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# Addition of seaweed flour (*Lithothamnium calcareum*) combined with homeopathic feed for broiler chickens decreases the toxic effects caused by aflatoxin B1

**Adição de farinha de algas marinhas (*Lithothamnium calcareum*) na ração combinada com homeopático para frangos de corte diminui os efeitos tóxicos causados pela aflatoxina B1**

**La adición de harina de algas marinas (*Lithothamnium calcareum*) no alimentos combinada con homeopáticos para pollos de engorde disminuye los efectos tóxicos causados por la aflatoxina B1**

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**Abstract**

The aim of this study was to determine whether a combination of seaweed flour (*Lithothamnium calcareum*) and a homeopathic added to broiler feed has a hepatoprotective effect capable of minimizing the negative impacts of aflatoxin B1 toxicosis. We used birds one day divided into four groups with six repetitions per group and 20 birds per repetition in a completely randomized design. The combination of seaweed flour + homeopathic product was identified as the test product (TP). The groups were identified as PC: intake of 300 µg/kg of AFB1 (positive control); PC+TP: intake of 300 µg/kg of AFB1 + 2 g/kg of TP; NC: birds that received control feed; and TP: supplemented with 2 g/kg TP. The birds in the PC+TP, NC and TP groups had the higher body weights on days 35 and 42 days than in the PC group. This same effect was also observed for daily weight gain and feed conversion for 1–42 days. A

higher villus: crypt relationship was observed in PC + TP, NC and TP compared to PC. The seaweed flour combined with the TP minimizes the negative effects of AFB1 on the liver health of broilers and consequently avoids losses in zootechnical performance.
**Keywords:** Homeopathy; Mycotoxin; Poultry; Seaweed.

**Resumo**
O objetivo deste estudo foi determinar se uma combinação de farinha de algas marinhas (*Lithothamnium calcareum*) e um homeopático adicionado à ração de frangos de corte tem um efeito hepatoprotetor capaz de minimizar os impactos negativos da intoxicação por aflatoxina B1. Foram utilizadas aves de um dia, divididas em quatro grupos com seis repetições por grupo e 20 aves por repetição em delineamento inteiramente casualizado. A combinação de farinha de algas + produto homeopático foi identificada como produto teste (TP). Os grupos foram identificados como PC: ingestão de 300 µg/kg de AFB1 (controle positivo); PC+TP: ingestão de 300 µg/kg de AFB1 + 2 g/kg de TP; NC: aves que receberam ração controle; e TP: suplementado com 2 g/kg de TP. As aves dos grupos PC+TP, NC e TP apresentaram os maiores pesos corporais nos dias 35 e 42 do que no grupo PC. Esse mesmo efeito também foi observado para ganho de peso diário e conversão alimentar por 1 a 42 dias. Uma maior relação vilo:cripta foi observada em PC+TP, NC e TP em comparação com PC. A farinha de alga combinada com o TP minimiza os efeitos negativos da AFB1 na saúde hepática de frangos de corte e consequentemente evita perdas no desempenho zootécnico.
**Palavras-chave:** Homeopatia; Micotoxina; Aves; Alga marinha.

**Resumen**
El objetivo de este estudio fue determinar si una combinación de harina de algas marinas (*Lithothamnium calcareum*) y un homeopático agregado al alimento para pollos de engorde tiene un efecto hepatoprotector capaz de minimizar los impactos negativos de la toxicosis por aflatoxina B1. Se utilizaron aves un día divididas en cuatro grupos con seis repeticiones por grupo y 20 aves por repetición en un diseño completamente al azar. La combinación de harina de algas marinas + producto homeopático se identificó como producto de prueba (TP). Los grupos fueron identificados como CP: ingesta de 300 µg/kg de AFB1 (control positivo); PC+TP: ingesta de 300 µg/kg de AFB1 + 2 g/kg de TP; NC: aves que recibieron alimento control; y TP: suplementado con 2 g/kg de TP. Las aves en los grupos PC+TP, NC y TP tuvieron pesos corporales más altos en los días 35 y 42 que en el grupo PC. Este mismo efecto también se observó para la ganancia de peso diaria y la conversión alimenticia durante 1 a 42 días. Se observó una mayor relación vellosidad: cripta en PC+TP, NC y TP en comparación con PC. La harina de algas marinas combinada con el PT minimiza los efectos negativos de AFB1 sobre la salud hepática de los pollos de engorde y, en consecuencia, evita pérdidas en el rendimiento zootécnico.
**Palabras clave:** Homeopatía; Micotoxinas; Aves; Algas.

## 1. Introduction

Mycotoxins are secondary metabolic low molecular weight produced by fungi. They occur in approximately 25% of cultures worldwide, causing substantial economic damage (Magnoli et al. 2017). Aflatoxin is a mycotoxin in the metabolism of *Aspergillus* spp., a fungus that produces four types of mycotoxins (B1, B2, G1 and G2) that can contaminate cereals (Buszewska-Forajta 2020). Aflatoxin B1 (AFB1) is produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and is considered the most toxic among the four types mentioned (Anater et al. 2020) because AFB1 causes hepatotoxic, carcinogenic, and teratogenic effects (Magnoli et al. 2017).

Birds are among the animals that are most sensitive to mycotoxins (Magnoli et al. 2017). When AFB1 is consumed by broilers, it reduces animal performance, in addition to causing changes in the intestinal mucosa, metabolism, and immunity (Abdolmaleki et al. 2019). The toxicity of mycotoxins in birds depends on factors such as exposure time, health status, contaminating dose, in addition to the sensitivity of the organism's exposure to toxins (Buszewska-Forajta 2020).

Adsorbents are molecules that adhere to mycotoxins to make them unavailable before they are absorbed into the bloodstream. Aflatoxins are thermostable, resistant to some chemicals, and soluble in solvents and insoluble in fats and oils; these features hinder the effectiveness of adsorbents (Khaleghipour et al. 2019). Therefore, natural alternatives have been sought that have an adsorbent effect and/or minimize the toxic effects caused by mycotoxins in birds. Souhaili et al. (2004) suggested that microalgae can inhibit the action of fungi and inactivate mycotoxins. *Lithothamnium calcareum* is a calcareous seaweed that belongs to the group of red or rhodophyte algae of the Coralineacea family (Melo & Moura 2009). This seaweed

is composed of calcite, aragonite and vaterite (Schlegel & Gutzwiller 2016), and has been studied in the feed of pigs and broilers as a possible source of calcium (Schlegel & Gutzwiller, 2016; Carlos et al. 2011). In addition, it is a renewable source of macro and micro minerals, and its growth depends on natural light (Carlos et al. 2011). Formulated as seaweed flour, we associated it with a homeopathic product produced and marketed for its protective action on the liver, as was described for another homeopathic product used to prevent acute carbon tetrachloride-induced hepatotoxicosis (Moncorvo et al. 1998).

In the field, poultry farmers and technicians have reported that the use of this seaweed meal in poultry feed has reduced the occurrence of problems with mycotoxins, as well as, the use of homeopathic has been indicated to protect the liver of broilers during the production cycle. The liver is of course an extremely important organ in metabolism and nutrition. Therefore, the objective of this study was to determine whether a combination of seaweed flour (*L. calcareum*) and a homeopathic added to broiler feed has a hepatoprotective effect capable of minimizing the negative impacts of aflatoxin B1 toxicosis.

## 2. Methodology

This project was approved by the Committee for the Use of Animals in Research (CEUA) of the Universidade do Estado de Santa Catarina (UDESC) under protocol number 7573270119. It complies with the rules issued by the National Council for Control of Animal Experimentation (CONCEA).

### 2.1 Products and mycotoxin

#### 2.1.1 Test product

OrgaAves (homeopathic product) was incorporated into commercial algae flour (*L. calcareum*) at a concentration of 10%, that is, 90% of the mixture was algae flour. Seaweed flour is a commercial product (*Lithothamnium*) from the company Oceana used as a vehicle for homeopathy in our study. It was chosen mainly for having mycotoxin adsorption characteristics described in the introduction. OrgaAves is a commercial hydroalcoholic solution formulated according to the recommendations of the Homeopathic Pharmacopeia, using the following ingredients: *Arsenicum album* (30CH), *Avena sativa* (6CH), *Calcarea carbonica* (30CH), *Colibacillinum* (30CH), *Echinacea angustifolia* (6CH), *Lachesis muta* (30CH), *Nux vomica* (30CH) and *Pyrogenium* (30CH). In our study, we combined the homeopathic product + seaweed flour to produce a test product (TP), in a powdered formulation.

#### 2.1.2 Aflatoxin B1

Aflatoxins were produced by fermentation in rice, converted under constant agitation and at controlled temperature. The NRLL 2999 strain of A. parasiticus was used according to the method described by West et al. 1973. After autoclaving with a valve opening, the material was dried in a forced ventilation oven and ground in a laboratory mill equipped with a 1-mm sieve. The concentration of aflatoxins was subsequently determined using HPLC (Thorpe et al. 1982). Based on this information, we stipulated a concentration of 300 µg aflatoxin/kg of feed to challenge the birds during the trial period.

### 2.2 Animals, accommodation and food

Was used male day-old chicks of the Cobb strain, acquired from a commercial hatchery located in the city of Chapecó, SC, Brazil. Upon reaching the experimental shed, the birds were immediately weighed and randomly distributed in a completely randomized experimental design including four treatments, with six repetitions per group and 20 birds per repetition. The birds were kept in pens of 1.88 m2, on beds of wood shavings and exposed to a light program in accordance with the lineage manual.

The basal feed used in the present study was formulated based on crushed corn and soybean meal (Table 1), according to the nutritional requirements for broilers described in the Brazilian Tables for Poultry and Swine (Rostagno et al. 2017). All groups received the same basal feed. The groups were identified as PC: birds that received feed contaminated with 300 µg/kg of AFB1 (positive control); PC+TP group: birds that received feed contaminated with 300 µg/kg of AFB1 and supplemented with 2 g/kg of TP; NC group: birds that received control feed (free AFB1 and TP); and TP group: birds that received feed supplemented with 2 g/kg TP.

**Table 1:** Ingredients and basal feed used for all experimental groups.

| Ingredients (kg) | Age (days) | | |
|---|---|---|---|
| | 1 – 21 | 22–35 | 36–42 |
| Corn | 58.4 | 62.2 | 65.9 |
| Soybean meal | 35.7 | 30.4 | 26.9 |
| Soy oil | 1.01 | 3.21 | 3.21 |
| Bicalcium phosphate | 2.05 | 1.81 | 1.66 |
| Calcitic limestone | 0.81 | 0.73 | 0.69 |
| Iodized salt | 0.53 | 0.49 | 0.46 |
| DL-Methionine | 0.37 | 0.24 | 0.23 |
| Lysine | 0.43 | 0.25 | 0.29 |
| Threonine | 0.16 | 0.05 | 0.06 |
| Kaolin (inert) | 0.20 | 0.20 | 0.20 |
| Premix of vitamins[1] | 0.15 | 0.15 | 0.15 |
| Premix of minerals[2] | 0.15 | 0.15 | 0.15 |
| Lincomycin (g) | 1.00 | 1.00 | - |
| Diclazuril (g) | 200 | 200 | - |
| Calculated chemical composition | | | |
| Metabolizable Energy (Mcal/kg) | 2.9600 | 3.1500 | 3.200 |
| Crude protein (%) | 22.1 | 19.7 | 18.5 |
| Calcium (%) | 0.94 | 0.83 | 0.77 |
| Available phosphorus (%) | 0.47 | 0.41 | 0.38 |
| Digestible lysine (%) | 1.36 | 1.09 | 1.04 |
| Chloride (%) | 0.38 | 0.83 | 0.35 |
| Digestible methionine + cysteine (%) | 0.96 | 0.79 | 0.75 |
| Digestible threonine (%) | 0.88 | 0.71 | 0.68 |
| Digestible tryptophan (%) | 0.23 | 0.21 | 0.19 |
| Sodium (%) | 0.22 | 0.20 | 0.19 |
| Linoleic acid (%) | 1.81 | 3.00 | 3.04 |

**Note:** [1] Minimal vitamin levels per kg of food: vitamin A (5000000 IU); vitamin D3 (1,000,000 IU); vitamin E (15000 IU); vitamin K3 (1,500 mg); vitamin B1 (1,500 mg); vitamin B2 (3,000 mg); vitamin B6 (2,000 mg); vitamin B12 (7,000 mcg); folic acid (500 mg); nicotinic acid (15 g); pantothenic acid (7,000 mcg); choline (80 g); biotin (100 mg); minimum humidity (40 g); maximum mineral matter (500 g).
[2] Minimal mineral levels per kg of feed: copper (10 g); iron (50 g); iodine (1.000 mcg); manganese (80 g); selenium (300 mg); zinc (70 g); minimum humidity (20 g); maximum mineral matter (980 g).Source: Authors.

Aflatoxin was pre-mixed in small amounts of soybean meal, and subsequently it was incorporated into the vehicle (mineral/vitamin premix with soybean meal), and then the feed was beaten with all the ingredients. The experimental period was 42 days, when the experimental feed was supplied according to the production phase (initial, growth and termination). Water was provided ad libitum.

The levels of aflatoxins in all feeds were measured using HPLC, with immunoaffinity purification, post-column derivatization and fluorescence detection; the methodology was described in detail by Muller et al. (2017). The analysis showed the real levels of aflatoxin in the feeds were: PC: 326 µg/kg, PC+TP: 320 µg/kg NC: 21 µg/kg, TP: 25 µg/kg.

**2.3 Performance**

The birds were weighed on days 1, 21, 35, and 42 of the experiment using a digital scale. From these data, the body weight of the birds, the daily weight gain were determined, by calculating the subtraction of the final weight of the group, less the initial weight divided by the number of birds. The feed intake (g/bird/day) was obtained through the difference between the feed provided at the beginning and the heavy leftovers at the end of each period. The feed conversion was calculated by the total amount of feed ingested divided by the live weight of the birds.

**2.4 Sample collection**

Blood was collected from six birds per group at 21 and 42 days of experiment. The birds were contained manually, and using an insulin syringe, blood was collected from the ulnar vein. This material was allocated in two tubes, one with EDTA to obtain whole blood for hematological analysis, and another tube without anticoagulant to obtain serum. Subsequently, blood was centrifuged at 3500 rpm for 10 minutes and the serum separated, collected and frozen (–20 ℃) for biochemical analysis.

**2.5 Histopathology**

Samples of liver and jejunum of six birds by group were collected and preserved in vials containing 10% formaldehyde on day 42. It is important to note that the location (fragment) of the intestine collected was standardized, in order to obtain similar and representative samples from the same segment. Slides with histological sections were generated and stained with hematoxylin and eosin. The length of the villi and the depth of the crypts were determined according to Caruso & Demonte (2005) and described in detail by Galli et al. (2020).

**2.6 Blood analysis**

**2.6.1 Serum biochemistries**

Biochemical variables were measured in the serum: protein total, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) using commercial analytical kits (Analisa®) and a semi-automatic biochemical analyzer BioPlus (Bio-2000®). Globulin values were calculated as total proteins – albumin.

**2.6.2 Serum oxidant/antioxidant status**

Liver fragments ere weighed and placed in test tubes. The liver was homogenized in 10 mmol Tris-HCl (pH 7.2) to measure oxidizing and antioxidant compounds. The samples were stored in tubes and frozen at –20 °C for further analysis, together with the serum.

The levels of free radicals (ROS) in the plasma and liver were determined according to the technique described by Halliwell and Gutteridge (2007). The samples were diluted 1:10 with 10 mM Tris (pH 7.4) and 5 µl of dichlorofluorescein diacetate (DCFH-DA). The result was expressed in U DCF/mg of protein. Levels of non-homogeneous lipid peroxidation in the liver were determined by levels of antibodies reactive to thiobarbituric acid (TBARS), measured by the absorbance of the red product at 532 nm, according to the method described by Ohkawa et al. (1978) and expressed in nmol MDA/mg protein. Hepatic carbonyl protein content was determined in supernatants as described by Reznick & Packer (1994), and the results were expressed as nmol of carbonyls formed/mg of protein.

The activity of glutathione S-transferase (GST) was determined in both the liver and serum according to Habig et al. (1974), with modifications. GST activity was determined as the rate of formation of dinitrophenyl-S-glutathione at 340 nm in a medium containing 50 mM potassium phosphate, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) as substrate

and tissue supernatants (approximately 0.045 mg protein). The results were expressed as U GST/mg protein. Catalase activity (CAT) was determined in the liver by the decomposition of H2O2 at 240 nm according to the method described by Nelson & Kiesow (1972). CAT results were expressed in nmol CAT/mg protein.

### 2.7 Statistical analysis

We used a completely randomized design. All variables were subjected to the normality test (Shapiro–Wilk); the variables that did not have normal distribution were transformed into logarithms. Subsequently, the data were subjected to analysis of variance (ANOVA) and Tukey's test, in which differences between treatments were considered when $P < 0.05$.

## 3. Results

### 3.1 Performance

The results of animal performance were presented in Table 2. At 21 days the body weight per bird was higher in the PC+TP and TP groups in relation to the PC ($P < 0.05$). On days 35 and 42, the body weight per bird was higher in all groups compared to PC ($P < 0.05$). This same effect was observed for daily weight gain ($P < 0.05$). Feed consumption over 1–42 days was higher in all groups in relation to PC ($P < 0.05$). The feed conversion of 1–35 days was lower in PC+TP compared to PC and TP ($P < 0.05$). Over 1–42 days, feed conversion was lower in the PC+TP, NC, and TP groups compared to the PC ($P < 0.05$). There was no difference in mortality between groups during the entire experimental period ($P > 0.05$).

### 3.2 Histopathology

No changes were observed in the liver of broilers for all treatments. Larger intestinal villus size, lesser crypt depth and greater villus:crypt ratio were observed in PC+TP, NC and TP treatments compared to PC ($P < 0.001$; Table 3).

### 3.3 Serum biochemistry

At 21 days, lower levels of ALT were observed in the NC and TP groups compared to the PC ($P < 0.05$). At 42 days, lower ALT levels were also observed in the TP group compared to PC ($P < 0.05$), whereas levels of AST and GGT were lower in the NC and TP groups compared to PC ($P < 0.001$). For the levels of total proteins, albumin, globulin, ROS, TBARS and GST, no differences were observed at 21 and 42 days ($P > 0.05$). While at 21 days there was no difference between groups for the AST and GGT enzymes ($P > 0.05$; Table 4).

**Table 2:** Growth of broiler chickens fed with experimental feed containing aflatoxin B1 and seaweed flour + homeopathic product.

| Variables | Days | PC | PC+TP | NC | TP | CV (%) | P-Value |
|---|---|---|---|---|---|---|---|
| | 1 | 38.9 | 39.0 | 38.3 | 38.8 | 1.69 | 0.989 |
| Body weight per | 21 | 553.9[b] | 659.7[a] | 604.5[ab] | 636.1[a] | 3.84 | 0.027 |
| broiler (g) | 35 | 1411[b] | 1606[a] | 1552[a] | 1554[a] | 4.96 | 0.018 |
| | 42 | 1935[b] | 2215[a] | 2318[a] | 2225[a] | 4.07 | 0.001 |
| Daily weight gain | 1-21 | 26.3 [b] | 29.5 [a] | 26.9 [ab] | 28.4 [a] | 1.54 | 0.035 |
| (g) | 1-35 | 65.3 [b] | 74.6 [a] | 72.1 [a] | 72.0 [a] | 2.03 | 0.001 |
| | 1-42 | 90.3 [b] | 103.6 [a] | 108.5 [a] | 104.1 [a] | 2.68 | 0.001 |
| Feed intake (g) | 1-42 | 4271 [b] | 4795 [a] | 4761 [a] | 4728 [a] | 5.63 | 0.025 |
| Feed: gain | 1-21 | 1.57 | 1.54 | 1.58 | 1.58 | 3.05 | 0.337 |
| ratio (FCR) | 1-35 | 1.73 [a] | 1.62 [b] | 1.67 [ab] | 1.71 [a] | 2.59 | 0.050 |
| | 1-42 | 1.86 [a] | 1.68 [b] | 1.72 [b] | 1.74 [b] | 3.64 | 0.001 |
| Mortality (%) | 1-42 | 10.0 | 7.50 | 11.25 | 6.25 | - | - |

* Different letters ([a, b]) within a line indicates significant differences among treatments using Tukey post-hoc test.
(PC): Feed with 300 µg/kg of AFB1 and 0 g/kg of test product (Algae Flour + homeopathic product);
(PC + TP): Feed with 300 µg/kg of AFB1 and 2 g/kg of test product (Algae Flour + homeopathic product);
(NC): Feed with 0 µg/kg of AFB1 and 0 g/kg of test product (Algae Flour + homeopathic product);
(TP): Feed with 0 µg/kg of AFB1 and 2 g/kg of test product (Algae Flour + homeopathic product);
Source: Authors.

**Table 3.** Size of intestinal crypts and villi of 42-day-old broiler chickens fed with an experimental feed containing aflatoxin B1 and seaweed flour + homeopathic product.

| **Treatment** | **Intestinal villi (µm)** | **Intestinal crypts (µm)** | **Villus/crypt ratio** |
|---|---|---|---|
| PC | 1354 [b] | 367.0 [a] | 3.68[b] |
| PC+TP | 1686 [a] | 268.6 [b] | 6.27[a] |
| NC | 1651 [a] | 253.1 [b] | 6.52[a] |
| TP | 1698 [a] | 244.9 [b] | 6.93[a] |
| P-Value | 0.001 | 0.001 | 0.001 |
| CV (%) | 8.06 | 11.7 | 5.35 |

*Different letters ([a, b]) within the same column indicates significant differences among treatments using Tukey post-hoc test.
(PC): Feed with 300 µg/kg of AFB1 and 0 g/kg of test product (Algae Flour + homeopathic product);
(PC + TP): Feed with 300 µg/kg of AFB1 and 2 g/kg of test product (Algae Flour + homeopathic product);
(NC): Feed with 0 µg/kg of AFB1 and 0 g/kg of test product (Algae Flour + homeopathic product);
(TP): Feed with 0 µg/kg of AFB1 and 2 g/kg of test product (Algae Flour + homeopathic product);
Source: Authors.

### 3.4 Hepatic biochemistry

At 42 days, lower levels of ROS were observed in the livers of the NC and TP groups compared to the PC (P> 0.05). The levels of TBARS and protein carbonyl were lower in the PC+TP, NC and TP groups compared to the PC (P <0.05). CAT activity was lower in NC compared to PC and PC+TP (P <0.05), while GST activity was lower in TP compared to PC (P <0.05; Table 5).

**Table 4:** Clinical biochemistry and oxidant and antioxidant status in serum of broiler chickens fed with an experimental feed containing aflatoxin B1 and seaweed flour + homeopathic product as an adsorbent.

| Variables | PC | PC+TP | NC | TP | CV (%) | P-Value |
|---|---|---|---|---|---|---|
| **Day 21** | | | | | | |
| Total proteins (g/dL) | 2.88 | 2.80 | 2.82 | 2.95 | 6.35 | 0.415 |
| Albumin (g/dL) | 1.08 | 1.02 | 1.00 | 1.07 | 2.96 | 0.781 |
| Globulin (g/dL) | 1.80 | 1.77 | 1.82 | 1.87 | 5.94 | 0.389 |
| ALT (U/L) | 13.8[a] | 5.00[ab] | 2.50[b] | 2.00[b] | 7.21 | 0.002 |
| AST (U/L) | 191.3 | 191.2 | 194.6 | 189.0 | 10.8 | 0.821 |
| GGT (U/L) | 20.0 | 17.2 | 16.3 | 18.5 | 5.02 | 0.485 |
| ROS (U DCF/mg of protein) | 68.3 | 71.3 | 63.2 | 67.3 | 16.7 | 0.568 |
| TBARS | 13.9 | 14.5 | 16.3 | 14.6 | 10.3 | 0.796 |
| GST (µmol CDNB/min/mg protein) | 95.4 | 102.7 | 95.8 | 100.4 | 9.06 | 0.830 |
| **Day 42** | | | | | | |
| Total proteins (g/dL) | 2.65 | 3.11 | 2.52 | 2.58 | 7.82 | 0.076 |
| Albumin (g/dL) | 1.21 | 1.45 | 1.22 | 1.27 | 3.85 | 0.142 |
| Globulin (g/dL) | 1.34 | 1.55 | 1.30 | 1.55 | 6.27 | 0.083 |
| ALT (U/L) | 23.6[a] | 15.0 [ab] | 17.5 [ab] | 11.5 [b] | 9.71 | 0.042 |
| AST (U/L) | 176.7 [a] | 182.0 [a] | 142.2 [b] | 140.0 [b] | 13.5 | 0.001 |
| GGT (U/L) | 28.8 [a] | 25.0 [a] | 8.75 [b] | 6.12 [b] | 12.1 | 0.001 |
| ROS (U DCF/mg of protein) | 79.3 | 67.3 | 74.2 | 78.3 | 21.8 | 0.605 |
| TBARS (nmol MDA/mL) | 16.9 | 17.5 | 15.3 | 15.6 | 11.7 | 0.893 |
| GST (µmol CDNB/min/mg protein) | 164.4 | 132.7 | 152.8 | 183.4 | 23.7 | 0.527 |

* Different letters ([a, b, c]) within a line indicates significant differences among treatments using Tukey post-hoc test. **Note:** ROS (reactive oxygen species); GST (glutathione S-transferase);
(PC): Feed with 300 µg/kg of AFB1 and 0 g/kg of test product (Algae Flour + homeopathic product);
(PC + TP): Feed with 300 µg/kg of AFB1 and 2 g/kg of test product (Algae Flour + homeopathic product);
(NC): Feed with 0 µg/kg of AFB1 and 0 g/kg of test product (Algae Flour + homeopathic product);
(TP): Feed with 0 µg/kg of AFB1 and 2 g/kg of test product (Algae Flour + homeopathic product);
Source: Authors.

**Table 5:** Clinical biochemistry and oxidant and antioxidant status in liver of broiler chickens fed with an experimental feed containing aflatoxin B1 and seaweed flour + homeopathic product as absorbent.

| Variables | PC | PC+TP | NC | TP | CV (%) | P-Value |
|---|---|---|---|---|---|---|
| ROS (U DCF/mg of protein) | 106.4[a] | 95.1 [ab] | 79.2 [b] | 78.1 [b] | 10.8 | 0.037 |
| TBARS (nmol MDA/g) | 45.8 [a] | 36.5 [b] | 29.0 [b] | 30.4 [b] | 6.42 | 0.001 |
| Protein Carbonyl (nmol carbonyl/mg protein) | 2.10 [a] | 2.19 [a] | 1.85 [b] | 1.67 [b] | 2.41 | 0.020 |
| CAT (nmol/mg protein) | 0.54 [a] | 0.62 [a] | 0.37 [b] | 0.47 [ab] | 3.96 | 0.050 |
| GST (µmol CDNB/min/mg protein) | 403.3 [a] | 385.2 [ab] | 314.3 [bc] | 280.0 [c] | 26.7 | 0.046 |

* Different letters ([a, b, c]) within a line indicates significant differences among treatments using Tukey post-hoc test. **Note:** ROS (reactive oxygen species); GST (glutathione S-transferase);
(PC): Feed with 300 µg/kg of AFB1 and 0 g/kg of test product (Algae Flour + homeopathic product);
(PC + TP): Feed with 300 µg/kg of AFB1 and 2 g/kg of test product (Algae Flour + homeopathic product);
(NC): Feed with 0 µg/kg of AFB1 and 0 g/kg of test product (Algae Flour + homeopathic product);
(TP): Feed with 0 µg/kg of AFB1 and 2 g/kg of test product (Algae Flour + homeopathic product);
Source: Authors.

## 4. Discussion

Mycotoxin adsorbent additives can act in several ways. The two main ways are adsorption, in which the additive binds to the mycotoxin surface (adsorption); the other involves the additives degrading or transforming the metabolically forms into less toxic substances (biotransformation) (Vila-Donat et al. 2018). Natural additives that are hepatopotectors have been sought that can minimize the toxic effects of mycotoxins, including lipid peroxidation and liver failure (Sakamoto et al. 2018). Liu et al. (2020) observed a reduction in body weight, average daily gain, and feed intake in broilers naturally contaminated with AFB1 and AFB2. Andretta et al. (2011) also found that aflatoxins reduced food intake by 11%, feed conversion by 6%, and weight gain by 11%. According to these authors, 65% of the variation in weight gain was due to lower feed consumption, and only 3% was attributable to the presence of mycotoxins in feeds. Therefore, we sought to solve this problem in broiler production by producing a test product combining seaweed flour and a homeopathic product.

AFB1 is biotransformed in the liver and intestine into toxic metabolites, the main one being AFB 1-exo-8,9-epoxide (AFBO) (Sergent et al. 2008). AFB1 also inhibits protein synthesis due to its interaction with DNA and RNA (Grenier & Applegate, 2013). Liu et al. (2020) observed that aflatoxin causes an inflammatory response in the liver that requires energy to produce cells of the immune system instead of muscle tissue. These negative effects of AFB1 were minimized in this study, evidenced by the finding that poisoned birds who consumed the test product had similar weight gain to the control group, and oxidative lesions in the liver were prevented.

Kulshreshtha et al. (2017) found that supplementation of red seaweed in the laying hen feed maintained animal performance and improved immune status. Abudabos et al. (2013) observed that seaweed improved broiler performance; they suggested that these results were related to the composition of seaweed flour that rich in crude protein being supplemented in the feed, with amino acids including methionine. These facts may explain the excellent result observed in chickens that consumed AFB1 and were supplemented with seaweed flour + homeopathic product, as it minimized the negative effects of AFBI and prevented losses in the growth of birds.

Anas et al. (2020) provided 100 and 500 µg/kg AFB1 to broilers and observed smaller of intestinal villi and villus:crypt ratios and greater depths of crypts. These findings suggest that AFB1 affects the absorption of nutrients, caused by smaller area of absorptive contact and by greater energy expenditure intended for the renewal of intestinal mucosa cells; this also affects animal performance. Wang et al. (2018) observed that 600 µg/kg of aflatoxin in the feed of broiler chickens caused damage to mitochondria and microvilli in the small intestine. In addition, they found a decrease in goblet cells that are responsible for mucin production. Bortoluzzi et al. (2016) found that an algae-based product improved the villus:crypt ratio. This may have occurred due to the product's antioxidant properties. These results corroborate the findings of the present study, and suggest that seaweed flour + homeopathic product improves the physical structure of the broiler intestine, and consequently the absorption of nutrients, which also explains the better animal performance in these birds in relation to the PC.

Jiang et al. (2014) observed an increase in levels of liver enzymes (ALT, GGT and AST) at 21 and 42 days (ALT and GGT) in broilers fed aflatoxin B1, zeralenone, fumonisin, and deoxynivalenol. Rosim et al. (2019) also observed an increase in GGT activity at 35 and 42 days of experiment in broilers poisoned with AFB1. This probably occurs due to the lesions caused by mycotoxin in the liver, which causes these enzymes to leak into the circulation. Andretta et al. (2012) found an increase of 17% in ALT levels, 14% in AST levels and 10% in GGT levels in broilers challenged with aflatoxin. These authors emphasize that this increase may be due to the damage caused to the integrity of the liver cell membrane, which must also have occurred in this study.

Oxidative stress was observed in this study in birds that consumed AFB1; a biochemical disorder characterized by an imbalance between the oxidant and antioxidant system. Therefore, antioxidant enzymes are responsible for eliminating the excess of free radicals produced in the body, thereby reducing cellular and tissue damage (Sakamoto et al. 2018). The liver is

the target organ of AFB1, and it is the primary involved in the response to poisoning, resulting in a series of changes in the metabolism of proteins, carbohydrates, and lipids (Liu et al. 2020). AFB1 in the liver causes oxidative disorders as seen in this study, i.e., increased levels of ROS and TBARS. Mughal et al. (2017) also observed that AFB1 caused oxidative stress due to excessive ROS production, in addition to playing an important role in hepatocyte apoptosis.

CAT is the first enzyme to act in the antioxidant defense system it is responsible for catalyzing H2O2 directly into H2O and O2 in the presence of GSH (Rajput et al. 2019). These facts may explain the increase in CAT activity, which occurred as a response to neutralize the toxic effects generated by free radicals and lipid peroxidation. The synthesis of glutathione depends on two amino acids, methionine and cysteine (Brown-Borg et al. 2018). Therefore, the increase in the activity of this enzyme in case of mycotoxicosis can mean an increase in the requirement or deviation of these amino acids. GST is responsible for causing the detoxification of AFBO, and formation of glutathione conjugates (Eaton & Gallagher, 1994). This fact also explains the increased activity of this enzyme when birds consumed aflatoxin during the production cycle.

The protein oxidative process involves the side chain of amino acids, with emphasis on threonine, proline, lysine, and arginine (Dalle-Donne et al. 2003); when metabolized, these amino acids form groups called carbonyls (aldehydes and ketones). Protein oxidation impairs the functioning of proteasomes and lysosomes, damaging macromolecules (Kolgiri & Patil 2017). For these reasons, carbonyl proteins are used as markers of protein oxidation. Changes in their levels reflect the functions of proteins (Kolgiri & Patil 2017). High levels of carbonyl proteins may indicate protein oxidation and protein dysfunction derived from a disease process (Kolgiri & Patil 2017), in this case mycotoxicosis. Chen et al. (2020) observed increases in carbonyl protein levels in shrimp that consumed AFB1, which can contribute to oxidative damage in the liver and other organs, as seen in the present study.

Homeopathy has as a principle the dilution of preventive and curative substances. In addition, it has been an alternative to replace antimicrobials, as it does not leave residues in animal tissues and the environment (Benez et al. 2004). Banerjee et al. (2011) verified the hepatoprotective effect of Arsenicum album in mice poisoned by arsenic due to the decrease in AST, ALT, glutathione reductase, CAT, succinate dehydrogenase, and SOD, all of which are used as biomarkers of liver toxicity. Debanath et al. (2018) provided an extract of A. sativa for mice and noted that it has the ability to donate the hydroxyls present in its molecule and, thereby, reduce the levels of malondialdehyde and liver damage marker enzymes (AST and ALT), as well as, decrease pro-inflammatory cytokines that also generate free radicals. In addition, A. sativa can protect the liver from alcohol-induced injuries (Mir et al. 2018). In other words, it has hepatoprotective and antioxidant effects.

*Pyrogenium* is used to reduce fever and blood poisoning (Kauser et al. 1992). This homeopathic product reduces the levels of prostaglandins and cytokines that are related to inflammation (Ahmad et al. 2018). Calcarea carbonica has been used to treat cancer patients and has shown an immunomodulatory effect during tumor apoptosis (Saha et al. 2013). Zacharias et al. (2008) tested a homeopathic based on Arsenicum album and Ferrum phosphoricum, alternately with Calcarea carbonica, and observed an antiparasitic effect and improvement in weight gain in sheep. Echinacea angustifolia is a medicinal plant native to the USA that possesses anti-stress activity due to its ability to decrease leukocytes and hormones such as adrenocorticotrophin and cortisol, both of which are associated with increased stress (Roshan et al. 2010).

*Nux vomica* are dried ripe seeds from a Chinese plant (Fan et al. 2018). It stimulates intestinal cells by stimulating the nervous system, in addition to being analgesic and anti-inflammatory for having terpenes, flavonoids, among other components (Guo et al. 2018). Gopalkrishna et al. (2010) observed that N. vomica seeds have hepatoprotective effects; however, this occurs only when the seeds undergo a detoxification process. Colibacillinum is mainly used for the treatment of urinary infections caused by E. coli (Joséa et al. 2018). It decreased the incidence of diarrhea in animals (Fortuoso et al. 2018). These findings suggest that the homeopathic product used in the present study may have acted in the various ways to reduce the negative effects, directly and/or indirectly, of AFBI in broilers.

## 5. Conclusion

This study is innovative because it seeks to understand the effects of the combination of seaweed flour (*L. calcareum*) combined with a homeopathic product in the feed contaminated with AFB1 in broilers. Thus, it is concluded that the test product was able to minimize the negative effects of AFBI on the health of broilers, and consequently avoided growth disorders.

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