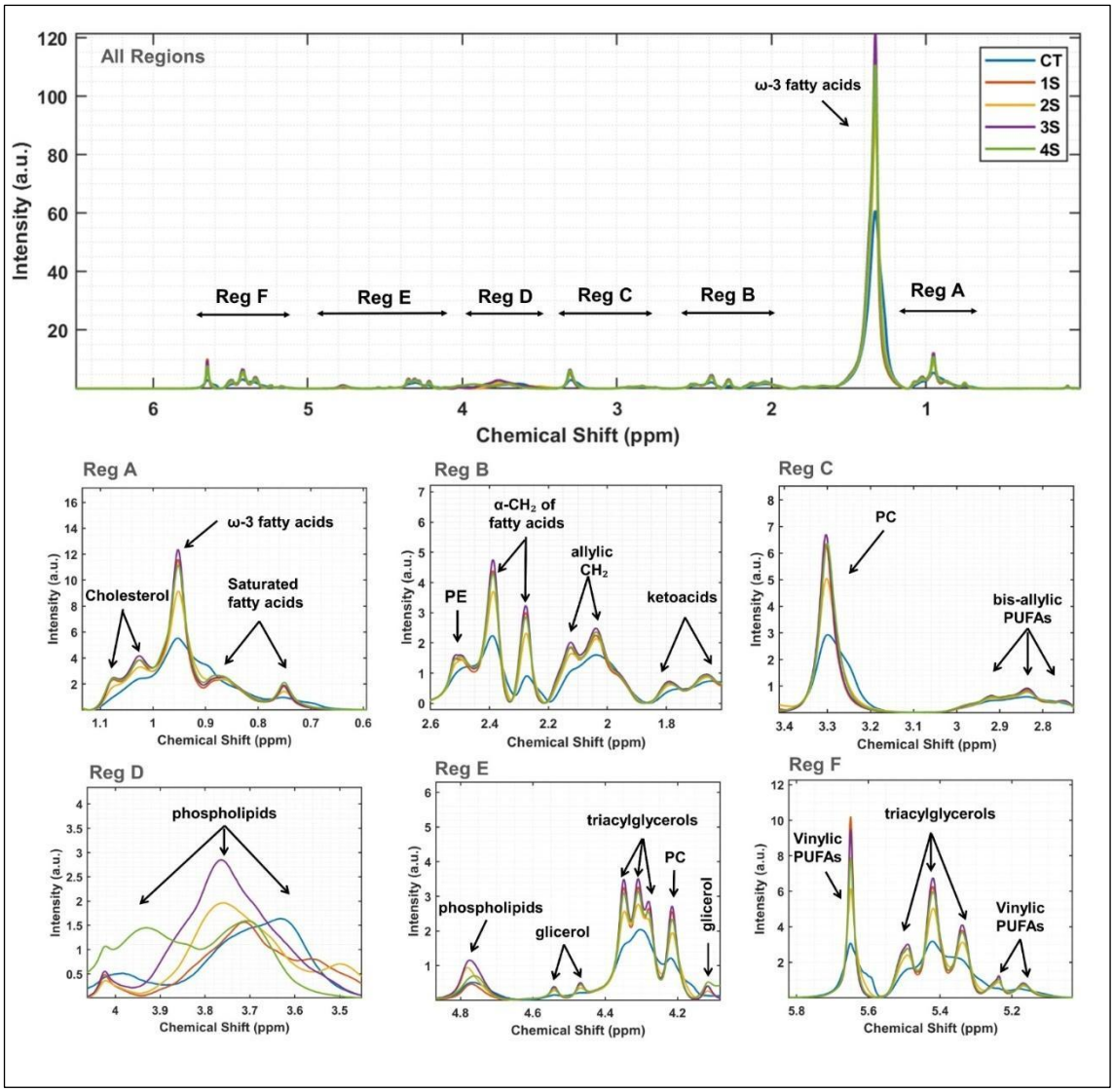

Figure 5. Average low-field ^{1}H NMR spectra (δ 0.00 – 6.50 ppm) of the lipid fraction of egg yolks from laying hens in the second production cycle, fed a control diet (CT) and diets containing different levels of tambaqui silage (BST) inclusion: 1% (1S), 2% (2S), 3% (3S), and 4% (4S).

20.1.1. Exploratory analysis using PCA

PCA was applied to explore metabolic differences in low-field ^{1}H NMR spectra obtained from the apolar metabolic profile of egg yolks from laying hens in the second production cycle, subjected to experimental diets. The first three principal components jointly explained 93.08% of the total variance in the data, with 81.19% attributed to PC1, 10.12% to PC2, and 1.77% to PC3, demonstrating the robustness of the model and its ability to represent most of the metabolic variation among treatments in a low-dimensional space.

In the score plot shown in Figure 6 (A), PC1 was the axis that best explained the separation between the control group (CT) and the silage-inclusion treatments. CT samples clustered more strongly in the negative region of PC1, while treatments with 1% (1S), 3% (3S), and 4% (4S) were positioned in the positive region of PC1, clearly distinguishing the control diet from silage-containing diets and indicating greater metabolic similarity among the 1–4% inclusion levels. PC2 contributed further to the separation, differentiating not only CT but also highlighting the 2% silage treatment (2S), which was positioned more clearly in the positive region of PC2. This pattern suggests that 2S presented a unique metabolic profile, more similar to the control group and distinct from the other inclusion levels. PC3 explained more specific variations, being particularly relevant for characterizing the 2S group, which was predominantly classified in the positive region of this component, reinforcing that this inclusion level exhibited an exclusive metabolic behavior compared with the other treatments.

Figure 6 (B, C, and D), corresponding to the loadings of PCs 1, 2, and 3, allow interpretation of the spectral metabolic profile underlying this separation. PC1 (Figure B) was strongly influenced by signals at δ ~0.95–1.35 ppm, attributed to Region A (methyl/methylene groups of fatty acids, cholesterol, and ω-3 fractions), with a secondary contribution at δ ~5.65 ppm. Thus, PC1 primarily represents variations in the overall content of apolar lipids. PC2 (Figure C) showed relevant loadings at δ ~5.16–5.23 and δ ~5.65 ppm, corresponding to vinyl PUFAs (Region F), as well as δ ~1.32 ppm, indicating that this component mainly describes differences in the degree of unsaturation of the lipid

fraction. PC3 (Figure D), which explained only 1.77% of the variance, retained residual variations and did not alter the main patterns observed.

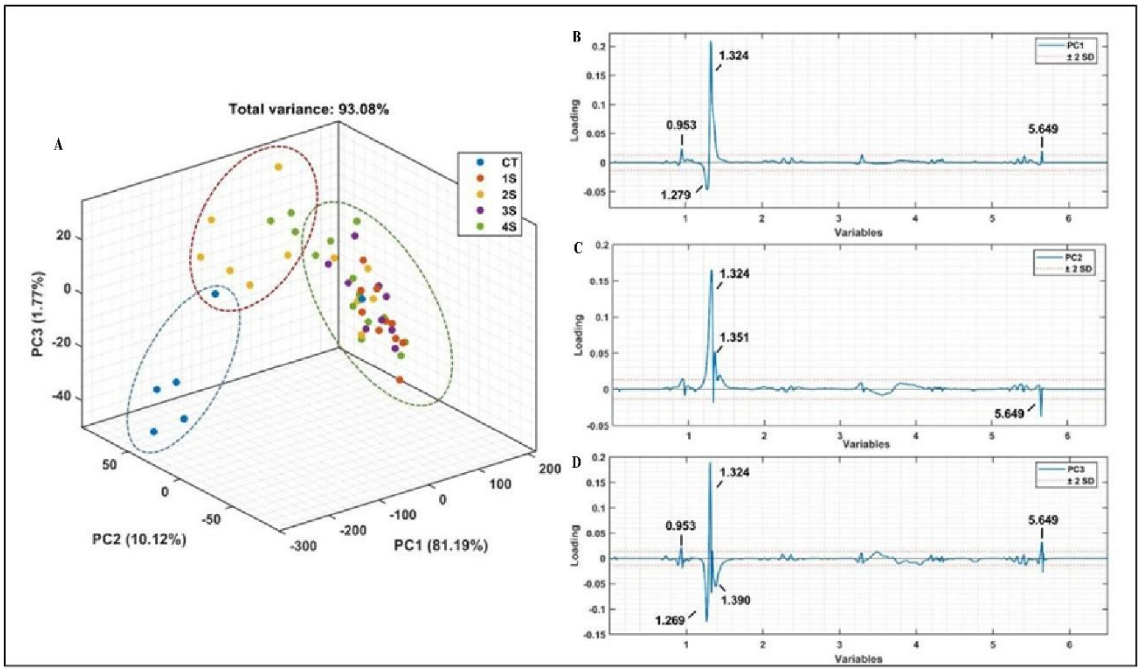

Figure 6. Principal Component Analysis (PCA) applied to low-field ^{1}H NMR spectra of the nonpolar metabolic profile of egg yolk from laying hens in the second production cycle, fed a control diet (CT) or with different levels of tambaqui biological silage inclusion: 1% (1S), 2% (2S), 3% (3S) and 4% (4S). (A) Score graph and (B _ D) Loading graphs correspond to PC1, PC2 and PC3, highlighting the spectral signals responsible for the separation between the groups.

20.2. ^{1}H NMR spectra for laying hens in the first production cycle

Figure 7 shows the average low-field ^{1}H NMR spectra obtained from the apolar fraction of egg yolks from laying hens in their first production cycle, subjected to the control diet (CT) and diets containing different levels of tambaqui biological silage: 1% (1S), 2% (2S), 3% (3S), and 4% (4S). As with second-cycle hens, the spectrum was segmented into six regions (A–F), but here some relevant differences were observed, reflecting the particular metabolic characteristics of younger hens.

In Region A (δ 0.60–1.20 ppm), in addition to the typical signals of saturated fatty acids (δ 0.70–0.87 ppm), ω-3 fatty acids (δ ~0.95 ppm), and cholesterol (δ 1.02–1.07 ppm), the evident presence of ketoacids (δ ~1.20 ppm) was highlighted, observed exclusively in the control group (CT). In the silage-inclusion treatments, these signals

disappeared, suggesting that supplementation may have contributed to reducing the formation or accumulation of ketoacids in the yolk. Signals attributed to ω-3 fatty acids (δ ~0.95 ppm) and cholesterol (δ 1.02–1.07 ppm) showed higher intensities specifically in the 3% silage group (3S), indicating enhanced deposition of these compounds at this supplementation level. In contrast, saturated fatty acids (δ 0.70–0.87 ppm) were more intense in the 4% treatment (4S), suggesting a dose-dependent differential response between saturated and unsaturated lipids.

Region B (δ 1.70–2.60 ppm) displayed characteristic signals of the α-CH_2 group of fatty acids (δ 2.27–2.38 ppm), allylic CH_2 (δ 2.03–2.12 ppm), phosphatidylethanolamine-PE (δ ~2.50 ppm), and additional ketoacids (δ 1.66–1.79 ppm). Signals for PE and allylic CH_2 were similar in intensity for the 3S and 4S groups, indicating that both supplementation levels favored the deposition of these unsaturated constituents. The α-CH_2 signal of fatty acids was more pronounced in the 3S treatment, suggesting that this level promoted greater incorporation of this specific structural group of fatty acids. In addition, ketoacids were detected in the control group and in the 3S and 4S levels, indicating that although reduced compared with CT, these compounds still appeared at higher silage inclusions, possibly reflecting metabolic adjustments in yolk oxidative balance.

In Region C (δ 2.70–3.40 ppm), signals were identified for phosphatidylcholine (PC) (δ ~3.30 ppm) and bis-allylic PUFAs (δ 2.70–2.90 ppm). Phosphatidylcholine showed higher intensity in the 3S treatment, suggesting that this silage inclusion level particularly favored the deposition of this structural phospholipid in the yolk. Meanwhile, the signals corresponding to bis-allylic PUFAs showed very similar behavior between the control and silage treatments, indicating that supplementation did not produce expressive alterations in this class of polyunsaturated fatty acids, which remained relatively stable among the experimental groups.

Region D (δ 3.50–4.00 ppm) exhibited marked differences compared with older hens, highlighting the specific metabolic behavior of first-cycle layers. Phospholipids appeared with higher intensity and definition, especially in the 2S group, but also clearly in 3S and 4S, indicating a more pronounced incorporation of phospholipids in young hens, possibly related to the more active metabolism characteristic of the early laying phase. It is important to note that the control group (CT) showed phospholipid peaks distinct from those observed in silage treatments, with relevant intensities, suggesting a differentiated deposition profile. Moreover, each silage inclusion level displayed a specific peak pattern

in this region, indicating that supplementation modulated not only the amount but also the type of phospholipids incorporated into the yolk.

In Region E (δ 4.10–4.80 ppm), in addition to the expected signals of triacylglycerols (δ ~4.28 ppm), phosphatidylcholine-PC (δ ~4.21 ppm), phospholipids (δ ~4.70 ppm), and glycerol (δ 4.10–4.54 ppm), the presence of new phospholipid peaks was observed, not as clearly detected in second-cycle hens. The 3S treatment showed the highest signal intensities in this region, followed by 4S, indicating that higher silage inclusion levels favor the deposition of different phospholipids. The control group also exhibited greater metabolite intensity in this region compared with the 1S and 2S treatments, suggesting that lower supplementation levels were insufficient to surpass the natural contribution of these compounds in the control diet.

Region F (δ 5.10–5.80 ppm), corresponding to vinyl PUFAs (δ 5.16–5.23 and δ 5.65 ppm) and triacylglycerols-TAGs (δ 5.34–5.42 ppm), showed a pattern partially similar to that observed in second-cycle hens. The 1S group displayed the highest vinyl PUFA signals, indicating that lower silage inclusion levels favored selective deposition of unsaturated fatty acids. Meanwhile, TAG signals predominated in the 3S and 4S treatments, reinforcing the trend of greater overall lipid accumulation at higher supplementation levels. This pattern suggests that in both Regions E and F, there is convergence toward the same metabolic dynamic: increased deposition of TAGs and phospholipids at higher silage levels (particularly 3S), whereas 1S is differentiated by the selective contribution of unsaturated compounds.

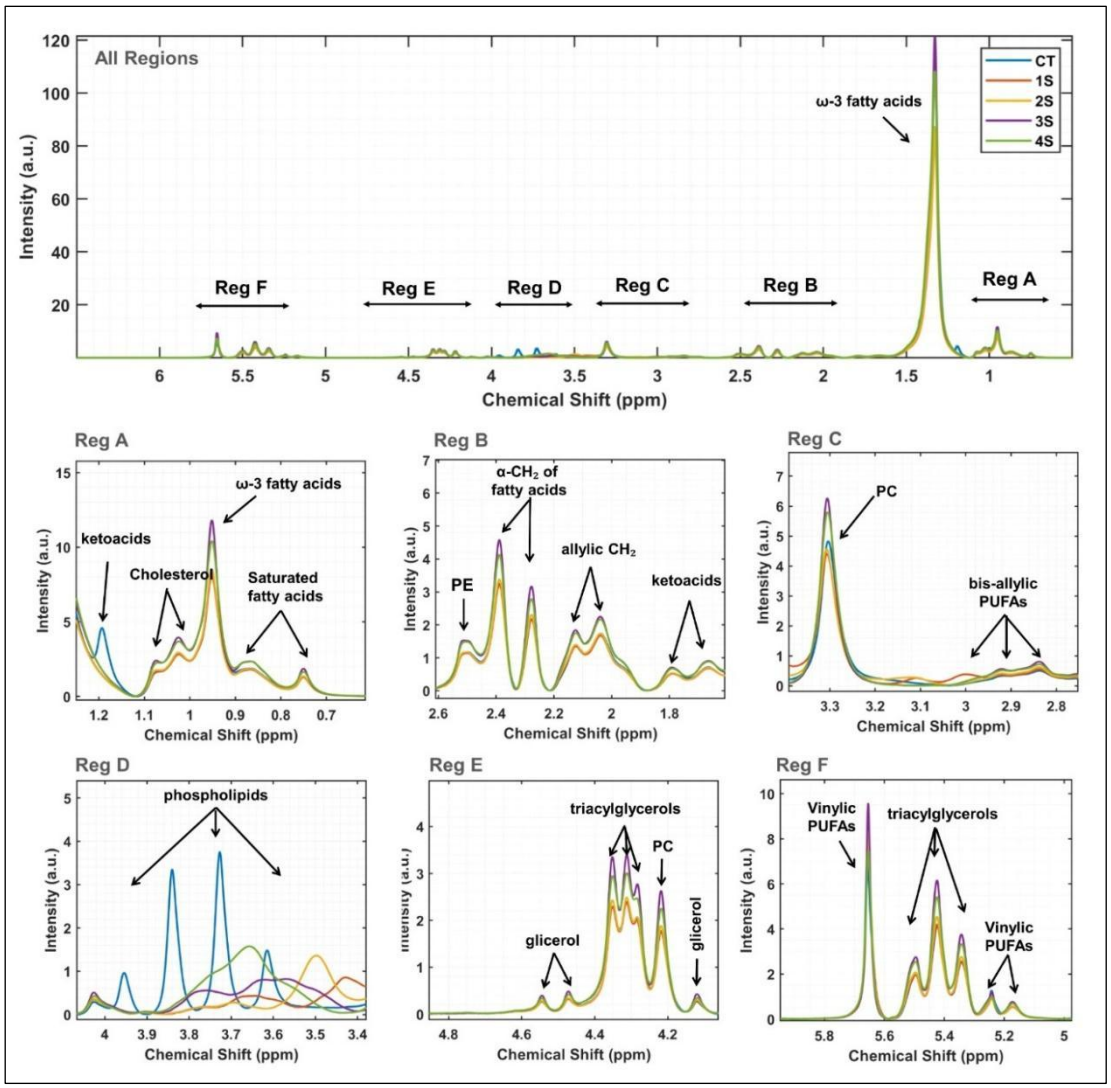

Figure 7. Average low-field ^{1}H NMR spectra (δ 0.00 – 6.50 ppm) of the lipid fraction of egg yolks from laying hens in the first production cycle, fed a control diet (CT) or with different levels of tambaqui biological silage (BST) inclusion: 1% (1S), 2% (2S), 3% (3S), and 4% (4S).

20.2.1. Exploratory analysis using PCA

PCA was applied to the ^{1}H NMR spectra of the apolar fraction of egg yolks from laying hens in their first production cycle, with the aim of exploring metabolic differences between the control diet (CT) and different levels of tambaqui silage inclusion (1S, 2S, 3S, and 4S). The first three principal components explained 96.24% of the total variance in the data, with 88.83% attributed to PC1, 6.47% to PC2, and 0.94% to PC3, evidencing the robustness of the model and its ability to describe virtually all variability of lipid profiles (Figure 8 – A).

In the score plot (Figure 8 – A), PC3 best characterized the control group (CT), positioned in the positive region and clearly separated from the other treatments. PC1, in turn, differentiated treatments 1S, 2S, and 4S, which were distributed in the negative quadrant, from treatment 3S, concentrated in the positive region, indicating that this component mainly explains the unique behavior of the 3% silage inclusion level. PC2 contributed to distinguishing treatments 3S and 4S, located in the negative region, from the other groups, including the control, which clustered in the positive region.

The PC1 loadings (Figure 8 – B) showed that signals at δ 0.95 and 1.32 ppm (fatty acids) and δ 5.65 ppm (vinyl PUFAs) were determinant for the separation of the 3S group (positive) in relation to 1S, 2S, and 4S (negative), reflecting differences in global fatty acid content and unsaturation. PC2 (Figure 8 – C) was explained by signals at δ 1.30–1.38 ppm (fatty acids), δ 3.72–3.85 ppm (phospholipids), and δ 5.65 ppm (vinyl PUFAs), distinguishing 3S and 4S (negative) from CT, 1S, and 2S (positive), indicating modulation of phospholipids and unsaturated lipids. Finally, PC3 (Figure 8 – D), although with lower variance (0.94%), separated the control (positive) from the other groups (negative), supported by signals of fatty acids (δ 0.95–1.32 ppm), phospholipids (δ 3.65–3.85 ppm), and vinyl PUFAs (δ 5.65 ppm), confirming the distinct lipid profile of the control group compared with the silage diets.

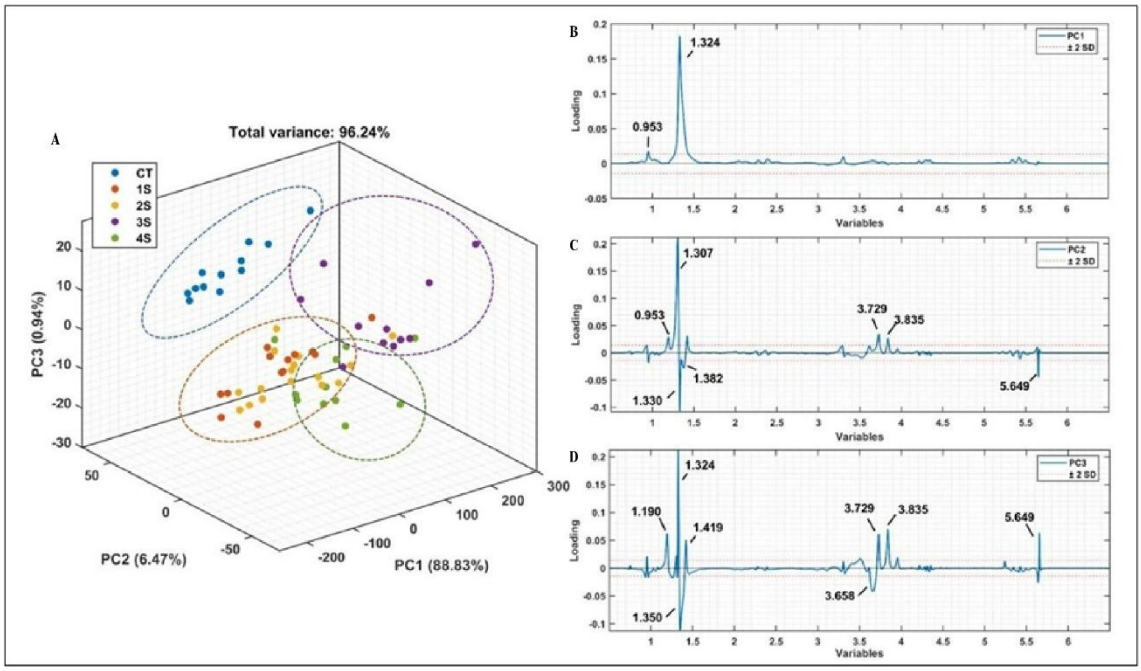

Figure 8. Principal Component Analysis (PCA) applied to ^{1}H NMR spectra of the lipid fraction of egg yolks from laying hens in the first production cycle, subjected to the

control diet (CT) and different levels of tambaqui silage (BST) inclusion: 1S, 2S, 3S, and 4S. (A) Score plot showing the separation between groups, and (B–D) loading plots corresponding to PC1, PC2, and PC3, highlighting the spectral signals responsible for the separation between groups.

20.3. PCA – Laying Hens in Second Production Cycle vs. First Production Cycle

To gain a deeper and more detailed understanding of the metabolic differences between young (N) and old (V) laying hens, a PCA was conducted integrating the ^{1}H NMR spectra of the apolar fraction of egg yolks at all levels of tambaqui silage inclusion (Figure 9). This approach allowed the evaluation not only of the dietary effect but also of how productive age influences yolk lipid metabolism under different experimental conditions.

The PCA explained 94.80% of the total variance, distributed as 69.70% by PC1, 19.83% by PC2, and 5.27% by PC3 (Figure 9 – A). PC1 was the most important component for separating the control group of old hens (CT_V, negative region) from all other groups, including both the birds fed tambaqui biological silage diets and the young hens, which clustered in the positive region. PC2, although accounting for a relevant portion of the variance, did not show a clear separation among groups, indicating that this axis was not decisive in distinguishing age or levels of tambaqui biological silage inclusion. PC3, although representing only 5.27% of the variance, played a key role, as it enabled the separation of young hens (N, negative region) from old hens (V, positive region), showing that age was an additional discriminant factor when considered together with dietary supplementation. Moreover, a closer proximity of the lower silage levels (1S_N and 2S_N) and the control group of young hens with the control group of old hens (CT_V) was observed, reflecting a metabolic similarity among these groups according to their relative distance in multivariate space.

The PCA loadings showed that PC1 was sustained by signals at δ 0.95–1.32 ppm (fatty acids) and δ 5.65 ppm (vinyl PUFAs), which were responsible for distinguishing the control of old hens (CT_V) from the other groups. PC2 presented contributions at δ 1.30–1.38 ppm (fatty acids) and δ 3.72–3.85 ppm (phospholipids), but without producing a clear separation among treatments. PC3 again highlighted signals at δ 0.95–1.32 ppm (fatty acids), δ 3.65–3.85 ppm (phospholipids), and δ 5.65 ppm (vinyl PUFAs), being determinant for differentiating young (N) from old (V) hens and explaining the similarity

between the lower silage levels of young hens (1S_N and 2S_N) and CT_V, due to resemblance in lipid patterns.

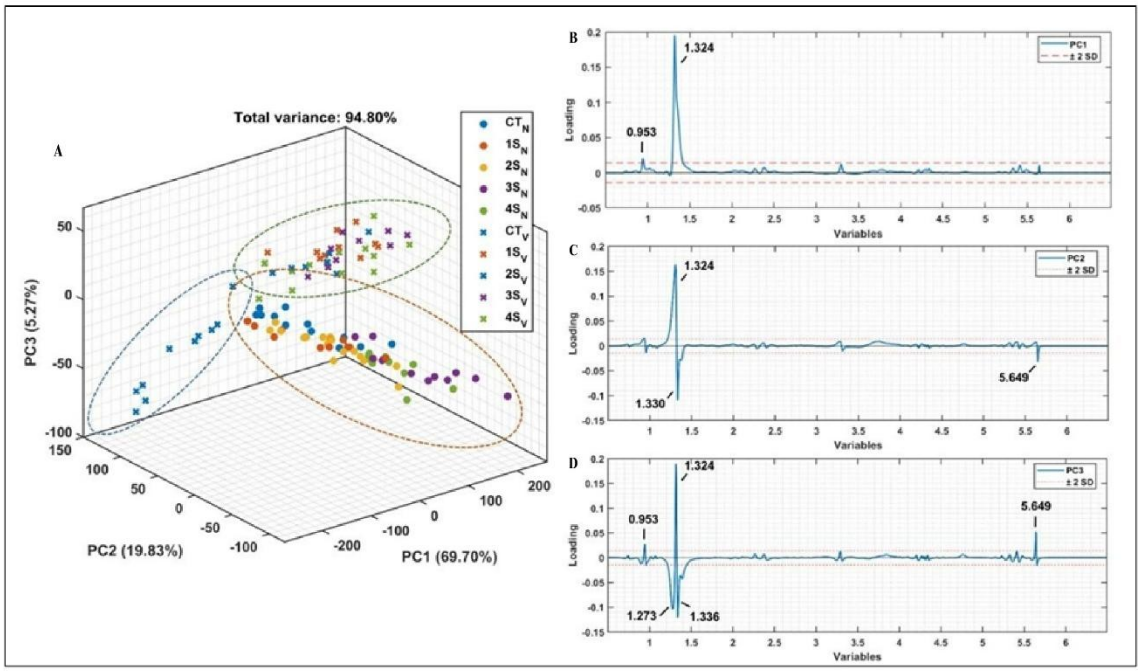

Figure 9. Principal Component Analysis (PCA) of ^{1}H NMR spectra of the lipid fraction of egg yolks from young (N, represented by "o") and old (V, represented by "x") laying hens subjected to the control diet (CT) and different levels of tambaqui silage inclusion (1S, 2S, 3S, and 4S). (A) Score plot showing the separation between groups, and (B–D) loading plots corresponding to PC1, PC2, and PC3, highlighting the spectral signals responsible for the separation between groups.

21. DISCUSSION

The lipid composition of the yolk is directly modulated by the diet of laying hens, since the liver is the main lipogenic organ in birds, responsible for the synthesis of yolk-specific very-low-density lipoproteins (VLDLy) and vitellogenin, which transport triglycerides, phospholipids, and cholesterol to the developing oocyte yolk (VAN ECK *et al.*, 2023; WALZEM *et al.*,1999). The inclusion of biological silage from tambaqui by-products in the diet of laying hens was an important factor, resulting in consistent alterations in the apolar metabolic profile of the yolk, as evidenced by ^{1}H NMR and multivariate analyses (PCA). These alterations reflect both the direct effect of lipid supplementation and the metabolic particularities associated with the productive age of the hens.

According to Ozyurt, *et al*, (2017) fish silage contains high concentrations of lipids, including monounsaturated and polyunsaturated fatty acids (oleic, linoleic, EPA, and DHA), and therefore represents a dietary source with potential to modify yolk lipid composition. In the present study, the inclusion of 3% tambaqui biological silage favored greater global deposition of apolar lipids, reflected in the intensification of signals attributed to triglycerides (δ ~5.3 ppm) and phospholipids (δ 3.5–4.3 ppm). This effect indicates that there is an optimal inclusion point at which the bioavailability of silage nutrients is maximized, corroborating Batalha *et al.* (2017), who, using acid silage meal from *Arapaima gigas* residues in diets for commercial light layers, reported that lipids in silage are bioavailable for absorption and subsequent incorporation into eggs, with efficient digestibility by hens and a significantly higher apparent digestibility coefficient for ether extract (74.36%) compared with the control diet (67.59%). On the other hand, lower levels (1%) promoted metabolic selectivity, particularly favoring vinyl PUFAs (δ 5.16–5.65 ppm), indicating that small amounts of tambaqui biological silage may direct preferential deposition of unsaturated fatty acids, though without globally increasing lipid accumulation.

PCA analysis associated with ^{1}H NMR spectra demonstrated that silage inclusion in the diet promoted significant alterations in the apolar metabolic profile of yolks from 83-week-old laying hens, partially balancing the adverse effects of aging on lipid metabolism. Aging in laying hens is associated with reduced digestive efficiency, hepatic accumulation of cholesterol and triglycerides, and a decline in antioxidant activity (GU *et al.*, 2023). Physiologically, this results from decreased circulating estradiol levels and reduced activity of antioxidant enzymes such as superoxide dismutase and glutathione

peroxidase, leading to greater hepatic and systemic oxidative stress (JULIAN, 2005). Consequently, eggs of inferior quality are produced, with reduced yolk height, greater susceptibility to lipid oxidation, and decreased structural firmness (SALEH *et al.*, 2013).

The intensification of signals from unsaturated fatty acids (δ 2.8 ppm) and triglycerides (δ 5.3 ppm), as well as the presence of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), indicates that silage promoted greater membrane fluidity and structural preservation of the yolk (SALEH *et al.*, 2019). According to Gu *et al.* (2023) supplementation with sources rich in phospholipids and polyunsaturated fatty acids may attenuate the effects of aging on hepatic and oocyte metabolism, preserving lipid deposition and delaying the decline in egg quality. In addition, the presence of signals related to PC and PE (δ 3.5–4.3 ppm) indicates maintenance of yolk structural integrity, balancing membrane and storage lipids, which are essential to sustaining internal quality even in older birds.

On the other hand, PCA analysis associated with ^{1}H NMR spectra demonstrated that silage inclusion in the diet showed a distinct behavior in the apolar metabolic profile of yolks from 23-week-old laying hens. In young birds, intense estrogenic activity stimulates hepatic synthesis of reduced-diameter VLDLy (25–44 nm), resistant to lipoprotein lipase and thus captured intact by oocyte membrane receptors (ABEYRATHNE *et al.*, 2022; SAH; MISHRA, 2018). This mechanism explains the greater intensity of signals attributed to PC and PE in NMR spectra of young hens, indicating that tambaqui biological silage inclusion stimulated hepatic synthesis of structural phospholipids. PC is the main yolk phospholipid (~78.5% of the phospholipid fraction), followed by PE (~17.5%) and sphingomyelin (~2.5%). These compounds are essential for the formation of stable VLDLy and efficient lipid deposition in the oocyte (JULIAN, 2005). The predominance of these phospholipids is crucial not only for yolk structure but also for highly relevant metabolic functions such as cholinergic neurotransmission and antioxidant activity associated with PC and PE, respectively (CHEN *et al.*, 2019; ABEYRATHNE *et al.*, 2022). Greater deposition of PC and PE was observed in the treatments, especially with 3% inclusion, along with enrichment in ω-3 fatty acids.

The presence of ω-3 fatty acids in signals at δ 0.9–1.3 ppm indicates that silage was a relevant source of these lipids, which were preferentially incorporated into yolk phospholipids and triglycerides. Studies with dietary sources rich in DHA and EPA demonstrate that direct supplementation results in higher concentrations of these fatty

acids in the yolk (ELKIN; HARVATINE, 2023). This metabolic remodeling in young hens reflects hepatic plasticity at the onset of laying, enabling efficient redirection of dietary lipids to the ovary.

Comparative PCA between hens in their first and second production cycles further revealed that low levels of tambaqui biological silage (1 and 2%) in young birds resulted in metabolic profiles similar to the control treatment of older birds. Although intriguing, this finding indicates that including lower levels of tambaqui biological silage in the diet of first-cycle hens produces yolk lipid deposition patterns resembling those of physiologically aged hens (second production cycle). Thus, intermediate levels (especially 3%) appear more suitable, as they maximize the deposition of beneficial lipids without inducing profiles associated with metabolic aging in laying hens.

From a practical standpoint, the results demonstrate that tambaqui silage has the potential to enrich eggs with ω-3, functional phospholipids, and unsaturated fatty acids, thereby adding nutritional value to the product, as also reported by Kassis *et al.* (2010) in diets enriched with DHA-rich oils. Furthermore, by preserving yolk quality in older hens, supplementation may help extend the productive lifespan of flocks, reducing early culling and increasing production system efficiency.

It is worth noting, however, that although low-field NMR is efficient in detecting global metabolic variations, it has resolution limitations that hinder the unequivocal identification of specific compounds. Future studies integrating high-field NMR, LC-MS/MS, or GC-MS could more precisely characterize and quantify the lipid metabolites involved, enabling a deeper understanding of the metabolic remodeling induced by tambaqui biological silage. Therefore, the use of fish silage as an alternative ingredient represents not only a nutritional innovation but also a practice aligned with the circular bioeconomy. The utilization of by-products from the fishing industry in diet formulation adds value to production chains, reduces environmental impacts, and contributes to the sustainability of egg production. Thus, the inclusion of tambaqui biological silage emerges as a viable and sustainable strategy capable of improving lipid metabolism, extending the productive performance of second-cycle laying hens, and producing eggs with greater functional and nutritional value.

22. CONCLUSION

The results demonstrated that the inclusion of tambaqui biological silage in the diet of laying hens promoted significant alterations in yolk lipid metabolism, as evidenced by PCA and ^{1}H NMR analyses. In 83-week-old birds, supplementation counteracted the physiological effects of advanced age, increasing the availability of unsaturated fatty acids and preserving yolk quality traits such as height and firmness. In young hens, at 23 weeks of age, silage enhanced the incorporation of ω-3, the accumulation of triacylglycerols, and the synthesis of structural phospholipids, reflecting the high metabolic capacity of the liver at the onset of laying.

Taken together, the findings indicate that biological silage modulated the yolk lipid profile at different production stages, increasing its nutritional value through greater deposition of polyunsaturated fatty acids and phospholipids, compounds of recognized relevance for human health. In this context, ^{1}H NMR proved to be a highly sensitive analytical tool for identifying modifications in lipid metabolism, establishing itself as a promising resource for productive evaluation applied to layer nutrition, as it enables metabolic characterization associated with both productive performance and egg quality.

Beyond its productive and nutritional impact, the use of fish residues for the formulation of functional ingredients represents an approach aligned with the principles of the circular bioeconomy, reducing environmental liabilities and promoting the sustainability of aquaculture and poultry production chains. Thus, the inclusion of tambaqui biological silage represents a sustainable nutritional strategy capable of providing eggs with higher added value from functional, economic, and environmental perspectives.

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CHAPTER VI – PRODUCTIVE PERFORMANCE AND ECONOMIC FEASIBILITY THE INCLUSION OF BIOLOGICAL SILAGE FROM TAMBAQUI BY-PRODUCTS IN DIETS FOR COMMERCIAL HENS

24. MATERIALS AND METHODS

The current experiment was conducted at the Faculty of Agrarian Sciences of the Federal University of Amazonas, located in Manaus (AM), Brazil. All experimental procedures were conducted in accordance with the guidelines of the Local Experimental Animal Care Committee and were approved by the UFAM ethics committee (protocol number 005/2022).

24.1. Processing of tambaqui residues and silage production

The residues, consisting of viscera, gills, scales, and fins, discarded during the processing of tambaqui (fish weighing between 800 and 1,300 g) were obtained from an industrial fish processing facility in Manaus, Amazonas. These residues were trans-ported to the laboratory in insulated containers and stored in a freezer until further processing. During the initial preparation, the residues were thawed at room temperature and subjected to thermal treatment at 65°C for 5 minutes. Subsequently, they were filtered through a sieve, and the remaining mass was pressed using a hydraulic press. The processed material was then weighed and transferred into 40-liter plastic buckets for later use.

For the production of silage, the residual fish mass was combined with the following ingredients in specific proportions: 10% (w/w) cassava trimmings (*Manihot esculenta* Crantz), obtained from local markets in Manaus. The trimmings were cleaned and ground to serve as a carbohydrate source for the silage. Additionally, 2.5% (v/w) of an inoculum consisting of pure cultures of Lactobacillus plantarum, a proteolytic bacterium, was added, along with 0.1% (w/w) benzoic acid as a fungicidal and bactericidal preservative, and 0.1% (w/w) ascorbic acid as an antioxidant. This process followed the methodology described by Vidotti *et al.* (2003) and Vidotti *et al.* (2011).

All ingredients were thoroughly mixed in the buckets until the silage components were completely uniform. The buckets were then hermetically sealed to ensure anaerobic conditions. To achieve this, a sterilized plastic bag was placed directly over the surface of the ensiled mass before sealing the buckets. The silage was stored and stirred every 24

hours to enhance the fermentation process and maintained for 14 days at room temperature under anaerobic conditions.

After fermentation, the silage was transferred to aluminum trays and placed in a forced-ventilation oven at 65°C (149°F) for 72 hours to reduce its moisture content. During drying, the biomass was periodically stirred to ensure uniform dehydration. Once dried, the tambaqui biological silage was subjected to proximate composition analysis, following the method-ologies described by Van Soest *et al.* (1991) and AOAC (2019). The results are presented in Table 22.

Table 22. Proximate composition of fresh residual mass and biological silage from tambaqui by-products.

Proximate composition	Fresh waste*	Biological silage*
Dry matter, %	44.61±2.87	87.69±0.17
Crude protein, %	22.67±1.81	44.98±0.59
Fats, %	7.38±0.35	17.89±0.47
Minerals, %	11.70±1.15	21.87±0.69
Crude fiber, %	1.62±0.28	2.44±1.24
Soluble carbohydrates, %	1.23±0.32	0.50±1.06

* All nutrient values were calculated on a dry matter basis. Mean ± standard deviation of three replicates.

24.2. Facilities, animals and experimental design

To evaluate the effects of tambaqui biological silage on productive performance and economic viability, two experiments were conducted. The first assessed the inclusion of tambaqui biological silage in diets for younger commercial hens (23 weeks old), while the second evaluated its effects in older commercial hens (83 weeks old) (Figure 10). A poultry house with a ceiling height of 3.25 meters was used, with structural adaptations aimed at improving bird welfare. Temperature and relative humidity were monitored using a small digital weather station, which recorded average values of 28.6 °C (83.48 °F) and 54.15%, respectively. Throughout the experimental period, the hens were monitored for potential signs of heat stress caused by environmental conditions; however, no such signs were observed during the entirety of either experiment.

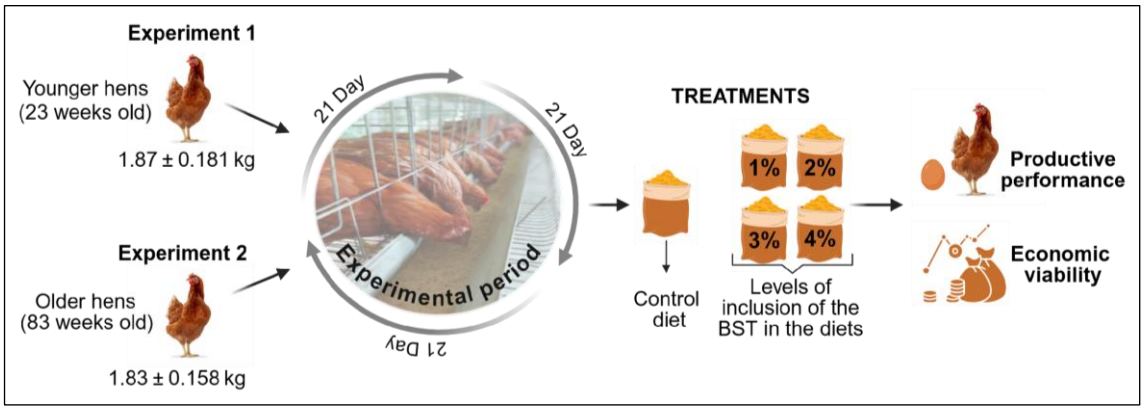

Figure 10. Experimental design showing the evaluation of tambaqui biological silage at four inclusion levels (1 – 4%) in diets for younger (23 weeks) and older (83 weeks) laying hens, with assessment of productive performance and economic viability over a 63-day period.

In both experiments, 120 commercial hens of the Hissex Brown strain were used. The experimental period lasted 63 days, divided into three 21-day phases, and was preceded by a seven-day adaptation period to the diets and housing. The birds were weighed at the beginning of the experimental period to standardize the experimental units, with an average weight of 1.87 ± 0.181 kg in Experiment 1 and 1.83 ± 0.158 kg in Experiment 2.

The hens were housed in galvanized wire cages (0.45 m high, 0.40 m wide, and 1.00 m long), each housing six birds, arranged in a single row. The cages were equipped with trough feeders and nipple drinkers. Birds received 16 hours of light per day (12 hours of natural light and 4 hours of artificial light) throughout the experimental period. Eggs were collected twice daily (at 9 a.m. and 3 p.m.), and daily records were kept for all relevant occurrences (mortality, egg production, among others).

Both Experiment 1 (younger hens) and Experiment 2 (older hens) were conducted using a completely randomized design. The treatments consisted of a control diet (without tambaqui biological silage) and four levels of tambaqui biological silage inclusion (1, 2, 3, and 4%), with four replicates per treatment and six birds per replicate. The experimental diets (Table 23) were formulated based on the reference values proposed by Rostagno *et al.* (2017; 2024), except for the tambaqui biological silage, which was based on prior compositional analyses. Metabolizable energy values followed the data reported by Guimarães *et al.* (2019). Tambaqui biological silage was treated as a fixed component in the diet formulation, and the levels of other feed ingredients were adjusted according to

the inclusion levels proposed in the experiments. The diets were formulated using the SuperCrac *software* (TD *Software*©, Viçosa, Brazil), and their proximate composition was analyzed to confirm nutrient values, following the methodologies described by Van Soest *et al.* (1991) and AOAC (2019).

Table 23. Composition of the experimental diets containing biological silage from tambaqui by-products.

Feedstuffs	Biological silage from tambaqui by-products levels, %				
	0.00	1.00	2.00	3.00	4.00
Corn (7.88%)[1]	65.80	65.30	64.42	64.29	63.79
Soybean meal (46%)[1]	21.41	20.76	20.11	19.46	18.81
Tambaqui biol. silage	0.00	1.00	2.00	3.00	4.00
Limestone	9.78	9.91	10.42	10.17	10.30
Dicalcium phosphate	1.93	1.94	1.95	1.95	1.96
Vit. min. supplement[2]	0.60	0.60	0.60	0.60	0.60
Salt	0.29	0.29	0.30	0.30	0.30
DL-methionine (99%)[3]	0.19	0.20	0.21	0.22	0.23
Total	100.00	100.00	100.00	100.00	100.00
Nutrient	Nutritional levels				
M.E., kcal.kg^{-1}	2,900.00	2,900.00	2,900.00	2,900.00	2,900.00
Crude protein, %	15.50	15.50	15.50	15.50	15.50
Calcium, %	4.32	4.37	4.42	4.47	4.52
Available phosphorus, %	0.45	0.45	0.45	0.45	0.45
Crude fiber, %	2.55	2.52	2.48	2.45	2.41
NDF, %	10.54	10.39	10.24	10.09	9.94
ADF, %	3.92	3.85	3.78	3.71	3.64
Methionine + Cystine, %	0.70	0.70	0.70	0.70	0.70
Methionine, %	0.43	0.44	0.45	0.45	0.46
Lysine, %	0.76	0.74	0.72	0.70	0.68
Threonine, %	0.60	0.58	0.57	0.56	0.54
Tryptophan, %	0.18	0.17	0.17	0.16	0.16
Sodium, %	0.15	0.15	0.15	0.15	0.15
Price, R$/kg	2.70	2.68	2.66	2.65	2.63

1 Values in parentheses indicate the protein content of these feedstuffs.

2 Guaranteed levels per kilogram of the product: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2 760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, Calcium Pantothenate 2,000 mg, Folic acid 50 mg, Coccidiostat 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg. Selenium 40 mg. Vehicle q.s.p. 1,000 g.

3 The value in parentheses indicates the purity of the amino acid.

24.3. Experimental analysis

In both experiments, the performance of the hens was assessed following the methodology outlined by Rufino *et al.* (2021). Every seven days, the following parameters were recorded: feed intake (g/bird/day), egg production (%), feed conversion ratio (kg of feed per kg of eggs produced – kg/kg), feed conversion ratio (kg of feed per dozen eggs produced – kg/dz), and egg mass (g) for each replicate.

For determining feed costs and production expenses, only the per-kilogram prices of the feedstuffs used and their updated prices in the region during the experiment were considered. The prices were as follows: corn, R$ 1.66; soybean meal, R$ 3.65; limestone, R$ 0.73; dicalcium phosphate, R$ 4.50; common salt, R$ 0.83; DL-Methionine 99%, R$ 51.00; and mineral and vitamin supplement, R$ 28.35/kg (average price). The cost of tambaqui biological silage was calculated considering only transportation and handling expenses (labor), with estimated price of R$ 1.50 per kilogram. Fixed costs included depreciation of facilities and equipment (maintenance, water, electricity, etc.), where interest on capital remained unchanged in the short term and was considered constant across all treatments. Variable costs included only bird feed expenses and labor.

The feed cost (FC, R$), the only production cost used as an analysis variable, was determined through the acquisition of ingredients and feed preparation, estimated by the formula:

$$FC=AFI*AP$$

Where:

AFI = accumulated feed intake of the plot (kg)

AP = average price per kilogram of feed (R$/kg)

For total egg production (PO, units), the total units of eggs produced by each pen was considered, as described by Brelaz *et al.* (2021). To calculate the production cost per egg (PC, R$/unit), the following formula was used:

$$PC=FC/PO$$

Where:

FC = feed cost (R$)

PO = total production of eggs (units)

The gross revenue (REV, R$) was calculated based on the relationship between egg production and the selling price per egg, using:

$$REV=Q*SP$$

Where:

Q = quantity of eggs produced by the plot (unit)

SP = selling price per egg produced (R$/unit)

It is important to note that the selling price per egg, applying a gross margin value-added calculation, was determined based on the market price in the region, fixed at R$ 0.75 per egg. Gross profit (PRO, R$) was calculated as the monetary difference between the total revenue from the estimated sale of the eggs and the discounted production cost, which derived from the feed cost, using the formula:

$$PRO=REV-FC$$

Where:

REV = gross revenue of the plot (R$)

FC = feed cost of the plot (R$)

The profitability index (PI, %), which indicates the capital available after covering costs (in this case, feed costs), was derived from the relationship between gross added value and gross revenue, using the formula:

$$PI=(PRO/REV)*100$$

Where:

PRO = gross profit of the plot (R$)

REV = gross revenue of the plot (R$)

For the break-even point (BE, kg), the quantity of production required to achieve zero return, covering all costs, was considered. In this case, it represents a partial break-even point, as it reflects the production volume necessary to cover feed costs. The formula used was:

$$BE=FC/SP$$

Where:

FC = feed cost of the plot (R$)

SP = selling price per egg produced (R$/kg)

Statistical analyses

The statistical model adopted was as follows:

$$Y\_ik=\mu+\alpha\_i+\epsilon\_ik$$

Where:

Yik = Observed value for the variable under study

μ = Overall experiment mean

αi = Effect of tambaqui biological silage levels

ϵik = Experimental error

In both experiments, all data were analyzed using one-way ANOVA with R *software* (2021). Commands were executed following Logan (2010) guidelines. Tukey's Honest Significant Difference (HSD) test was used to examine significant differences among the tambaqui biological silage levels (independent variable) for each dependent variable evaluated. Results are presented as means, and the significance level for differences was set at 0.05.

Subsequently, results for significant variables ($p<0.05$) were subjected to correlation and polynomial regression analysis to evaluate the influence of the independent variable on the dependent variables (CHATTERJEE; HADI, 2006; LOGAN, 2010). The mathematical model, either linear (Y = a + bx) or quadratic (Y = c + bx + ax2), was selected based on the influence of the independent variable on the dependent variable analyzed (DORMANN *et al.*, 2013). R-squared values were also considered as a criterion to determine the best model (CHATTERJEE; HADI, 2006; DORMANN *et al.*, 2013).

25. RESULTS

25.1. Experiment 1

The inclusion of tambaqui biological silage in the diets of young laying hens significantly influenced productive performance variables, as shown in Table 24. Feed intake exhibited a significant quadratic effect ($p<0.01$), increasing up to the 2% inclusion level, followed by a decline at higher levels. The fitted model ($Y = 119.36 + 4.377x – 2.055x^2$; $R^2 = 0.82$) indicates that moderate inclusion levels favored intake, possibly due to improved palatability resulting from compounds generated during silage fermentation.

Egg production showed a linear decline with increasing levels of silage inclusion ($p=0.03$), as described by the model ($Y = 91.362 – 6.012x$; $R^2 = 0.90$). This decrease may be associated with nutritional imbalances or the presence of secondary metabolites generated during fermentation, which may negatively affect the birds' reproductive physiology. Feed efficiency per kilogram of eggs was significantly affected ($p<0.01$), exhibiting a quadratic response ($Y = 2.6294 + 0.2301x – 0.0793x^2$; $R^2 = 0.95$). The results indicate that inclusion levels between 1% and 2% of silage optimized the conversion of feed into eggs.

Feed efficiency per dozen eggs was also significantly affected ($p=0.05$), showing a linear increase ($Y = 1.59 + 0.092x$; $R^2 = 0.85$), indicating that larger amounts of feed were required to sustain egg production at higher levels of silage inclusion. This finding aligns with the observed decline in egg production and may suggest reduced metabolic effi-ciency in response to excessive silage levels. Egg mass showed a linear decreasing trend ($p = 0.04$), as described by the fitted model ($Y = 40.37 – 2.626x$; $R^2 = 0.88$), indicating impaired nutrient deposition into the egg content as silage levels increased.

The economic analysis of including tambaqui biological silage in diets for young laying hens, based on the data presented in Table 25, revealed progressively adverse effects on the main financial viability indicators as inclusion levels increased. Feed cost exhibited a significant quadratic trend ($p<0.01$), increasing up to the 2% inclusion level and decreasing there-after. Egg production peaked at the 1% inclusion level, followed by a continuous decline. Consequently, gross revenue declined linearly ($p = 0.03$), following the trend of reduced egg production. Gross profit and the profitability index declined linearly ($p = 0.05$), reflecting the combined effect of higher costs and reduced economic returns. The break-even point, in turn, increased significantly with higher silage inclusion levels, indicating greater production requirements to achieve economic sustainability.

Table 24. Performance of of younger commercial hens fed diets containing increase levels of biological silage from tambaqui by-products[1].

Variables[2]	Biological silage from tambaqui by-products levels, %					p-value[3]	CV[4] %	MM[5]	R^2
	0.00	1.00	2.00	3.00	4.00				
FI	118.69±12.25[a]	121.79±16.13[a]	123.61±10.12[a]	108.94±11.25[b]	105.90±12.12[b]	<0.01	7.51	$Y = 119.36 + 4.377x - 2.055x^2$	0.82
EP	88.24±5.32[a]	90.02±9.97[a]	80.05±7.74[b]	70.38±6.64[c]	68.00±12.19[c]	0.03	6.71	$Y = 91.362 - 6.012x$	0.90
FEKG	2.61±0.63[b]	2.81±0.52[a]	2.80±0.45[a]	2.54±0.41[b]	2.31±0.25[c]	<0.01	8.76	$Y = 2.6294 + 0.2301x - 0.0793x^2$	0.95
FEDZ	1.60±0.32[c]	1.61±0.41[c]	1.84±0.52[b]	1.91±0.43[a]	1.91±0.22[a]	0.05	5.77	$Y = 1.59 + 0.092x$	0.85
EM	39.98±4.12[a]	38.94±8.12[a]	35.30±7.33[ab]	30.10±10.12[b]	31.27±11.11[b]	0.04	7.22	$Y = 40.37 - 2.626x$	0.88

[1] All data represent the average of 24 replicates (hens) per treatment.

[2] FI = Feed intake (g/bird/day). EP = Egg production (%). FEKG = Feed efficiency, kg/kg (Quantity of feed (kg) to produce one kilogram (kg) of egg). FEDZ = Feed efficiency, kg/dz (Quantity of feed (kg) to produce one dozen (dz) of eggs). EM = Egg mass (g).

[3] The means followed by lowercase letters in the lines differ using the Tukey test ($p<0.05$). not significant = $p>0.05$.

[4] CV = Coefficient of variation.

[5] MM = Mathematical model adjusted according to the influence of the independent variable on the dependent variable.

Table 25. Economic analysis of the performance of younger commercial hens fed diets containing increase levels of biological silage from tambaqui by-products[1].

Var.[2]	Biological silage from tambaqui by-products levels, %					*p*-value[3]	CV[4], %	MM[5]	R^2
	0.00	1.00	2.00	3.00	4.00				
FC	161.57±8.26[a]	164.57±5.72[a]	166.27±7.47[a]	145.67±5.27[b]	140.80±8.94[b]	<0.01	8.06	$Y = 162.43 + 4.8246x – 2.7171x^2$	0.85
PO	444.75±13.72[a]	453.75±23.79[a]	403.50±30.34[b]	354.75±76.65[c]	342.75±84.26[c]	0.03	6.71	$Y = 454.71 + 18.729x – 2.8929x^2$	0.90
PC	0.36±0.01[b]	0.36±0.02[b]	0.41±0.02[a]	0.42±0.10[a]	0.42±0.07[a]	0.05	5.31	$Y = 0.358 = 0.018x$	0.82
RE	333.56±10.29[a]	340.31±17.84[a]	302.62±22.76[b]	266.06±57.49[c]	257.06±63.19[c]	0.03	6.71	$Y = 345.37 – 22.725x$	0.89
PR	171.98±10.55[a]	175.49±22.58[a]	136.34±17.35[b]	120.38±53.63[bc]	116.26±55.34[c]	0.05	9.02	$Y = 177.4 – 16.655x$	0.87
PI	51.54±2.41[a]	51.42±3.81[a]	44.92±2.74[b]	43.02±13.75[b]	43.34±10.21[b]	0.05	7.37	$Y = 51.808 – 2.48x$	0.84
BE	215.43±11.01[a]	219.75±7.63[a]	221.70±9.96[a]	194.23±7.03[b]	187.73±11.92[b]	<0.01	8.06	$Y = 216.66 + 6.4966x – 3.6471x^2$	0.85

[1] All data represent the average of 24 replicates (hens) per treatment.

[2] FC = Feed Cost (R$). PO = Total Egg Production (units). PC = Production Cost per egg (R$/unit). RE = Gross Revenue (R$). PR = Gross Profit (R$). PI = Profitability Index (%). BE = Break-even point (units).

[3] The means followed by lowercase letters in the lines differ using the Tukey test ($p<0.05$). not significant = $p>0.05$.

[4] CV = Coefficient of variation.

[5] MM = Mathematical model adjusted according to the influence of the independent variable on the dependent variable.

25.2. Experiment 2

Based on the data presented in Table 26, which evaluates the productive performance of older commercial laying hens fed diets containing different levels of tambaqui biological silage, a dose-dependent adverse effect was observed on the main productive indicators. Feed intake initially increased with the inclusion of 1% silage, reaching 111.48 ± 11.25 g/bird/day, compared to the control group, which consumed 97.63 ± 10.13 g/bird/day. However, from the 2% inclusion level onward, a progressive and significant reduction in intake was observed, with the lowest value recorded at 4% inclusion (57.29 ± 10.49 g/bird/day; $p < 0.01$). Statistical analysis revealed a negative linear trend ($R^2 = 0.38$), suggesting that high silage concentrations may compromise diet palatability or alter energy density, directly affecting voluntary intake.

Regarding egg production, the results showed stability across treatments with 0, 1, and 2% silage, with production rates ranging from 66.50% to 67.25%. However, significant reductions were observed at 3% and 4% inclusion levels, with values of 40.75 ± 4.96% and 36.25 ± 5.12%, respectively ($p = 0.04$). This indicates that silage inclusion up to 2% does not significantly affect reproductive performance, whereas higher levels impair laying capacity. The adjusted linear model ($Y = 57.9 – 0.975x$; $R^2 = 0.24$) supports this negative trend.

Feed efficiency, assessed both per kilogram of eggs produced and per dozen eggs, improved at initial inclusion levels (1% and 2%) but declined significantly in the 3% and 4% treatments. The lowest feed efficiency per kilogram of eggs produced (2.08 and 2.17 kg/kg) and per dozen eggs (1.36 and 1.59 kg/dz) values were observed in the control and 1% groups, while the highest values were recorded at 4% inclusion (3.95 and 2.95, respectively), with statistical significance of $p = 0.03$ and $p = 0.05$. These increases indicate that more feed was required to maintain equivalent egg production, reflecting reduced feed efficiency.

Egg mass followed a similar pattern to egg production, remaining stable up to 2% inclusion and decreasing significantly from 3% onward. The control group showed an average egg mass of 35.57 ± 9.12 g, while the lowest value was observed at 4% silage inclusion (17.83 ± 7.66 g; $p = 0.02$). The fitted linear model ($Y = 30.0825 – 0.0236312x$; $R^2 = 0.17$) reinforces the linear decline in egg mass with increasing silage levels.

In the final phase of the production cycle, the inclusion of tambaqui biological silage, according to the data presented in Table 27, demonstrated a positive effect on economic indicators at moderate inclusion levels, highlighting its potential as a viable

alternative ingredient for older laying hens. Feed cost increased up to the 3% inclusion level, followed by a reduction at 4%. This variation may reflect both formulation costs and adjustments in birds' feed in-take.

Egg production increased significantly from 1% to 3% inclusion, with a peak observed at the 3% level. This result indicates that the silage contributed positively to the productive performance of older birds. The unit production cost per egg was significantly reduced at the 1% and 2% in-clusion levels, indicating greater economic efficiency.

Gross revenue increased from 1% to 3% inclusion levels, consistent with the rise in productivity. Gross profit and the profitability index (PI) improved significantly at 1% and 2% inclusion levels. Finally, the break-even point was significantly reduced at the 1% inclusion level, indicating a lower production requirement to cover variable costs, thus favoring the financial viability of the system.

Table 26. Performance of older commercial hens fed diets containing increase levels of biological silage from tambaqui by-products[1].

Variables[2]	Biological silage from tambaqui by-products levels, %					p-value[3]	CV[4] %	MM[5]	R^2
	0.00	1.00	2.00	3.00	4.00				
FI	97.63±10.13ab	111.48±11.25a	89.05±13.15b	71.28±10.28b	57.29±10.49c	<0.01	2.30	Y = 93.4459 - 4.0473x	0.38
EP	67.25±3.58a	69.00±6.28a	66.50±6.12a	40.75±4.96b	36.25±5.12b	0.04	3.87	Y = 57.9 - 0.975x	0.24
FEKG	2.08±0.45c	2.17±0.43c	2.43±0.33c	3.36±0.66b	3.95±0.52a	0.03	5.35	Y = 3.73 - 0.164984x	0.17
FEDZ	1.36±0.44c	1.59±0.52c	1.99±0.33b	2.70±0.42a	2.95±0.36a	0.05	5.25	Y = 2.24 - 0.0587018x	0.15
EM	35.57±9.12a	37.49±8.33a	39.40±8.42a	29.86±7.77b	17.83±7.66c	0.02	4.41	Y = 30.0825 - 0.0236312x	0.17

1 All data represent the average of 24 replicates (hens) per treatment.

2 FI = Feed intake (g/bird/day). EP = Egg production (%). FEKG = Feed efficiency, kg/kg (Quantity of feed (kg) to produce one kilogram (kg) of egg). FEDZ = Feed efficiency, kg/dz (Quantity of feed (kg) to produce one dozen (dz) of eggs). EM = Egg mass (g).

3 The means followed by lowercase letters in the lines differ using the Tukey test ($p<0.05$). not significant = $p>0.05$.

4 CV = Coefficient of variation.

5 MM = Mathematical model adjusted according to the influence of the independent variable on the dependent variable.

Table 27. Economic analysis of the performance of older commercial hens fed diets containing increase levels of biological silage from tambaqui by-products[1].

Var.[2]	Biological silage from tambaqui by-products levels, %					*p*-value[3]	CV[4], %	MM[5]	R^2
	0.00	1.00	2.00	3.00	4.00				
FC	96.46±12.46^{c}	119.79±15.27^{b}	132.91±22.52^{ab}	149.07±33.45^{a}	76.18±47.52^{c}	<0.01	13.91	$Y = 90.081 + 52.986x - 13.529x^2$	0.76
PO	217.98±11.17^{b}	338.94±98.88^{a}	347.76±70.91^{a}	360.36±103.49^{a}	182.70±41.63^{c}	<0.01	14.68	$Y = 214.6 + 164.65x - 42.39x^2$	0.92
PC	0.61±0.03^{a}	0.30±0.09^{c}	0.35±0.07^{c}	0.44±0.16^{b}	0.43±0.12^{b}	0.01	13.33	$Y = 0.5614 - 0.2049x + 0.0457x^2$	0.71
RE	163.48±8.38^{b}	254.20±74.16^{a}	260.82±53.18^{a}	270.27±77.62^{a}	137.02±31.22^{b}	<0.01	14.68	$Y = 160.94 + 123.49x - 31.794x^2$	0.92
PR	30.57±8.38^{c}	157.74±64.15^{a}	141.02±45.19^{a}	121.20±57.25^{b}	60.84±11.29^{c}	0.02	8.54	$Y = 43.451 + 110.45x - 27.011x^2$	0.87
PI	18.53±4.37^{c}	59.21±13.11^{a}	52.61±9.55^{a}	40.19±21.86^{b}	41.49±17.04^{b}	0.01	5.26	$Y = 24.943 + 26.856x - 6.0414x^2$	0.70
BE	177.21±21.15^{b}	128.61±17.52^{c}	159.72±22.35^{b}	198.75±15.15^{a}	101.57±13.66^{c}	<0.01	13.13	$Y = 156.65 + 17.383x - 6.3743x^2$	0.71

[1] All data represent the average of 24 replicates (hens) per treatment.

[2] FC = Feed Cost (R$). PO = Total Egg Production (units). PC = Production Cost per egg (R$/unit). RE = Gross Revenue (R$). PR = Gross Profit (R$). PI = Profitability Index (%). BE = Break-even point (units).

[3] The means followed by lowercase letters in the lines differ using the Tukey test ($p<0.05$). not significant = $p>0.05$.

[4] CV = Coefficient of variation.

[5] MM = Mathematical model adjusted according to the influence of the independent variable on the dependent variable.

26. DISCUSSION

The reduced intake at higher inclusion levels suggests lower feed acceptance by the younger commercial hens, likely due to the accumulation of undesirable organoleptic compounds. Highly hydrolyzed silages may impair animal performance because of the excess of free amino acids and short-chain peptides, which at high concentrations can cause metabolic competition, reducing protein synthesis and growth (GOOSEN *et al.*, 2016). Moreover, intensified protein degradation during silage processing favors the formation of volatile compounds that negatively affect feed acceptability and palatability (BOITAI *et al.*, 2017).

Similar results were reported by Batalha *et al.* (2017), who observed reduced crude protein digestibility in lightweight laying hens fed acid silage from *Arapaima gigas* (pirarucu) at inclusion levels of 3% or higher. These findings suggest that even moderate inclusion levels of silage may impair the utilization of essential nutrients, directly impacting egg production.

This positive response may be attributed to greater nutrient bioavailability resulting from the fermentation process used in silage preparation. According to Goosen *et al.* (2014), fish silage is highly digestible and has a favorable nutritional profile, making it a promising ingredient in animal nutrition. During fermentation, proteins are hydrolyzed into simpler compounds such as free amino acids and short-chain peptides, which are more readily absorbed in the digestive tract (OZYURT *et al.*, 2017). However, the decline in feed efficiency at higher inclusion levels reinforces the existence of a safe threshold, above which adverse metabolic effects and impaired productive performance may occur.

The reduction in egg mass may be associated with lower intake of essential nutrients such as sulfur-containing amino acids and calcium, or with the presence of metabolites produced during fermentation. Although the hydrolysis process releases functional peptides with bioactive properties and potential benefits for animal health (DADKHODAZADEH *et al.*, 2024), the excessive accumulation of these compounds may lead to adverse metabolic effects (GOOSEN *et al.*, 2014). In this scenario, the birds' metabolic energy may be redirected toward maintenance and detoxification processes, compromising nutrient deposition in the eggs. Therefore, the reduction in egg mass constitutes a direct indicator of reproductive physiological dysfunction.

This may be related to adjustments in feed intake in response to palatability or dietary energy density. A similar result was reported by Rufino *et al.* (2015), who

observed a reduction in feed cost after 5% inclusion of tucumã residue meal. Likewise, Melo *et al.* (2017) found that including 5% of yam (Dioscorea spp.) resulted in the lowest feed cost, representing the optimal level. Batalha *et al.* (2019), using acid silage from pirarucu (Arapaima gigas) residues, also reported a significant reduction in feed cost with 3% inclusion.

This response may be linked to reduced nutrient availability or the presence of antinutritional compounds that impair birds' physiological performance at higher levels. Similar findings were reported by Melo *et al.* (2017), who observed a sharp drop in production after 10% inclusion of yam, and by Batalha *et al.* (2019), who reported maximum productive performance at 2.63% inclusion of acid silage meal.

This relationship was also observed by Rufino *et al.* (2017), who found higher revenue in treatments with up to 5% inclusion of buriti flour, followed by a decline. Similarly, Batalha *et al.* (2019) reported the highest revenue (R$ 185.17) at 2.63% inclusion of acid silage. The production cost per unit increased significantly in the 3% and 4% inclusion treatments (R$ 0.42/unit), indicating reduced productive efficiency. This pattern was also noted by Melo *et al.* (2017), who reported economic unfeasibility beyond 15% inclusion of yam, and by Batalha *et al.* (2019), who found the lowest cost per unit with 3.11% inclusion of acid silage from pirarucu.

According to Rufino *et al.* (2017), inclusion levels above 10% of buriti flour led to a significant drop in profitability, supporting the findings of Batalha *et al.* (2019), who reported maximum profitability (58.56%) with 3.1% inclusion of acid silage. A similar situation was described by Rufino *et al.* (2015) for tucumã meal, and by Batalha *et al.* (2019), who estimated the optimal break-even point at 1.17% inclusion. Therefore, the use of tambaqui biological silage in diets for young laying hens should be approached with caution, as inclusion levels above 1% result in economic, productive, and operational losses, com-promising the profitability of poultry systems during the early laying phase.

These findings suggest that older commercial hens are more sensitive to organoleptic and energetic changes introduced by silage, which negatively affect their feed intake at higher inclusion levels. In addition to the effects of diet composition and palatability, it is important to consider that older birds experience physiological changes that reduce their ability to respond to alternative ingredients. In aging hens, there is a progressive decline in digestive efficiency, including reduced enzymatic secretion, decreased intestinal motility, and lower absorptive capacity of the intestinal epithelium

(SAKOMURA; ROSTAGNO, 2016). These factors may explain the more pronounced performance decline in older birds fed diets with high silage inclusion.

The increased feed efficiency values at higher inclusion levels indicate a greater feed requirement per unit of product, reflecting a lower utilization of ingested nutrients. This compromised feed conversion is directly linked to the observed decline in both egg production and egg mass starting from the 3% inclusion level. These results can be partially explained by the physiological limitations inherent to older laying hens (ROSTAGNO *et al.*, 2024). In aging birds, physiological processes increasingly prioritize body maintenance over egg production, particularly when nutrient availability or utilization is limited.

Batalha *et al.* (2019), using acid silage from pirarucu, observed a similar pattern, with the lowest feed cost estimated at 3% inclusion. Similarly, Rufino *et al.* (2015), evaluating tucumã meal, reported a reduction in feed cost starting from the optimal inclusion point of 5%. A similar trend was reported by Melo *et al.* (2017) with 5% yam inclusion, and by Batalha *et al.* (2019), who observed an estimated production of 462.93 eggs with 2.63% inclusion of acid silage from pirarucu.

This result aligns with the findings of Rufino *et al.* (2015) and Batalha *et al.* (2019), who identified the lowest production cost per unit at 3.11% inclusion of acid silage. Studies by Rufino *et al.* (2017) and Batalha *et al.* (2019) also reported increased revenue at moderate inclusion levels (up to 5%), reinforcing the economic potential of protein-rich fish by-products in poultry feeding during the late laying phase.

The highest profit and PI values observed in the present study are consistent with findings by Melo *et al.* (2017), who reported greater economic return with 5% yam inclusion, and by Batalha *et al.* (2019), who obtained 58.56% profitability with 3.1% inclusion of acid silage from pirarucu. Batalha *et al.* (2019) estimated the optimal break-even point at 1.17% inclusion, rein-forcing the importance of respecting physiological limits to maximize profitability. Therefore, the inclusion of tambaqui biological silage in the diets of older laying hens proved to be economically viable and productively promising at inclusion levels of 1 to 2%, representing an efficient and sustainable alternative for the valorization of fishery residues in late-phase poultry farming.

27. CONCLUSION

The inclusion of tambaqui biological silage in diets for commercial laying hens had distinct impacts on productive indicators and, more notably, on the economic parameters evaluated across the two production cycles. In young hens, inclusion levels of up to 1% resulted in pro-ductive performance and economic return comparable to the control treatment, with reduced production costs, satisfactory gross profit, and a competitive profitability index. However, increasing silage levels beyond this threshold significantly impaired egg production, raised the cost per unit produced, and reduced net profit and profitability, resulting in economic infeasibility from the 2% inclusion level onward.

In contrast, older laying hens demonstrated greater physiological and productive tolerance to silage inclusion. Levels between 1 and 2% were the most efficient in terms of economic return, notably reducing the unit cost per egg, increasing gross revenue, and maximizing profit and profitability. The economic results in this group indicated more efficient utilization of silage as an alternative protein ingredient, particularly when con-sidering break-even point and profitability index, which reached values higher than those observed in other treatments. Nevertheless, as in the first cycle, inclusion levels above 2% had detrimental effects, reflected in reduced production, increased feed cost per unit, and decreased profit margins.

Therefore, tambaqui biological silage proved economically viable at inclusion levels of up to 1% for young hens and up to 2% for older hens, representing a promising alternative for diet formula-tion in production systems that aim to reduce costs, valorize regional residues, and enhance economic sustainability in Amazonian poultry farming. The judicious use of this ingredient, respecting the physiological and productive limits of each phase of the birds' cycle, is essential to ensure the technical efficiency and financial viability of the pro-duction system.

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29. FINAL CONSIDERATIONS

The central objective of this thesis was to investigate the feasibility of using biological silage from tambaqui by-products in the diet of commercial laying hens, guided by the articulation of productive, physiological, qualitative, economic, and metabolomic analyses. The chapters that compose the work addressed, in a complementary manner, essential dimensions for understanding the potential of this alternative ingredient, consolidating an integrated view of its practical applicability and scientific relevance. Overall, it was observed that tambaqui biological silage represents a sustainable alternative for reducing conventional ingredients such as corn and soybean meal, without compromising the productive performance of the birds. The results obtained in hens of different ages demonstrated maintenance or even improvement of productive parameters, indicating good efficiency in feed conversion and physiological adaptation to diets with tambaqui biological silage inclusion. These findings were corroborated by hematological and biochemical analyses, which showed stability in health indicators and the absence of relevant adverse effects, indicating metabolic compatibility in the birds.

Regarding the quality of the final product, the egg, it was found that the inclusion of tambaqui biological silage did not impair physical, chemical, or sensory characteristics. On the contrary, under certain conditions, the modulation of the yolk lipid fraction was observed, a feature of great nutritional and functional importance. The use of low-field ^{1}H NMR, combined with multivariate statistical analysis, proved to be an innovative and effective tool for detecting metabolic changes in the yolk as a function of diet, expanding the understanding of the biochemical mechanisms underlying the nutritional response of hens. This approach gives the study a distinctive methodological character, reinforcing the integration between animal science and analytical biotechnologies.

From a scientific and technological perspective, the results contribute to consolidating the concept of the circular bioeconomy, by demonstrating that fish processing residues can be transformed into value-added inputs for poultry production. In addition, the research advances in three main dimensions: scientific, by filling gaps regarding the effects of fish silage on commercial laying hens, especially at different production stages; methodological, by integrating productive, physiological, economic, and metabolomic analyses; and technological and practical, by demonstrating the feasibility of including tambaqui biological silage as a strategy to reduce costs, add value to regional production chains, and mitigate environmental impacts from the improper disposal of fish residues.

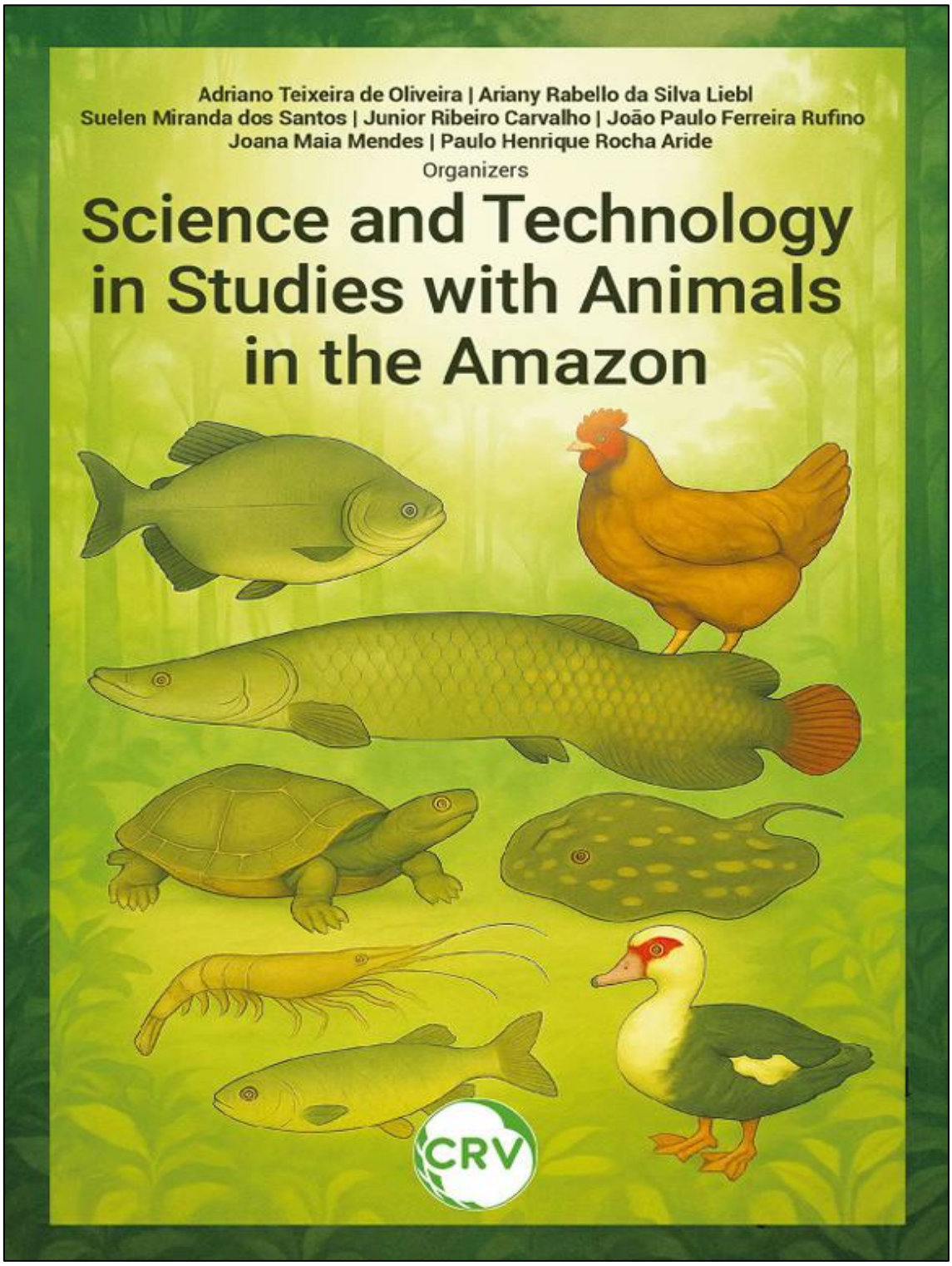
Despite the advances achieved, global limitations of the study are recognized. The main one refers to the experimental scale, conducted under controlled conditions, which may not fully reflect the dynamics observed in large-scale commercial systems. Furthermore, seasonal and technological variation in the composition of residues used in silage preparation may influence the nutritional quality of the product (egg), requiring standardization and continuous monitoring for large-scale adoption. The absence of long-term evaluations also constitutes a limitation, as it was not possible to estimate the cumulative effects of tambaqui biological silage inclusion on the health and productive longevity of hens.

Therefore, it is recommended that future research advance in three main directions: application at a commercial scale, with cost-benefit analyses in different regional and productive contexts; in-depth evaluations of the nutritional and functional quality of eggs, especially regarding lipid composition and its potential effects on human health; and complementary omics approaches, such as lipidomics and proteomics, which could expand the metabolomic findings and more comprehensively elucidate the physiological mechanisms modulated by diet.

In summary, this study confirms the technical, economic, and environmental feasibility of using tambaqui biological silage in the nutrition of commercial laying hens, establishing itself as a milestone of innovation in the sustainable utilization of Amazonian resources. By integrating experimental results, advanced analyses, and critical reflections, the study demonstrates scientific maturity and contributes to strengthening more resilient, sustainable production systems aligned with contemporary demands for food security, cost reduction, and the valorization of regional resources.

30. APPENDICES

Published Book Chapter



USING FISH BY-PRODUCTS AS SILAGE FOR ALTERNATIVE FOOD IN POULTRY DIETS

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João Paulo Ferreira Rufino[2]
Pedro de Queiroz Costa Neto[3]
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Ariany Rabello da Silva Liebl[13]
Adriano Teixeira de Oliveira[14]

Introduction

The search for alternative feeds in animal production aims to optimize diet formulation, reducing dependence on traditionally used ingredients and mitigating the financial impacts of commodity price fluctuations, particularly for protein and energy inputs, which represent the most costly components of animal feed (Cruz; Rufino, 2017; Silva et al., 2023). In this context, the integration of production chains has emerged as a

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Animal Use Ethics Committee Protocol Approval

Poder Executivo
Ministério da Educação
Universidade Federal do Amazonas
Comissão de Ética no Uso de Animais

UFAM

CERTIFICADO

Certificamos que a proposta intitulada **"Parâmetros fisiológicos e produtivos de poedeiras comerciais leves, com inclusão da silagem biológica de resíduos de tambaqui na alimentação"** sob a responsabilidade da pesquisadora **Cristiane Cunha Guimarães** (doutoranda PPG EM CIÊNCIA ANIMAL E RECURSOS PESQUEIROS – PPG-CARP/UFAM), orientada pelos professores Dr. Adriano Teixeira de Oliveira (docente/IFAM) e Dr. João Paulo Ferreira Rufino (docente/FCA/UFAM) – que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – e por encontrar-se de acordo com os preceitos da Lei n. 11.794, de 8 de outubro de 2008, do Decreto n. 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), após análise pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA UNIVERSIDADE FEDERAL DO AMAZONAS, foi aprovada *ad hoc* sob o N. 005/2022.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	De 05/2022 a 07/2025
Espécie/linhagem/raça	*Isa brown* (Galinha poedeira)
N. de animais	180
Peso/Idade	1800 g / 40 semanas
Sexo	Fêmeas
Origem e detalhamento de manutenção	Setor de Avicultura, Faculdade de Ciências Agrárias da Universidade Federal do Amazonas situada no Setor Sul do Campus Universitário, Manaus, Amazonas, Brasil. Aviário com cobertura de fibrocimento medindo 17,0m de comprimento, 3,5m de largura e 3,20m de pé-direito, com uma fileira de gaiolas de arame de cada lado; e alojadas em 30 gaiolas com dimensões de 1,0 m x 0,45 m x 0,45 m com divisores internos possuindo em comprimento 0,50m; bebedouros tipo nipple e ração em comedouros lineares de alumínio em regime *ad libitum*. Durante o período experimental, as aves receberão 16 horas de luz (12 horas natural + 4 horas artificial).

Manaus, 11 de maio de 2022.

Profa. Dra. Cinthya Iamile Frithz Brandão de Oliveira
Coordenadora da CEUA-UFAM

Published Scientific Article

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REGULAR ARTICLES

Biological silage from tambaqui (*Colossoma macropomum*) by-products on the productive performance, hematological parameters and egg quality of older commercial hens

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Received: 7 May 2024 / Accepted: 20 December 2024

Abstract
This study evaluated the effects of incorporating biological silage from tambaqui (*Colossoma macropomum*) by-products (BST) on the performance, hematological and plasma biochemical parameters, and egg quality (physical, proximate composition and sensory characteristics) of older commercial hens. The BST was prepared by ensiling tambaqui by-products with lactic acid bacteria, cassava trimmings, and preservatives, producing a nutrient-rich, high-protein feed ingredient. One hundred and twenty Hisex Brown hens (83 weeks old) were divided into five treatment groups, each receiving diets with 0, 1, 2, 3, or 4% BST. Performance metrics, egg quality parameters, and blood samples were collected over 63 days. The results showed that diets with 1 to 2% BST improved egg weight, eggshell quality, and feed efficiency without adverse effects on hen health. However, higher BST inclusion levels led to declines in feed intake, egg production, and mineral content in eggs, possibly due to the sensory properties of the tambaqui-based diets. Hematological analysis indicated an increase in MCV and MCH values with higher BST levels, while triglycerides increased and cholesterol decreased, suggesting potential benefits for lipid metabolism at moderate inclusion levels. Sensory analysis of eggs showed greater acceptance in diets with 3% to 4% BST due to yolk color enhancement, although higher levels were not optimal for hen productivity. These findings support BST as a viable alternative feed in poultry diets, promoting waste utilization in a sustainable, circular economy model for the agribusiness sector.

Keywords Alternative food · Fish by-product · Freshwater fish · Poultry science · Sensory quality

Introduction

Fish farming has become a rapidly growing global animal production activity (FAO 2022). In Brazil, a major player in animal production, there's been significant growth in fish farming, particularly focusing on native species, which now constitute over 30% of the country's production (Cyrino et al. 2010; Alhazzaa et al. 2019; FAO 2022). Among these, *Colossoma macropomum*, or tambaqui, is the most cultivated, predominantly in the Brazilian Amazon and Northeast regions (Cyrino et al. 2010; Boscolo et al. 2011). Tambaqui is favored for its high productivity, adaptability, and appealing taste to consumers (Ferreira et al. 2021; Souza Cornélio 2022). However, this increased tambaqui production also leads to significant waste generation, often with inadequate disposal methods (Rossetto and Signor 2021).

Studies have explored the reuse of fish processing by-products in animal nutrition, particularly in poultry diets (Ramírez et al. 2013; Batalha et al. 2017; Cândido et al. 2017; Guimarães et al. 2019), offering an alternative to reduce costs, utilize nutritional properties efficiently (Nascimento et al. 2014; Guimarães et al. 2021; Mevliyaoğulları et al. 2021, 2023; Silva et al. 2023), and

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Published online: 08 January 2025

Sandwich Phd Program In Brazil

UFRJ institutodequímica Universidade Federal do Rio de Janeiro NAL

DECLARAÇÃO – DOUTORADO SANDUÍCHE

Rio de Janeiro, 09 de setembro de 2025

Declaro para os devidos fins que, **Cristiane Cunha Guimarães**, CPF:
permaneceu no Núcleo de Análise de Alimentos (NAL) do Instituto de Química da Universidade Federal do Rio de Janeiro (UFRJ) no período de 16 de outubro de 2024 a 15 de abril de 2025 (6 meses), realizando experimentos e escrita de artigo científico em seu doutorado sanduíche, sob orientação do Professor Adriano Teixeira de Oliveira e Coordenação do Prof. Dr. Carlos Adam Conte Junior.

Prof. Dr. Carlos A. Conte Junior
Coordenador
Núcleo de Análise de Alimentos
NAL/LADETEC - IQ/UFRJ
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SALITAS

CERTIFICADO

A **Equipe SALITAS** com supervisão do Médico Veterinário Gustavo Nogueira (CRMV-PI 01764 VP) certifica que o trabalho intitulado **PARÂMETROS FISIOLÓGICOS DE POEDEIRAS ALIMENTADAS COM SILAGEM BIOLÓGICA DE SUBPRODUTOS DE TAMBAQUI NA DIETA** de autoria de **Cristiane Cunha Guimarães, Adriene Núzia De Almeida Santos, Maria Fernanda da Silva Gomes, Maiko Willas Soares Ribeiro, Pedro Henrique Santos Pedreno Beltrão, RAYANA MELO PAIXÃO, Alexandre Augusto Barai, Tiago Cabral Nóbrega, Ana Clara Nogueira Alves e Adriano Teixeira de Oliveira**, foi submetido e aprovado no evento **CONGRESSO INTERNACIONAL DE ZOOLOGIA**, realizado em **26/02/2024 a 27/02/2024**, contabilizando carga horária total de **10** horas.

26/02/2024 a 27/02/2024

Leonardo da Vinci Bragia Conceição
LEONARDO DA VINCI B. CONCEIÇÃO
COMISSÃO ORGANIZADORA

Gustavo Nogueira Silva
Médico Veterinário
CRMV PI 01764 VP
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Certificamos que o trabalho intitulado "***SILAGEM BIOLÓGICA DE RESÍDUOS DE PESCADO AMAZÔNICO: UMA ALTERNATIVA SUSTENTÁVEL PARA ALIMENTAÇÃO ANIMAL***" foi apresentado na forma de **POSTER** no CONCARP "***Congresso Brasileiro em Ciência Animal e Recursos Pesqueiros***", promovido pelo Programa de Pós-Graduação em Ciência Animal e Recursos Pesqueiros - PPGCARP, na Universidade Federal do Amazonas, realizado nos dias 9 a 12 de junho de 2025.

Autores: Larissa Dolzane Fonseca, Cristiane Cunha Guimarães, João Paulo Ferreira Rufino, Tiago Cabral Nóbrega, Ana Clara Nogueira Alves, Adriene Núzia de Almeida Santos, Alexandre Augusto Barai, Maria Fernanda da Silva Gomes, Shirlen Teixeira Soares, Isadora Catunda Almeida, Wilson de Souza Ferreira, Joana Maia Mendes, Ariany Rabello da Silva Liebl, Antônio José Inhamuns, Adriano Teixeira de Oliveira.

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Presidente da Comissão Organizadora

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Presidente da Comissão Científica

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Certificamos que o trabalho intitulado "***SILAGEM BIOLÓGICA DE RESÍDUOS DE TAMBAQUI NA DIETA DE POEDEIRAS EM SEGUNDO CICLO: EFEITOS SOBRE O DESEMPENHO ZOOTÉCNICO***" foi apresentado na forma de **POSTER** no CONCARP "***Congresso Brasileiro em Ciência Animal e Recursos Pesqueiros***", promovido pelo Programa de Pós-Graduação em Ciência Animal e Recursos Pesqueiros - PPGCARP, na Universidade Federal do Amazonas, realizado nos dias 9 a 12 de junho de 2025.

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CERTIFICADO

Certificamos que o trabalho intitulado "***INFLUÊNCIA DA INCLUSÃO DE SILAGEM BIOLÓGICA DE TAMBAQUI (Colossoma macropomum) NA QUALIDADE MICROBIOLÓGICA DE RAÇÕES***" foi apresentado na forma de **POSTER** no CONCARP "***Congresso Brasileiro em Ciência Animal e Recursos Pesqueiros***", promovido pelo Programa de Pós-Graduação em Ciência Animal e Recursos Pesqueiros - PPGCARP, na Universidade Federal do Amazonas, realizado nos dias 9 a 12 de junho de 2025.

Autores: Isadora Catunda Almeida, Cristiane Cunha Guimarães, Adriene Núzia de Almeida Santos, Ana Clara Nogueira Alves, Marcelo Lima de Barros, Shirlen Teixeira Soares, Geovana Frazão Leite, Joana Maia Mendes, Suelen Miranda dos Santos, Adriano Teixeira de Oliveira.

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