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Acid-preserved broiler carcass silage in the diets of Nile tilapia (*Oreochromis niloticus*)

Silagem ácida de carcaça de frango em dietas para Tilápia-do-Nilo (*Oreochromis niloticus*)

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Highlights

Acid-preserved broiler carcass silage is a low-cost technology.
The addition of 2% phosphoric acid and 3% acetic acid inhibits *Salmonella*.
The inclusion of up to 30% acidic silage did not harm the performance of Tilapia.

Abstract

This study aimed to evaluate the efficiency of acid fermentation in eliminating *Salmonella* and other pathogenic microorganisms from broiler carcass silage and assess its use in the diet of Nile tilapia (*Oreochromis niloticus*). The silage was produced using whole, uneviscerated broiler carcasses with feathers, and by addition of 2% phosphoric acid, 3% acetic acid, 0.1% butylated hydroxytoluene (BHT; antioxidant) and 0.1% sorbic acid (antifungal). In Experiment I, the silages were placed in mini-silos according to the following treatments (T): T0, control (without inoculum); T1, with 10^2 cfu g^{-1} *Salmonella*; T2, with 10^3 cfu g^{-1} *Salmonella*; and T3, with 10^4 cfu g^{-1} *Salmonella*, and turned once daily. The silage was monitored for 45 days with daily pH and temperature (°C) measurements. Chemical analysis and microorganism evaluations (*Salmonella*, coliforms at 30 and 45°C) were carried out. In Experiment II, the ensiled mass was turned once daily and stored for 30 d, with daily temperature and pH monitoring. The silage was included as 0, 10, 20, and 30% diet (dry matter basis) of Nile tilapia to evaluate its productive performance. Briefly, 120 fish, with initial average weight of 146.8 g, were divided into 12 boxes of 500 l in a completely randomized design with four treatments and three repetitions per treatment. Survival

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rate, weight gain, feed conversion, specific growth rate, yield, and visceral percentage were evaluated. In Experiment I, the average pH and temperature of the silage was 4.2 and 26.6°C, respectively, at the end of 45 storage days. Microbiological results showed that three days after of ensiling has not observed the presence of *Salmonella* in all silages. The coliform number was <0.3 MLN g^{-1} in all silages after 20 d of storage. In Experiment II, no difference (P>0.05) was observed in the productive performance of tilapia, presenting weight gain of 236.6 to 263.1 g, apparent feed conversion of 1.3 to 1.5, specific growth rate (SGR) of 2.8 to 3.1, fillet yield of 34.28 to 35.36%, and visceral percentage of 9.8 to 10.9%. The results indicated that the acid treatment on silage fermentation effectively inhibited microbial growth, making it a safe product for animal feed, and the inclusion of up to 30% broiler carcass acid silage in formulating rations for Nile tilapia does not affect their productive performance.
Key words: Animal feed. Coliforms. Microbial inhibition. Pathogenic microorganisms. Productive performance.

Resumo

Este estudo teve como objetivo avaliar a eficiência da fermentação ácida na eliminação de *Salmonella* e outros microrganismos patogênicos da silagem de carcaça de frango de corte e avaliar sua utilização na dieta de tilápia do Nilo (*Oreochromis niloticus*). A silagem foi produzida utilizando carcaças de frango de corte inteiras, não evisceradas e com penas, e pela adição de 2% de ácido fosfórico, 3% de ácido acético, 0,1% de butil-hidroxitolueno (BHT; antioxidante) e 0,1% de ácido sórbico (antifúngico). No Experimento I, as silagens foram acondicionadas em minissilos de acordo com os seguintes tratamentos (T): T0, controle (sem inóculo); T1, com 10^2 ufc g^{-1} de *Salmonella*; T2, com 10^3 ufc g^{-1} de Salmonella; e T3, com 10^4 ufc g^{-1} de *Salmonella*, e reviradas uma vez ao dia. A silagem foi monitorada por 45 dias com medições diárias de pH e temperatura (°C). Análises químicas e avaliações de microrganismos (Salmonella, coliformes a 30 e 45°C) foram realizadas. No Experimento II, a massa ensilada foi revolvida uma vez ao dia e armazenada por 30 dias, com monitoramento diário de temperatura e pH. A silagem foi incluída como 0, 10, 20 e 30% da dieta (base matéria seca) de tilápia do Nilo para avaliar seu desempenho produtivo. Resumidamente, 120 peixes, com peso médio inicial de 146,8 g, foram divididos em 12 caixas de 500 l em um delineamento inteiramente casualizado com quatro tratamentos e três repetições por tratamento. Taxa de sobrevivência, ganho de peso, conversão alimentar, taxa de crescimento específico, rendimento e porcentagem visceral foram avaliados. No Experimento I, o pH e a temperatura médios da silagem foram de 4,2 e 26,6°C, respectivamente, ao final de 45 dias de armazenamento. Os resultados microbiológicos mostraram que três dias após a ensilagem não foi observada a presença de Salmonella em todas as silagens. O número de coliformes foi <0,3 MLN g^{-1} em todas as silagens após 20 dias de armazenamento. No Experimento II, não foi observada diferença (P>0,05) no desempenho produtivo da tilápia, apresentando ganho de peso de 236,6 a 263,1 g, conversão alimentar aparente de 1,3 a 1,5, taxa de crescimento específico (TCE) de 2,8 a 3,1, rendimento de filé de 34,28 a 35,36% e porcentagem visceral de 9,8 a 10,9%. Os resultados indicaram que o tratamento ácido na fermentação da silagem inibiu efetivamente o crescimento microbiano, tornando-a um produto seguro para alimentação animal, e a inclusão de até 30% de silagem ácida de carcaça de frango na formulação de rações para tilápia do Nilo não afeta seu desempenho produtivo.
Palavras-chave: Coliformes. Desempenho zootécnico. Inibição microbiana. Microrganismos patogênicos. Tilápia.

Introduction

Brazilian broiler production in 2023 was 14.833 million tons and is considered one of the most consumed proteins worldwide. In Brazil, approximately 90%, 7%, and 3% poultry farming is dedicated to chicken production, egg production, and breeding stock, respectively (Associação Brasileira de Proteína Animal [ABPA], 2024).

The pre-slaughter period is one of the most critical factors in poultry farming (Sakamoto, 2017), and the waste generated, together with environmental issues, is the main problem faced by the poultry industry. Despite developments in production, large volumes of waste, such as manure and hatchery waste, are generated. Moreover, the high mortality rates require immediate and regular waste disposal (Blake et al., 2008).

The disposal of carcasses can result in a considerable daily volume that must be treated before it can be used in the environment (Stentiford & Bertoldi, 2011).

Silage is a low-cost technology that allows for using production waste. The principle of this technique is to reduce pH, hydrolyze proteins, and obtain lactic acid as the final product (Bringas-Alvarado et al., 2018; E. R. Silva et al., 2020). The resulting silage has characteristics such as being odorless, not requiring refrigeration, and being kept for a long time (Carmo et al., 2008). Acid fermentation of carcasses prevents environmental contamination and inhibits the growth of pathogenic bacteria (Blake et al., 2008). Silage can also be prepared by adding organic acids (formic, propionic, acetic, and citric acids) and inorganic acids (sulfuric, hydrochloric, and phosphoric acids) (Batista, 1987; Vidotti & Gonçalves, 2006).

Various acids and combinations, including formic acid (Cai et al., 1995), formic acid and citric acid (Gao et al., 1992), acetic acid (Seibel & Souza-Soares, 2003), formic acid and propionic acid (Ferraz de Arruda et al., 2006), and phosphoric acid and acetic acid (Fernandes et al., 2007) can be used to prepare acid silage.

Several studies have been conducted using the acid silage technique with various animal production residues. Banze et al. (2017) determined the composition and quality of acid silage with tuna viscera, Batalha et al. (2017) evaluated the physicochemical and nutritional characteristics of acid silage flour from pirarucu waste, E. R. Silva et al. (2020) evaluated acid silage from pig viscera, Pessoa et al. (2011) evaluated the physicochemical and microbiological characteristics of whole fish acid silage, and Díaz-Cachay et al. (2023) evaluated the effect of chicken blood and offal silage as a substitute for fishmeal in Nile tilapia feed. According to Kompiang (1981), silage has high biological value and practically the same composition as its raw material, making it a good alternative for preparing diets for aquatic organisms.

This study aimed to evaluate the efficiency of eliminating *Salmonella* and other pathogenic microorganisms in chicken carcass silage and its use in feeding Nile tilapia (*Oreochromis niloticus*).

Material and Methods

Raw materials and silage production

Silage was prepared from the carcasses of broiler chickens that died during rearing on a commercial farm located in the municipality of Cambé-PR. The carcasses were collected daily immediately after death by a farm employee and stored in a freezer. Subsequently, the carcasses were brought to the laboratory of the Fish Farming Station of the State University of Londrina (UEL) for grinding, preparation, and production of silage, in accordance with prior approval by the Ethics Committee on Animal Experimentation (CEUA) of the UEL (Process No. 24359/2010).

Ensiling was carried out according to experimental conditions previously optimized by Response Surface Methodology, with an incomplete factorial design 3^3 with three repetitions at the central point (Box & Behnken, 1960), and the effects of acid concentration, storage time, and number of daily turns of the ensiled mass on pH, protein content, lipid oxidation, and mesophile count in the ensiled mass were evaluated as published by Inocente et al. (2024).

Whole carcasses, without evisceration and with feathers, were thawed and ground in an electric meat grinder (model 1970) using an 8 mm sieve. The product obtained by grinding the chicken carcasses was mixed to obtain a homogeneous mass, divided into 12 batches of 1 kg each, and packed in polyethylene silos. Phosphoric acid (2%), acetic acid (3%), the antioxidant BHT (hydroxybutylanisole) (0.1%), and sorbic acid (0.1%) were added to the silos as antifungal agents.

The ensiling process lasted for 45 days. The silage was turned once daily using a stick. The pH and temperature were measured using a potentiometer and a rod thermometer, respectively, twice daily at 7 am and 5 pm throughout the ensiling process. The silos were kept at room temperature and the average minimum and maximum temperature of the ensiled mass was 17.7 and 30.9°C, respectively.

Experiment I

Inoculum

To prepare the inoculum, *Salmonella enteritidis* ATCC 13076 culture was diluted in trypticase soy broth (TSB) to 10^8 CFU/ mL^{-1} using the 0.5 Mac Farland scale (approximately $1.5x10^8$ CFU mL^{-1}) and rediluted to 10^5, 10^6, 10^7 CFU mL^{-1}. To confirm the concentration, the inocula were sown on PCA agar (the standard for counting).

Different inoculum concentrations were added to 1 kg silage to make up the experimental treatments: T0, without *Salmonella*; T1, with 10^2 CFU g^{-1}; T2, with 10^3 CFU g^{-1}; and T3, with 10^4 CFU g^{-1}, in a completely randomized design. The trials were repeated three times, and each repetition was analyzed as an individual sample.

Chemical analysis

At the Animal Nutrition Laboratory of the Department of Animal Science/UEL, dry matter (DM), mineral matter (MM), crude protein (CP), and ether extract (EE) were determined 45 d after ensiling, according to

Association of Official Analytical Chemists [AOAC] (2016). A digital potentiometer (Hanna®) was used to determine the pH of the silage. The measurements were performed daily throughout the silage storage period.

Microbiological analysis

Microbiological analyses of the most probable number of coliforms (MPN g−1) at 30°C and 45°C and Salmonella detection were carried out in the Animal Products Inspection laboratory of the Department of Preventive Veterinary Medicine/UEL on samples collected when the carcasses were ground, considered day 0 before the acids were added, and after 3, 10, 20, and 30 days of ensiling. The methodologies used are described in Instrução Normativa no 62, de 26 de agosto de 2003. (MAPA, 2003).

MPN at 30 and 45°C

Presumptive test

Using the previously prepared dilutions, 10 mL of the 10^{-1} dilution was distributed into a series of three tubes containing sodium lauryl sulfate broth at double concentrations (corresponding to the tenth dilution). Next, 1 mL of the 10^{-1} dilution was inoculated into a series of three tubes containing sodium lauryl sulfate broth at a single concentration. The other dilutions were inoculated into a series of three tubes containing sodium lauryl sulfate broth at a single concentration up to a decimal dilutions of 10^{-6}. All the tubes were incubated at 36°C for 24-48 h.

The readings were taken after 24 h of incubation and repeated after 48 h when the presence of negative tubes. Tubes with cloudy media and gas in the Durhan tube were suspected to contain coliforms.

Confirmatory test

Coliforms at 30°C

Samples from the tubes containing sodium lauryl sulfate broth considered positive were transferred to tubes containing bile brilliant green broth with 2% lactose and incubated at 36°C for 24-48 h. The presence of coliforms at 30°C was confirmed by gas formation in the Durhan tubes. The MPN g^{-1} of coliforms was calculated (Basic Counting Procedures) as included Instrução Normativa no 62, de 26 de agosto de 2003. (MAPA, 2003).

Coliforms at 45°C

Samples from the tubes containing sodium lauryl sulfate broth considered positive were transferred to tubes containing EC broth and incubated in a water bath at 45°C for 24–48 h. The presence of coliforms at 45°C was confirmed by gas formation in the Durhan tubes. The MPN g^{-1} of coliforms was calculated (Basic Counting Procedures) as included in Instrução Normativa no 62, de 26 de agosto de 2003. (MAPA, 2003).

Salmonella spp. detection

Samples of the raw material were collected before the experiment and after 3, 10, 20, and 30 d of experimentation. A 25 g sample from each repetition/treatment was collected and added to 225 mL 1% peptone-buffered saline. The samples were then homogenized in a stomacher and incubated at 36°C for 20 h, which corresponds to the pre-enrichment phase.

After the pre-enrichment phase, 0.1 and 1 mL culture in 1% peptone-buffered saline was transferred to tubes with 10 mL Rappaport–Vassiliadis (RV) broth and 10 mL selenite cystine (SC) broth, respectively, and incubated for 24 h at 41°C. The cultures in RV and SC broths were streaked on plates with bright green phenol red lactose sucrose (BPLS) agar and xylose lysine deoxycholate (XLD) agar and incubated at 36°C for 24 h, observing the presence of typical *Salmonella* colonies.

On BPLS agar, the colonies, when present, were colorless or pinkish and translucent to slightly opaque. When surrounded by lactose-fermenting microorganisms, they may become yellowish-green in color. On XLD agar, the colonies were pink, with or without a black center. Strong H2S-producing strains can produce colonies with a large, shiny black center, or can even be entirely black (Instrução Normativa no 62, de 26 de agosto de 2003, MAPA, 2003).

When results indicated the presence of *Salmonella*, Gram staining was performed on at least three typical colonies. Confirmation was verified by visualization of gram-negative bacilli. The results of each analysis are expressed as the absence or presence of *Salmonella* spp. in 25 g silage.

Experiment II

The experiment was conducted at the State University of Londrina (UEL) Fish Farm for 86 days. All animal management practices were approved by the State University of Londrina Animal Experimentation Ethics Committee (CEUA) (Process No. 24359/2010).

We used 120 juvenile Nile tilapia (*Oreochromis niloticus*) with an average weight of 147.92±3.8 g, distributed in a completely randomized experimental design with four treatments and three replications. The fish were housed in 12 500-L polyethylene boxes with constant water renewal from an artesian well and continuous aeration, with each polyethylene box representing an experimental unit with 10 fish each.

Feed was analyzed at the Animal Nutrition and Soil Laboratories of the Department of Animal Science and Agronomy/UEL. The DM, MM, CP, and EE were determined according to the AOAC (2016), and mineral macroelements (Ca and P) were determined according to Malavolta et al. (1997). Crude energy (CE) was analyzed using an adiabatic calorimetric pump at the Animal Nutrition Laboratory of the State University of Maringá.

The diets were calculated to meet the nutritional requirements of the species, being isoproteic (35% CP in DM) and isoenergetic (4,600 kcal kg^{-1} in DM). The treatments (T) comprised increasing chicken silage levels in the diets T0, T1, T2, and T3, with 0, 10, 20, and 30% acidic silage inclusion, respectively (Table 1).

Table 1
Percentage and chemical composition of experimental rations with the inclusion of acid broiler silage using phosphoric (2%) and acetic (3%) acids, the antioxidant 0.1% (hydroxybutylanisole, BHT) and antifungal 0.1% (sorbic acid), on a dry matter basis

Ingredient (g kg^{-1})	Acid-preserved broiler carcass silage inclusion levels			
	0	10	20	30
Soybean meal	346.00	349.00	339.20	250.00
Corn	293.00	247.00	203.00	238.00
Meat meal	6.30	16.00	30.00	50.00
Wheat bran	49.20	80.00	95.00	39.20
Guts meal	246.30	135.00	40.00	5.00
Fish meal	51.40	65.20	85.00	110.00
Acid-preserved broiler carcass silage	0	100.00	200.00	300.00
Premix[1]	4.50	4.50	4.50	4.50
Salt	3.30	3.30	3.30	3.30
TOTAL	100	100	100	100
Dry matter	933.60	926.60	922.10	918.70
Crude energy	46217.30	47026.40	48139.30	48517.10
Crude protein	390.60	390.40	395.30	393.10
Ether extract	55.00	68.90	88.60	115.20
Crude fiber	22.90	26.40	23.40	18.60
Mimeral matter	87.80	87.70	89.60	101.90
Calcium	19.10	12.30	12.00	16.20
Phosphorus	25.50	26.20	34.80	43.10

[1]Premix, guarantee levels per kg product: vitamin A, 2,500,000 IU; vitamin D3, 300,000 IU; vitamin E, 40,000 IU; vitamin K3, 2,260 mg; vitamin B1, 2,500 mg; vitamin B2, 5,000 mg; vitamin B6, 2,500 mg; vitamin B12, 7,500 mcg; Niacin, 20,000 mg; folic acid, 500 mg; pantothenic acid, 12,500 mg; biotin, 150 mg; choline, 200,000 mg; vitamin C (protected), 75,000 mg; methionine, 5%; lysine, 4%; selenium, 75.15 mg; iodine, 125 mg; cobalt, 50 mg; iron, 15,000 mg; copper, 2,000 mg; manganese, 3,750 mg; zinc, 20,000 mg.

To prepare the diets, the ingredients were homogenized, and when necessary, water was added to bind the dough. The dough was then processed in a meat grinder, dried in a forced air circulation oven at 55±5°C for 24, and broken into small pieces to form a pellet. The animals were fed twice daily at 900 and 1600 h, with the amount supplied ranging from 2 to 3% of their live weight.

Sediments containing manure and food waste were removed daily via siphoning. Dissolved oxygen and temperature were measured daily using a YSI 55 oximeter. Other water quality parameters, such as ammonia, nitrite, alkalinity, and pH, were measured every two weeks as recommended by Sipaúba-Tavares (1994).

Performance evaluation

Productive performance was evaluated monthly by measuring feed consumption, survival parameters (%), weight gain (WG = final weight - initial weight), feed conversion (FC = feed consumption/weight gain), and specific growth rate SGR (%) = 100 × [Ln final weight–Ln initial weight]/number of days, where Ln = Neperian logarithm). To monitor performance and adjust the diet, monthly biometrics were performed, preceded by analgesia of the animals by immersion in an aqueous solution containing a eugenol-based anesthetic.

After 86 days of experimentation, the final biometrics were collected and the animals were euthanized. The skin was removed with special pliers and filleting was performed (skinless fillet), obtaining the fillet from the dorsal muscles on both sides of the fish in a longitudinal direction along the entire length of the spine and ribs (Souza, 2002). The fillets were removed at a slaughterhouse in the Rolândia-PR region, in accordance with the guidelines in the Manual for the Humane Slaughter of Fish (Ministério de Agricultura, Pecuária e Abastecimento [MAPA], 2022) and quantified to calculate the yield.

Statistical analysis

The means of the production performance parameters and limnological variables were subjected to analysis of variance (ANOVA, P<0.05). The STATISTICA 7.0 program (Statsoft, 2004) was used to perform the analyses.

Results and Discussion

Experiment I

Salmonella enteritidis and >10^6 MLN g^{-1} coliforms at 30 and 45°C were detected in the ground raw material, which indicates high contamination. *Salmonella* is common in commercial farms and has been isolated from slaughterhouse fat, scalding and cooling water, chicken carcasses, litter, chicken feed, and breeding stock (Park et al., 2015).

Although the T0 treatment was not inoculated with *Salmonella enteritidis*, its presence was confirmed by microbiological analysis of the raw material. According to Ferrari et al. (2019), the *enteritidis* serovar is the most common in Latin America and can infect various hosts. The main causes for the presence of this serovar are changes in the production chain and increases in population density.

The DM, CP, EE, crude fiber, and MM contents of silages containing different *S. enteritidis* concentrations as the inoculum were not different (P>0.05; Table 2).

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Table 2
Chemical analysis of acid-preserved broiler carcass silage with different concentrations of bacterial inoculum (Salmonella)

Variables (%)	Concentration of inoculum (CFU g^{-1})				SD
	0	10^2	10^3	10^4	
Dry matter	34.4	34.7	34.3	33.8	0.71
Protein*	48.7	47.3	47.5	46.8	1.32
Ether extract*	27.5	27.6	26.0	27.1	0.9
Mineral matter*	13.6	13.4	13.4	12.3	0.68

*dry basis; SD=Standart Deviation.

The DM content can vary according to the volume of acid used as well as the methodology and addition of flour when preparing the silage. The DM content found were higher than those found by Díaz-Cachay et al. (2023) in chicken intestine and blood silage and close to those observed by Kherrati et al. (1998) in biological silage of chicken viscera, offal, feathers, head, and feet (slaughterhouse waste) prepared with the addition of 15% molasses (35–37.3%). Boscolo et al. (2010) found 27–32.5% DM in acid silage from tilapia filleting waste. Cai et al. (1994) reported that the DM content of organic silage from chicken carcasses with the addition of molasses or brewer's yeast was close to 30%. Vidotti and Gonçalves (2006) reported that the DM content of acidic and biological fish waste silages was 37.5–43%.

The average CP content was similar to that observed by Kherrati et al. (1998) in organic silage from chicken slaughterhouse waste. However, Cai et al. (1994) found 36% protein in organic silage from chicken carcasses with the addition of molasses or brewer's yeast.

The high percentage of fat was attributed to the inclusion of whole chickens with viscera and skin, which are naturally lipid-rich. The fat percentage can also vary with the age of the chicken used, since animals close to slaughter accumulate more fat. According to Alves et al. (2016), carcass composition can be modified by age, sex, handling, and diet, thereby influencing poultry meat quality and fat accumulation.

The average MM content (13.1%) was considered high because of the presence of bones and feathers in acidic chicken silage. Kherrati et al. (1998) found close to 10% MM in organic silage from chicken slaughterhouse waste; however, these values may be higher or lower than those reported above depending on the waste type.

With the addition of acids in the first few days, the pH of broiler silage decreased from 6.5 in the ground mass to 4.2 on average at the end of the experiment. The silage stabilizes at pH below 4.5 (Fagbenro & Jauncey, 1995). Poveda-Parra and Ramos-Embus (2001) stated that a rapid drop in pH in the first 72 h allows the nutrients in the silage to be maintained or to improve their characteristics.

In the microbiological analyses, the coliform counts at 30 and 45°C were not significantly different (P>0.05; Table 3). On day three of silage storage, the number of coliforms decreased. Treatments T0 and T2 had 0.5 and 0.37 MLN g^{-1} coliforms, respectively, while the others had <0.3 MLN g^{-1} coliforms.

Table 3
Microbial profile of acid-preserved broiler carcass silage with different concentrations of Salmonella inoculum at different storage times

	Days	Concentration of inoculum (CFU g^{-1})			
		0	10^2	10^3	10^4
Coliforms at 30°C (MLN g^{-1})	3	0.5	<0.3	0.4	<0.3
	20	<0.3	<0.3	<0.3	<0.3
	30	<0.3	<0.3	<0.3	<0.3
	45	<0.3	<0.3	<0.3	<0.3
Coliforms at 45°C (MLN g^{-1})	3	<0.3	<0.3	<0.3	<0.3
	20	<0.3	<0.3	<0.3	<0.3
	30	<0.3	<0.3	<0.3	<0.3
	45	<0.3	<0.3	<0.3	<0.3
Salmonella	3	absent	absent	absent	absent
	20	absent	absent	absent	absent
	30	absent	absent	absent	absent
	45	absent	absent	absent	absent

MLN g^{-1} = Most likely number.

To analyze the presence or absence of *Salmonella*, the pathogen was completely eliminated on the third day of ensiling. The decrease in pH, anaerobiosis, and the production of some antibacterial substances by bacteria, such as *Lactobacillus*, prevent the development of pathogenic bacteria such as *Salmonella* (Nascimento et al., 2014; Maia & Sales, 2013). According to Jones (2011), the addition of organic acids can control pathogens in the raw materials used in animal feed.

Similar results were observed by Kherrati et al. (1998) in organic silage of chicken waste (offal, viscera, feathers, head and feet), where after 10 days of ensiling, the pH of the silage was close to 4.2 and microbiological analysis showed a reduction in coliforms at 30 and 45°C from 10^6 to 1 CFU g^{-1}, and no *Salmonella.* Banze et al. (2017) observed <1.0×10 CFU g^{-1} *Staphylococcus coagulase* and *Thermotolerant coliforms* and no *Salmonella* acidic tuna silage after 30 days in. Ferreira et al. (2018) did not observe *Staphylococcus* in fish acid silage after 14 days of fermentation.

Experiment II

Limnological evaluation and production performance

The limnological parameters were not different (P>0.05) between the treatments. The average temperature, dissolved oxygen content, nitrite level ammonia level, pH, and alkalinity were 22.5±0.4°C, 4.3±0.2 mg L^{-1}, 0.15±0.09 mg L^{-1}, 0.27±0.08 mg L^{-1}, 6.1±02, and 51.7±1.8 mg L^{-1}, respectively. The results of the limnological variables met the recommendations of the National Environment Council (CONAMA 357/2005) according to Class 2, which classifies waters intended for natural and/or intensive breeding (aquaculture) (Resolução N. 357 do Ministério do Meio Ambiente, Conselho Nacional do Meio Ambiente, 2005).

Increasing acidic broiler silage levels in the diet did not significantl influence the productive performance of tilapia under the experimental conditions evaluated (P>0.05; Table 4). The pelletized experimental diets provided fish with good feed conversion and SGRs. Tilapia performance values close to those found in this study have been reported by Abimorad et al. (2009), who studied tilapia in the same weight range (approximately 390 g). The fish were fed fish silage-based feed (58%), and feed conversion of 1.72 and specific growth rate of 1.07 were observed.

Table 4
Productive performance of tilapia fed different levels of acid-preserved broiler carcass silage

Variables[1]	Levels of acid-preserved broiler carcass silage in the diet *, %				
	0	10	20	30	CV, %
Initial weight, g	147,9	145,1	146,4	147,9	11,4
Final weight, g	411,0	398,1	394,6	384,6	14,5
Weight gain, g	263,1	253,1	248,2	236,6	5,9
Feed comsumption, g	3289	3417	3359	3460	2,3
Feed conversion	1,3	1,4	1,5	1,5	8,2
Survival, %	96,7	93,3	93,3	100	5,4
SGR, %	3,1	2,9	2,9	2,8	5,9
Fillet yield, %	35,4	35,8	34,3	34,3	13,7
Guts, %	9,8	10,2	10,9	10,6	14,2

* Dry basis; SGR - specific growth rate; 1P>0,05.

The fillet yield was within the recommended values; however, it varied between 25 and 42% depending on the filleting method, the average weight of the animals, the skill of the filleter, and the equipment used (Pinheiro et al., 2006; Souza & Maranhão, 2001). The visceral percentage showed no significant difference (P>0.05) between the treatments. It was similar to that reported by Santos et al. (2007); however, F. V. Silva et al. (2009) found 13.26% viscera.

As a part of the original project that also resulted in this work, a patent was applied to optimize the process of obtaining acid silage from broiler carcasses, which was granted on July 14, 2020, under registration number PI000215 at the National Institute of Industrial Property (INPI).

Conclusion

Silage process with 2% phosphoric acid and 3% acetic acid promotes microbial inhibition, mainly of *Salmonella*, from the third day of silage storage, and can be added by up to 30% in formulating tilapia feed without damaging their productive performance.

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