



## Research article

Effects of chemical silage of red tilapia viscera (*Oreochromis spp.*) as a source of protein on the productive and hematological parameters in isa-brown laying hens (*Gallus gallus domesticus*)Yhoan S. Gaviria G<sup>a</sup>, Luis F. Londoño F<sup>b</sup>, José E. Zapata M<sup>a,\*</sup><sup>a</sup> Nutrition and Food Technology Group, Universidad de Antioquia, Medellín, Colombia<sup>b</sup> Politécnico Jaime Isaza Cadavid, Medellín, Colombia

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## ABSTRACT

This paper evaluates the inclusion of chemical silage from viscera of red tilapia (*Oreochromis spp.*) in diets of Isa-Brown laying hens (*Gallus gallus domesticus*), and its influence on productive variables and hematological parameters. A total of 56, 16-week-old laying hens were randomly divided into two groups (one per diet), which in turn were subdivided into 7 replicates of 4 birds each. All test groups were fed for 16 weeks. During this period, the evaluation of the productive variables was carried out, and at the end, random blood samples were taken from 3 birds per diet. The results indicated that the inclusion of chemical silage from red tilapia viscera with a proportion of 17.18% dry matter, does not present statistically significant differences in the productive variables which were evaluated ( $p > 0.05$ ) with respect to the control. Moreover, the chemical silage did not modify the hematological parameters and blood the chemistry in the hens. This allowed us to conclude that silage can be used as a substitute for conventional protein raw materials such as fishmeal and soybean meal in the preparation of diets for laying hens, without altering their productive performance.

## 1. Introduction

In recent years, human beings have acquired an advanced knowledge about the health benefits associated with eating fish. This has led to a permanent growth of aquaculture production throughout the world. In 2016, global fish production reached 171 million tons, of which 86% was for direct human consumption (FAO, 2018). In 2017, the Colombian aquaculture sector underwent a growth in production of 9% with a total of 120,230 tons, of which the red tilapia species held the most significant contribution with 62%, followed by white cachama at 16%, silver tilapia at 14%, trout at 5%, and a 3% of remaining species (Ministerio de Agricultura, 2017). This growth has been followed by an equivalent volume of waste, which can generate significant environmental impacts, given that it represents between 60-70% (w/w) of total production (Martínez-Alvarez et al., 2015). These residues are mainly composed of fillet remains (15–20%), skin and fins (1–3%), bones (9–15%), heads (9–12%), viscera (12–18%) and flakes (5) (Martínez-Alvarez et al., 2015), which, despite being important sources of protein, are most often discarded without any attempt at reutilizing them. However, due to how rich this waste is in terms of nutrients, its inadequate disposal can affect

ecosystems at different levels. This can generate problems in ecosystems such as biomass reduction, the density and diversity of benthos and plankton, as well as the modification of natural trophic networks. These residues can be used for animal feed, to produce fishmeal, fats, or fertilizers, among others (Chalamaiah et al., 2012).

One of the most widely used strategies for nutrient recovery from fish by-products consists of adding acids, enzymes, or lactic acid bacteria to raw fish, or parts of it. This is done in order to cause the pH to drop and generates a liquefaction of the mass by effect of endogenous enzymes which act under acidic conditions (Fernández Herrero et al., 2013). This process is called silage (Suarez et al., 2018). The product obtained from said process is a grayish-brown colored, semi-liquid, pasty substance, with a characteristic fishy odor. The product provides partially hydrolyzed proteins, with nutritional properties which are very similar to those of the fish (Botello, 2010) they come from. These proteins could improve the digestibility of diets in animal feed, which is one of the factors that positively influences growth. The poultry sector is possibly the fastest growing and the most flexible of all the livestock sectors. Mainly driven by a strong demand, this sector has been expanded, consolidated and globalized in the last 15 years in countries

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of all income levels (FAO, 2013). However, the high cost of poultry feeding is one of the main problems that plagues the production chain, since approximately 95% of the chain is used to satisfy protein and energy needs. Fishmeal and soy meal are the main sources of protein and corn flour is the main source of energy (Ravindran, 2013). The objective of this paper was to evaluate the effect of the inclusion of chemical silage made from red tilapia (*Oreochromis spp.*) viscera, in the elaboration of diets for Isa Brown laying hens. The study takes the productive variables and hematological parameters into account in order to evaluate the effects of this dietary modification.

## 2. Material and methods

### 2.1. Handling of the materials

The red tilapia (*Oreochromis spp.*) viscera were supplied by the El Gaitero fish farm, located in the municipality of San Jerónimo, Antioquia-Colombia. Initially, the viscera were degreased, for which they were brought to 67 °C for 30 min, with a subsequent drop to 45 °C and the fat was decanted. After this, they were frozen (at -18 °C) for 24 h. This allowed the separation of the oil from the aqueous-protein phase, since when phases solidify they do so separately (Arias et al., 2017). Then, the lipid phase was detached from the frozen aqueous-protein phase, by means of a cross-section, and the aqueous-protein phase was stored for the silage. The aqueous-protein phase was homogenized with a crushing mill. Then, it was mixed with 0.03% sulfuric acid at 97% (Merck, Germany) and 1.16% formic acid at 85% (Merck, Germany) to start the hydrolysis and acidification process. The BHT antioxidant was then added (Butyl hydroxy toluene) (Tecnas SA, Colombia) along with an antifungal - potassium sorbate (Tecnas SA, Colombia). Finally, the mixture was stored at room temperature for a minimum time of 8 days. During this period the product was homogenized by stirring, and a pH reading was taken every two days until it stabilized.

### 2.2. Poultry feed

Fifty-six, 16-week-old Isa Brown laying hens were randomly divided into two equal groups called the control group (CT) and the silage group (ENS), which were fed from week 16 to week 32. The isoproteic diets were prepared following the nutritional requirements established by Rostagno et al. (2011) (Table 3). The CT group was fed with the diet without the viscera silage, and the silage group (ENS) was fed with the diet of which 32% of the protein was from the viscera silage. The hens were fed twice a day (morning and afternoon) with a 90 g/day ration for each bird, and a constant supply of water.

### 2.3. Physicochemical and microbiological analysis

The physicochemical characterization of the silage was carried out in accordance with the provisions of the AOAC (*Association of Official Analytical Chemists*). The moisture content was determined using the methodology established in standard 930.15 (AOAC, 2000b), by drying the sample at 105 °C for 8 h. The protein was determined by the Kjeldahl method (standard 954.010) (AOAC, 2000a). The ashes were analyzed according to standard 942.05 (AOAC, 1995). Fat analysis was conducted according to standard 920.39 (AOAC, 2003). The carbohydrate determination was performed by subtracting one hundred percent of the previously mentioned components from the sample (Spanopoulos-Hernandez et al., 2010).

The microbiological analyzes were carried out according to the Colombian Technical Standard NTC 3688 (ICONTEC, 1999), which prescribes the analysis of aerobic mesophylls, total and fecal coliforms, *Salmonella spp.*, *Clostridium* spore reducing sulfites and molds and yeasts for these types of substrates.

### 2.4. Determination of productive variables

Both diets were evaluated for weight gain by performing weekly weighing rounds of the fasted animals with a 1g precision analytical balance (TxB220-1L from Shimadzu, Japan). Eggs were collected, counted and weighed daily for 13 weeks to determine the laying percentage and their average weight.

### 2.5. Blood chemistry and hemogram

The complete hemogram was performed using the methodology proposed by Clark et al., in 2012, which counts red blood cells or erythrocytes, white blood cells or leukocytes, and thrombocytes or platelets, which are all structures produced in the bone marrow by the cytoplasmic fragmentation process (Clark et al., 2012). Hematological analyses are among the methods which may contribute to the detection of some changes in health status and can be a useful aid for diagnosis diseases in birds (Schmidt et al., 2009). Blood samples were collected from the wing vein, with or without anticoagulants, at from the 32th week of age from all of the groups. Plasma samples were analyzed for total cholesterol, total protein, triglycerides, alkaline phosphatase and the enzyme alanine aminotransferase (ALT) concentrations.

### 2.6. Statistical analysis

The data collected was evaluated at a confidence level of 95% ( $P < 0,05$ ) by means of the hypothesis test to determine the difference in means using Fisher's LSD (Least significant difference) test with *statgraphics centurion XVI* software. A Completely Randomized Design was used in this study. There were seven replications for each treatment, and four layer hens in each replication. The treatments were 0 (control), and 32% silage of the viscera red tilapia substitution of basal diet.

### 2.7. Ethical approval

The experiments carried out in this manuscript were submitted and approved by the ethics committee for experimentation with animals of the University of Antioquia - Colombia.

## 3. Results and discussion

### 3.1. Proximal composition of the silage

Table 1 shows the bromatological composition of the viscera and the silage obtained from them. The viscera showed the levels of protein and fat typical to this species (Arias et al., 2017; Suarez et al., 2018). However, the values tended to be higher than those reported for acidic silage from freshwater fish residues, with a dry basis protein of 44.38% (Vidotti et al., 2003) and that of biological silage of tuna (*Thunnus albacares*) with 30.52% crude protein and 14.25% lipids (Spanopoulos-Hernandez et al., 2010).

In addition, the silage obtained showed an increase in protein content and a decrease in the level of fat, which is due to the degreasing process to which the viscera were subjected. Said process is necessary to be able to balance diets such as the ones which were evaluated. A similar reduction was reported by Ramírez et al. (2013) authors who managed to reduce the ether extract from 24.5% to 14.5% on a wet basis.

In this study, the product presented the appropriate characteristics of an optimal silage: the substance was a grayish-brown colored, semi-liquid paste with a characteristic fishy odor (Botello, 2010; Suarez et al., 2018). The product's protein and fat levels allowed it to be an ideal substrate for animal feed, since it provides partially hydrolyzed proteins, with nutritional properties very similar to those of the original fish (Botello, 2010). This helps improve the digestibility of animal feed, which is one of the factors that positively influences productive variables. Proteins and lipids from tilapia waste silage have been found to have an

**Table 1.** Proximate and composition analysis of entrails and chemical silage.

	Viscera*	Chemical silage*
Moisture (%)	61,36 ± 0,29	82,73 ± 0,10
Crude protein (%)	10,45 ± 0,10	50,89 ± 0,51
Carbohydrate (%)	2,56 ± 0,09	0,48 ± 0,04
Crude fat (%)	85,24 ± 0,04	39,78 ± 0,13
Ash (%)	1,75 ± 0,04	8,85 ± 0,02

\* Dry matter.

appropriate nutritional profile for herbivorous and omnivorous species mainly, which can be used as a partial substitute for fishmeal (Goosen et al., 2014; Güllü et al., 2014), which is the most important source of protein used in animal feed (precisely due to its digestibility and protein content (Chalamaiah et al., 2012)). In this vein, this study is part of the current trend of evaluating the effects of using mixtures of agricultural and aquaculture sources in order to reduce the environmental impact generated by fish farming and thus reuse food sources to make it sustainable (Smárason et al., 2017). Our results coincide with Kjos et al. in (2001) who reported similar values of protein in silage of Salmon residues (*Salmo*), which was used to feed laying hens (Kjos et al., 2001).

This study was able to include a considerable fraction of viscera silage (43.4%), which corresponds to 17.8% on a dry basis. This represents a substitution of 57.37% and 23% of soybean meal and fishmeal, respectively. Other studies have used between 15% and 40% of the silage produced, with similar physicochemical parameters with regard to protein and fat, in food for tilapia (Llanes et al., 2010). This is important to highlight because in this case the use of silage would be maximized and possibly reduce the costs of the production system.

The microbiological composition of the chemical silage of red tilapia viscera (Table 2), is within the parameters established by the national technical directive for raw materials and inputs for animal feed of the Colombian Institute of Agriculture (Directivas técnicas de alimentos para animales y sales mineralizadas, 1999). This shows that the chemical silage of tilapia viscera can be used as a raw material in the elaboration of food for Isa-Brown laying hens without causing any negative effect or pathology in the species.

Two dietary treatments were used. The CT group was fed with the diet without the viscera silage, and the silage group (ENS) was fed with the diet of which 32% of the protein was from the viscera silage (Table 3).

### 3.2. Hemogram and blood chemistry

Table 4 shows the results of the hematological examinations with their respective reference values according to Schmidt et al. (2009). It is important to highlight that the literature reports few reference parameters for the species which was studied (Gutiérrez Castro and Corredor Matus, 2017). However, blood is essential for electrolyte and water balance, for temperature control and for the functioning of the immune system, among other factors (Voigt, 2003). Similarly, blood values can be influenced by nutritional conditions, sex (estrogens in birds tend to depress erythropoiesis), age, habitat, season, reproductive status, trauma, management and environmental stress (Campbell, 2004).

**Table 2.** Microbiological composition of SQ.

	Result	Reference
Mesophilic aerobic (ufc/ml)	$5 \times 10^3$	$10 \times 10^5$
Total coliforms (ufc/ml)	$3,5 \times 10^2$	$10 \times 10^4$
Fecal coliformes	ABSENCE	ABSENCE
<i>Salmonella spp</i>	ABSENCE	ABSENCE
Spore <i>Clostridium</i>	ABSENCE	$20 \times 10^1$
Molds and yeasts (ufc/ml)	$8,2 \times 10^2$	$10 \times 10^4$

Table 4 shows that the values for the parameters of the red series (erythrocytes, hemoglobin and hematocrits) and the white series (leukocytes and lymphocytes), obtained for the control diet are above those of the silage diet. This suggests an increase in metabolism and, consequently, a better physiological behavior for the transport of oxygen and nutrients to the tissues, thus promoting improvements in the productive variables and a better response disposition of the immune system in animals fed with the silage diet. The normal hematocrit of birds is known to vary from 35 to 55%. As mentioned above, the hematocrit can suffer alterations with relation to the sex and age of the birds (Schmidt and dos, 2007; Thrall, 2004). The white series or leukocytes are closely related to the response of the animal's defense or immune system. Heterophilia (increased neutrophils or heterophiles), generally occurs in response to inflammatory or infection processes. Mild to moderate leukocytosis with heterophilia and lymphopenia indicates a stress response, with excess endogenous or exogenous glucocorticoid. This could be the case of the birds with slight leukocytosis in the study's silage group (Latimer and Bienzle, 2000; Thrall, 2004). For example, physiological lymphocytosis represents a transitory phenomenon in birds after excitement, fear or "struggle" during handling (for example, during the blood withdrawal procedure), which coincides with what was observed in the silage group (Latimer and Bienzle, 2000). Similar results were reported by (Cardoso et al., 2002), in White Leghorn birds, using a vegetable substrate in their diet.

This study also obtained higher than normal values for basophils, possibly associated with physiological stress during the handling of birds and with stress from the laying cycle of chickens (Schmidt et al., 2009). Basophilia also occurs in respiratory diseases and severe tissue injuries, associated with stress due to internal and external parasitism and is generally accompanied by eosinophilia (Latimer and Bienzle, 2000; Schmidt and dos, 2007). In summary, the results show that the substitution of the protein in the diet of laying hens with viscera silage did not significantly change the blood concentration of the parameters evaluated.

Cholesterol is an important precursor to cholesterol ester, bile acids, and steroid hormones and can be synthesized by various tissues in the body, but the liver is the main organ of endogenous cholesterol synthesis (Maldonado Saavedra et al., 2012). Hypercholesterolemia can be caused by diet or by liver failure (Kaneko et al., 2008). Due to the fact that cholesterol is eliminated in the form of bile acids, its increase in plasma is associated with extrahepatic biliary obstruction, liver fibrosis, or bile duct hyperplasia in birds (Campbell, 2004). Plasma concentrations for most bird species range from 100 to 250 mg/dL (Lumeij, 1997). In this

**Table 3.** Proximate composition analysis of diets.

	Control Diet (%)	Silage Diet (%)
Fish meal	13	10,06
Soybean meal	16,00	6,82
Corn meal	39	39,27
Rice flour	16	13
Fish entrails silage	0	17,18
Fish oil	4	0
Calcium carbonate	8	8,05
Dicalcium phosphate	2,5	4,02
Vit. min. supplement <sup>1</sup>	0,5	0,4
Lysine	0,25	0,3
Methionine	0,25	0,3
Tryptophan	0,25	0,3
Threonine	0,25	0,3
<b>Chemical composition</b>		
Protein	20,3	20,6
Crude fiber	2,7	2,2
Carbohydrate	37,66	36,21
Crude fat	9	7,09
Metabolizable energy (kcal/kg)	3600	3500
Calcium	4	4,06
Phosphorus	0,9	1,1

<sup>1</sup> Mineral vitamin supplement (composition per 250 g of product): vit. A - 1,400,000 IU; vit. B1 - 500 mg; vit. B12 - 300 mg; vit. B2 = 500 mg; vit. B6 - 1,6 g; vit. D3 - 2,500,000 IU; vit. E - 6,000 IU; vit. K3 = 1,000 mg; biotin - 30 mg; niacin -12 g; folic acid - 1 g; cobalt - 50 mg; Copper - 3,000 mg; Iron - 25 g; Iodine - 500 mg; Manganese - 32.5 g; Selenium - 100.50 mg; Zinc - 22.49 g.

**Table 4.** Hemogram and blood cell count.

	Unit	Control	Silage	Reference
Leukocytes	Cell x 10 <sup>3</sup> /μl	7,4 ± 0,2 <sup>a</sup>	11,6 ± 0,12 <sup>b</sup>	3,0–11,0
lymphocytes	Cell x 10 <sup>3</sup> /μl	2,37 ± 0,09 <sup>a</sup>	4,87 ± 0,11 <sup>b</sup>	-
Intermediate cell count	Cell x 10 <sup>3</sup> /μl	0,07 ± 0,01 <sup>a</sup>	0,12 ± 0,05 <sup>b</sup>	-
granulocytes	Cell x 10 <sup>3</sup> /μl	4,96 ± 0,04 <sup>a</sup>	6,61 ± 0,02 <sup>a</sup>	-
Lymphocytes	%	32 ± 0,7 <sup>a</sup>	42 ± 0,5 <sup>b</sup>	20–65
Monocytes	%	1 ± 0,2 <sup>a</sup>	1 ± 0,08 <sup>a</sup>	0–5
Heterophils	%	49 ± 0,8 <sup>a</sup>	47 ± 0,3 <sup>a</sup>	30–75
Eosinophils	%	6 ± 0,3 <sup>a</sup>	2 ± 0,7 <sup>b</sup>	0–5
Basophiles	%	12 ± 0,07 <sup>a</sup>	8 ± 0,04 <sup>b</sup>	0–5
Bands	%	0 <sup>a</sup>	0 <sup>a</sup>	-
Erythrocytes	Cell x 10 <sup>6</sup> /μl	3,97 ± 0,03 <sup>a</sup>	4,3 ± 0,06 <sup>a</sup>	2,5–4,5
Hemoglobin	g/dl	9,7 ± 0,2 <sup>a</sup>	10,6 ± 0,2 <sup>a</sup>	11,0–19,0
Hematocrit	%	29 ± 0,37 <sup>a</sup>	32 ± 0,57 <sup>a</sup>	23,0–55,0
Platelets	Cell x 10 <sup>3</sup> /μl	44,3 ± 0,83 <sup>a</sup>	49 ± 0,36 <sup>b</sup>	-
Plasmatic proteins	g/dl	3,03 ± 0,06 <sup>a</sup>	3,8 ± 0,07 <sup>a</sup>	3,0–4,9

<sup>a-b</sup> represent a significant difference  $p < 0,05$  for the groups;  $n = 3$  in each group.

study, the cholesterol levels of the silage group were 71.33 mg/dL vs 114 mg/dL of the control group, and the triglycerides were 63 mg/dL vs 170 mg/dL for each, respectively. In this regard, the levels of cholesterol and triglycerides in silage diet were decreased by 37.4% and 62.9% compared with those in control group. These differences in cholesterol and triglycerides can be attributed to the percentage of crude fat in the diets.

Similarly, omega 3 and omega 6 lipids increase high-density lipoproteins (LAD) and decrease atherogenic conditions and endothelial dysfunction in the blood vessels of birds (Palou et al., 2005), which improves blood supply and consequently the quality of the egg as a final product for the benefit of the end consumer (Buitrago et al., 2005). Similar results to those of the present work have been reported by Salma

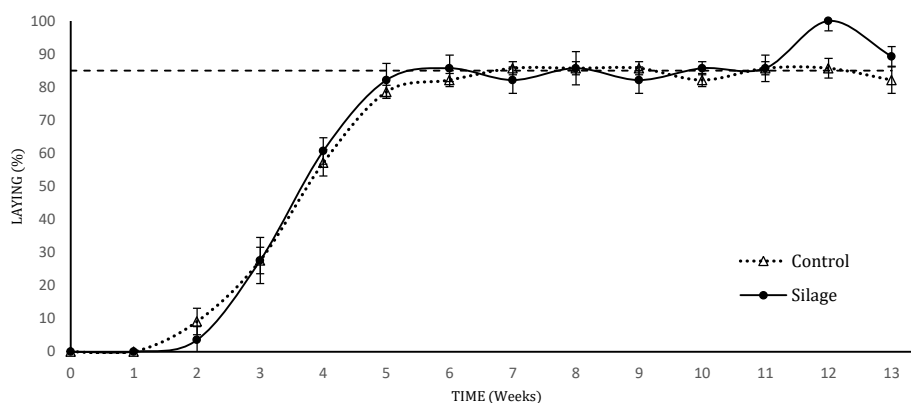
et al. (2007), who observed a decrease in cholesterol and serum triacylglycerides in laying hens, when using 0.04% Rhodobacter capsules (bacteria, probiotic). Nogueira et al. (2003) also found similar values, when supplementing diets with shark cartilage (rich in polyunsaturated fatty acids). The reverse effect is seen in diets with high percentages of saturated fatty acids, since they stimulate the hepatic production of very low-density lipoproteins (LMBD), by stimulating the expression of apolipoprotein B-100 (Bennett et al., 1995).

Likewise, high levels of saturated fatty acids affect the lipid profile of broilers with compatible signs of ascites syndrome, where a depression of the energy metabolism was observed due to decreased energy sources, since the animal gradually loses its appetite. The loss of appetite is generated by the pressure caused by abdominal edema and may lead to

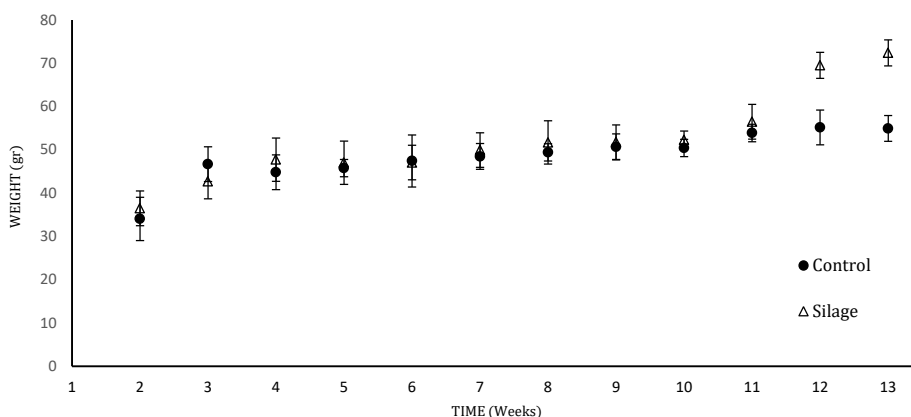
**Table 5.** Blood chemistry.

	Unit	Control	Silage	Reference
ALT	U/L	1,3 ± 0,1 <sup>a</sup>	4,8 ± 0,2 <sup>b</sup>	
Alkaline phosphatase	U/L	44,69 ± 0,17 <sup>a</sup>	39,25 ± 0,13 <sup>a</sup>	10–106
Total protein	g/dL	3,56 ± 0,27 <sup>a</sup>	4,84 ± 0,21 <sup>a</sup>	3–4,9
Cholesterol	mg/dL	114 ± 0,8 <sup>a</sup>	71,33 ± 1,7 <sup>b</sup>	129–279
Triglycerides	mg/dL	170 ± 1 <sup>a</sup>	63 ± 1,2 <sup>b</sup>	79–517

<sup>a-b</sup> represent a significant difference  $p < 0,05$  for the groups;  $n = 3$  in each group.



**Figure 1.** Laying percentage as a function of time during feeding of Isa-Brown line laying hens (*Gallus gallus domesticus*).



**Figure 2.** Egg weight as a function of time during feeding of Isa-Brown line laying hens (*Gallus gallus domesticus*).

decreased glucose, cholesterol and triglyceride levels (Buitrago et al., 2005).

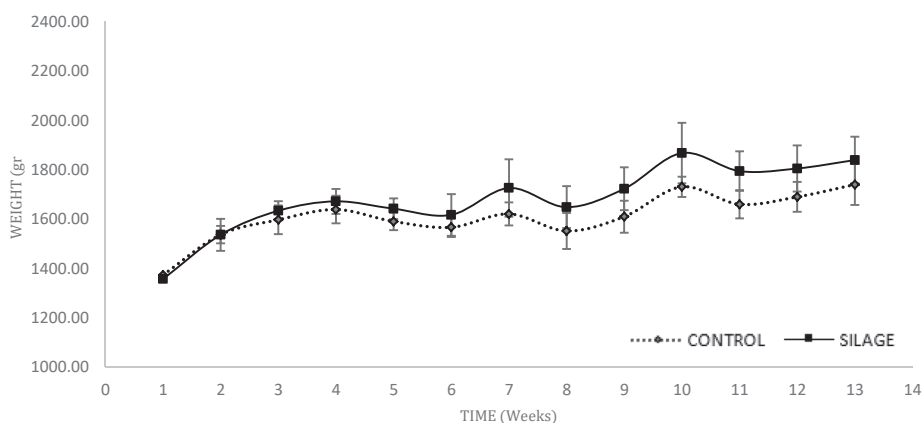
Table 5 shows that the triglyceride and cholesterol values are lower in the blood of animals fed with silage feed, compared to the control, possibly due to the presence of monounsaturated fatty acids in the viscera of fish (red tilapia), according to Clarke et al. (2002). This phenomenon as yet to be well documented in birds. However, we know that foods that are rich in monounsaturated fat promote cardiovascular health in humans, because it causes a reduction in postprandial triglycerides with respect to the intake of saturated fat (Palou et al., 2005).

Similarly, Table 5 shows that the levels of the enzyme alanine aminotransferase (ALT) for both the control and silage group were below the reference levels. The ALT enzyme is found both in the hepatocyte cytosol and in muscle and other tissues in birds. ALT activity in most bird species varies from 19 to 50 U/L (Campbell, 2004; Lumeij, 1997), when this enzyme is above this range, it indicates possible liver damage, especially in its functionality. However, no possible negative effects are reported for low ALT levels (Sanchez, 2009).

Plasma (Table 4) and total (Table 5) proteins are within the reference ranges, which indicates an adequate performance of both diets (control and test). Total plasma proteins in birds vary from 3.0 to 4.9 g/dL, of which albumin comprises 40–50% (Kaneko et al., 2008). In general, the main factors that affect total protein concentrations in birds are: age, seasonality, handling conditions, diseases and egg production (by albumin and globulins) (Lumeij, 1997). In this vein, the results show proper handling according to the age and production stage of the eggs, in addition to the absence of protein deficiency diseases in the birds which were studied. Furthermore, animal sources of protein are known to have a higher quality and biological value than plant protein (Rosenfeld et al., 1997).

### 3.3. Productive variables

Figure 1 shows that there were no statistically significant differences ( $P > 0.05$ ) in the percentage of egg-laying between the group fed with the control diet and the diet made with silage. The data shows that the



**Figure 3.** Weight gain over time of Isa-Brown line laying hens (*Gallus gallus domesticus*) during their feeding.

stability in the laying percentage (at 85%) was reached in week 5 for both diets and was maintained during the following weeks of study. This means that the quantity and quality of degreased silage in laying hens' feed did not have an adverse effect on the laying percentage of the birds, which shows that the nutritional requirements were adequately adjusted for the expected level of laying. Similar results were found by Kjos et al. (2001), however, this study only used 5% silage in the feed. Additionally (Al-Marzooqi et al., 2010), these authors showed that replacing soybean meal with fish silage protein improved the growth performance of broilers. In general, the amino acid content and protein quality of animal sources tend to be of better biological quality and consequently obtain better responses in weight and production compared to diets from plant sources (Rosenfeld et al., 1997).

Figure 2 shows that the average weight of the eggs increased gradually over time, until constant values were maintained between the sixth and seventh week, without showing statistically significant differences between the diets ( $P > 0.05$ ). Padhi et al. (2013) reported a similar behavior in the increase of egg weight depending on the age of the birds, concluding that the egg increases in weight until week 52, after which it maintains its uniform weight (Padhi et al., 2013). In this study both diets maintained a similar trend until week 12 of laying, after which the silage diet achieved a significant increase in egg weight ( $P < 0.05$ ), possibly due to the fact that the diet with silage had higher levels of plasma (Table 4) and total (Table 5) proteins. These proteins are the primordial precursors of the yolk (vitellogenin and lipoproteins), are synthesized in the liver and transported to the ovaries where they are incorporated into the oocyte in the ovary (Campbell, 2004; Lumeij, 1997; Rosenfeld et al., 1997).

Egg weights were consistent those obtained by Batalha et al. (2018), who used a lower concentration of silage in the diet. This may suggest that using higher concentrations of silage does not adversely affect the weight of eggs over time, and could actually favor it.

On the other hand, the weight gains of the birds in both diets did not show statistically significant differences during the weeks of the study ( $P > 0.05$ ) (Figure 3). Abelti (2018) obtained similar results by feeding broilers by adding different levels of *Barbus paludinosus* silage (Abelti, 2018) in the feed. Additionally (Al-Marzooqi et al., 2010), these authors showed that replacing soybean meal with fish silage protein improved the growth performance of broilers. In general, the amino acid content and protein quality of animal sources tend to be of better biological quality and consequently obtain better responses in weight and production compared to diets from plant sources (Rosenfeld et al., 1997).

#### 4. Conclusions

The chemical silage of red tilapia viscera has the appropriate microbiological conditions in accordance with Colombian national regulations

to be used as a protein raw material in the feeding of different species of birds. For this reason, this kind of silage can be used as an alternative protein raw material in the elaboration of diets for laying hens, thus substituting conventional raw materials such as fishmeal and soybean meal, without modifying the productive variables, percentage of laying, or weight gain. Likewise, the diets that include silage have positive effects on the health and nutrition of the birds within the established parameters, and do not show any negative alterations in blood chemistry or hemograms (from the red and white series). The inclusion of acidic silage from red tilapia viscera in the diet of laying hens significantly decreased the percentage of blood cholesterol and triglycerides in the birds.

#### Declarations

##### Author contribution statement

Yhoan S. Gaviria G.: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Luis F. Londoño F.: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

José E. Zapata M.: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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##### Data availability statement

Data included in article/supplementary material/referenced in article.

##### Declaration of interests statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

#### References

- Abelti, A.L., 2018. Evaluation of small barbus silage through inclusion into commercially formulated poultry feed. *Int. J. Poult. Sci.* 2 (1), 1–7.

- Al-Marzooqi, W., Al-Farsi, M.A., Kadim, I.T., Mahgoub, O., Goddard, J.S., 2010. The effect of feeding different levels of sardine fish silage on broiler performance, meat quality and sensory characteristics under closed and open-sided housing systems. *Asian Austral. J. Anim.* 23 (12), 1614–1625.
- AOAC, 1995. Determination of ash in animal feed AOAC 942.05. *Assoc. Offic. Anal. Chem.* 1.
- AOAC, 2000a. Crude protein AOAC 954.01. *Assoc. Offic. Anal. Chem.* 1.
- AOAC, 2000b. Moisture content AOAC 930.15. *Assoc. Offic. Anal. Chem.* 1.
- AOAC, 2003. Ether extract in animal feed AOAC 920.35. *Assoc. Offic. Anal. Chem.* 1.
- Arias, L., Gómez, L.J., Zapata, J.E., 2017. Efecto de temperatura-tiempo sobre los lípidos extraídos de Vísceras de tilapia roja (*Oreochromis sp.*) utilizando un proceso de calentamiento-congelación. *Inf. Tecnol.* 28 (5), 131–142.
- Batalha, O. de S., Alfaia, S.S., Cruz, F.G.G., Jesus, R.S., Rufino, J.P.F., Silva, A.F., 2018. Pirarucu by-product acid silage meal in diets for commercial laying hens. *Rev. Bras. Cienc. Avic.* 20 (2), 371–376.
- Bennett, J., Billett, A., Salter, M., Mangiapane, H., Bruce, S., Anderton, L., Marenah, B., Lawson, L., 1995. Modulation of hepatic apolipoprotein B, 3-hydroxy-3-methylglutaryl-CoA reductase and low-density lipoprotein receptor mRNA and plasma lipoprotein concentrations by defined dietary fats. *Biochem. J.* 167.
- Botello, A.J., 2010. Conservación in vitro de Tres ensilajes de Pescado (opisthionema oglinum) caracterización físico-química. *Rev. Electrón. Granma Ciencia* 14 (1027-975X), 1–15.
- Buitrago, L., Delfadillo, A., Guzmán, N., Fernández, N., Mejía, I., González, M., del P., Montes, F., Echeverri, D., 2005. Disfunción endotelial inducida por hipercolesterolemia: estudio in vitro en un modelo animal. *Rev. Med. Vet.* 13 (1), 37–44.
- Clarke, Steven, et al., 2002. Fatty acid regulation of gene expression a genomic explanation for the benefits of the mediterranean Diet. *Ann. N. Y. Acad. Sci.* 283–298.
- Campbell, T.W., 2004. Clinical chemistry of birds. In: Williams, Lippincott, Wilkins (Eds.), *Veterinary Hematology and Clinical Chemistry*, first ed., pp. 479–492.
- Cardoso, G.R., Lopez, E.M., Solarana, F.R., Peon, A.M., Rodriguez, N., Mendoza, M., 2002. White Leghorn con baja productividad inoculadas parenteralmente con un bioestimulador vegetal (*Eichornia crassipea*). III. Evaluación del suero sanguíneo y cuadro hemático. *Rev. Prod. Anim.* 4.
- Chalamaiah, M., Hemalatha, R., Jyothirmayi, T., 2012. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. *Food Chem.* 135, 3020–3038.
- Clark, P., Boardman, W., Raidal, S., 2012. Atlas OF clinical avian HEMAYOLOGY. In: W. B., Phillip Clark, S.R., Blackwell (Eds.), Vol. 66. Wiley-Blackwell.
- Directivas técnicas de alimentos para animales y sales mineralizadas, 1999, p. 13.
- FAO, 2013. Revisión del Desarrollo Avícola. In: *Revisión del desarrollo avícola*. <http://www.fao.org/docrep/019/i3531s/i3531s.pdf>.
- FAO, 2018. El estado mundial de la pesca y la acuicultura. <http://ftp.fao.org/docrep/fao/007/y5600s/y5600s00.pdf>.
- Fernández Herrero, A., Tabera, A., Agüeria, D., Manca, E., 2013. Obtención, caracterización microbiológica y fisicoquímica de ensilado biológico de anchoita (*Engraulis anchoita*). *Rev. Electrón. Vet* 14, 1695–7504.
- Goosen, N.J., de Wet, L.F., Görgens, J.F., Jacobs, K., de Bruyn, A., 2014. Fish silage oil from rainbow trout processing waste as alternative to conventional fish oil in formulated diets for Mozambique tilapia *Oreochromis mossambicus*. *Anim. Feed Sci. Technol.* 188, 74–84.
- Güllü, K., Acar, Ü., Tezel, R., Yozukmaz, A., 2014. Replacement of fish meal with fish processing by-product silage in diets for the rainbow trout, *Oncorhynchus mykiss*. *Pak. J. Zool.* 46 (6), 1697–1703.
- Gutiérrez Castro, L.L., Corredor Matus, J.R., 2017. Parámetros sanguíneos y respuesta inmune en pollos de engorde alimentados con probióticos. *Vet. Anim. Sci.* 11 (2), 81–92.
- ICONTEC, 1999. NTC 3688 Alimentos Para Animales, p. 1.
- Kaneko, J., Harvey, J., Bruss, M., 2008. Clinical biochemistry of domestic animals. In: Press, A. (Ed.), *Clinical Biochemistry of Domestic Animals*, 6.
- Kjos, N.P., Herstad, O., Skrede, A., Øverland, M., 2001. Effects of dietary fish silage and fish fat on performance and egg quality of laying hens. *Can. J. Anim. Sci.* 81 (2), 245–251.
- Latimer, K.S., Bienzle, D., 2000. Determination and interpretation of the avian leukogram. In: Williams, C.M. (Ed.), *Schalm's Veterinary Hematology*, fifth ed.
- Llanes, J.E., Bórquez, A., Toledo, J., De, M.L., 2010. Digestibilidad aparente de los ensilajes de residuos pesqueros en tilapias rojas (*Oreochromis mossambicus* x *O. niloticus*). *Zootec. Trop.* 28, 499–505.
- Lumeij, J.T., 1997. Avian clinical biochemistry. In: Press, A. (Ed.), *Clinical Biochemistry of Domestic Animals*, fifth ed., p. 962.
- Maldonado Saavedra, O., Ramírez Sánchez, I., García Sánchez, J.R., Ceballos Reyes, G.M., Méndez Bolaina, E., 2012. Colesterol: función biológica e implicaciones médicas Cholesterol: biological function and medical implications. *Rev. Mex. Cienc. Farm.* 43 (2), 7–22.
- Martínez-Alvarez, O., Chamorro, S., Brenes, A., 2015. Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: a review. *Food Res. Int.* 73 (1069), 204–212.
- Ministerio de Agricultura, 2017. Cadena de la Acuicultura 2017. <https://sioc.minagricultura.gov.co/Acuicultura/Documentos/002%2520-%2520Cifras%2520Sectoriales/002%2520-%2520Cifras%2520Sectoriales%2520-%25202017%2520-%2520Junio%2520Acuicultura.pptx+&cd=1&hl=es-419&ct=clnk&gl=co>.
- Nogueira, C.M., et al., 2003. The effect of supplementing layer diets with shark cartilage or chitosan on egg components and yolk lipids. *Br. Poult. Sci.* 218–223.
- Padhi, M.K., Chatterjee, R.N., Haunshi, S., Rajkumar, U., 2013. Effect of sulfonamides on egg quality in colour layers. *Indian J. Vet. Pathol.* 48–1 (2), 122–125.
- Palou, A., Catalina, O., Segura, P., Luisa, M., Piña, B., Vara, P.O., Vich, F.S., María, A., Guerrero, R., Riutort, J.R., 2005. El libro blanco de los Esteroles Vegetales, second ed. [http://www.nutricion.org/publicaciones/pdf/libro\\_blanco\\_esteroles\\_vegetales.pdf](http://www.nutricion.org/publicaciones/pdf/libro_blanco_esteroles_vegetales.pdf).
- Ramírez, J.C.R., Ibarra, J.I., Romero, F.A., Ulloa, P.R., Ulloa, J.A., Matsumoto, K.S., Cordoba, B.V., Manzano, M., Ángel, M., 2013. Preparation of biological fish silage and its effect on the performance and meat quality characteristics of quails (*Coturnix coturnix japonica*). *Braz. Arch. Biol. Technol.* 56 (6), 1002–1010.
- Ravindran, V., 2013. Disponibilidad de piensos y nutrición de aves de corral en países en desarrollo - avances en la nutrición de las aves de corral. *Fao* 1–4.
- Rosenfeld, D.J., Gernat, A.G., Marcato, J.G., Lopez, G.H., Flores, J.A., 1997. The effect of using different levels of shrimp meal in broiler diets. *Int. J. Poult. Sci.* 76, 581–587.
- Rostagno, H.S., Teixeira, L.F., Lopez, J., Cezar, P., Flávila de Oliveira, R., Clementino, D., Soares, A., Luiz de Toledo, S., Frederico, R., 2011. Tablas Brasileñas Para Aves Y Cerdos, pp. 157–166.
- Sanchez, G., 2009. Función Hepática Y Parámetros Analíticos. Laboratorio de Analisis Veterinario Arturo Soria, p. 6. [http://www.lav-asoria.com/content/781927/funci\\_n\\_hep\\_tica.pdf](http://www.lav-asoria.com/content/781927/funci_n_hep_tica.pdf).
- Schmidt, E.M., dos, S., 2007. Hematological and serum chemistry values for the ring-necked pheasant (*Phasianus colchicus*): variation with sex and age. *Int. J. Poult. Sci.*
- Schmidt, E.M., dos, S., Paulillo, A.C., Martins, G.R.V., Lopera, I.M., Testi, A.J.P., Junior, L.N., Denadai, J., Fagliari, J.J., 2009. Hematology of the bronze Turkey (*Meleagris gallopavo*): variations with age and gender. *Int. J. Poult. Sci.* 8 (8), 752–754.
- Smárason, B.Ö., Ögmundarson, Ó., Árnason, J., Björnsdóttir, R., 2017. Life cycle assessment of Icelandic arctic char fed three different feed types. *Turk. J. Fish. Aquat. Sci.* 17 (1303–2712), 79–90.
- Salma, U., et al., 2007. Effect of dietary rhodobacter capsulatus on egg-yolk cholesterol and laying hen performance. *Poult. Sci.*
- Spanopoulos-Hernandez, M., Ponce-Palafox, J.T., Barba-Quintero, G., Ruelas-Inzunza, J.R., Tiznado-Contreras, M.R., Hernández-González, C., Shirai, K., 2010. Production of biological silage from fish waste, the smoked yellowfin tuna (*Thunnus albacares*) and fillet of tilapia (*Oreochromis sp.*), for feeding aquaculture species. *Rev. Mex. Ing. Quim.* 9 (2), 167–178. <http://www.scopus.com/inward/record.url?eid=2-s2.0-79960206448&partnerID=40&md5=aebc679fc5dee73f2cd9d052af5db5c>.
- Suarez, L.M., Montes, J.R., Zapata, J.E., 2018. Optimización del Contenido de Ácidos en Ensilados de Vísceras de Tilapia Roja (*Oreochromis spp.*) con Análisis del Ciclo de Vida de los Alimentos Derivados. *Inf. Tecnol.* 29 (6), 83–94.
- Thrall, M.A., 2004. In: *Veterinary Hematology and Clinical Chemistry*. Williams & Wilkins, p. 518.
- Vidotti, R.M., Viegas, E.M.M., Carneiro, D.J., 2003. Amino acid composition of processed fish silage using different raw materials. *Anim. Feed. Sci. Tech.* 105 (1–4), 199–204.
- Voigt, G.L., 2003. *Conceptos y técnicas hematológicas para técnicos veterinarios* (ACRIBIA).