https://doi.org/10.22319/rmcp.v16s4.6709

Technical note

Effects of *Bryophyllum daigremontiana* in the broiler diet on productive performance and liver enzymes and serum troponin T in broilers

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Abstract:

The experiment was conducted to determine the effect of adding *Bryophyllum daigremontiana* to the feed of broiler chickens on their productive performance and their blood concentration of liver enzymes and troponin T. For this purpose, 120 1-d-old Cobb chicks were randomly assigned to two treatments: a control group and the addition of 20 g of *B. daigremontiana* per ton of feed. The experiment lasted 49 d, and the body weight, feed intake, and feed conversion were recorded. At the end, blood samples were obtained, and the concentrations of troponin T, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were determined. The data were analyzed in a mixed model of random

and fixed components. Chicks fed with *B. daigremontiana* had higher (P<0.05) daily weight gain and final weight, better feed conversion, and lower (P<0.05) liver enzyme and troponin T concentrations, compared to the control. The authors conclude that *B. daigremontiana* improves the productive performance and liver indicators in broilers.

Keywords: Cardioprotector, Production assay, Enzymes.

Received: 30/05/2024

Accepted: 11/03/2025

Growth promoters are chemical substances added to feed to improve broiler growth, increase feed efficiency, productivity, and increase financial indicators. The most commonly used are antibiotics, whose use is controversial and has even been banned in European Union countries as a measure to prevent resistance of various pathogenic bacteria, which may endanger human and animal health[1,2]. This has led to the exploration of natural alternatives to replace them ―such as the use of leaves, stems, roots or whole specimens of plants with medicinal properties, their essential oils in liquid form[3] or in micro particles[4]; their dried leaves, transformed into flours[5]; their organic acids[6], or their polar and non-polar extracts[7,8,9]― that have shown antioxidant and anti-inflammatory effects[10,11,12], among others, and can improve production rates[13,14]. Among its active principles are bufadienolides, the compounds with the highest proportion (17 to 41 mg/100 g dry weight of the leaf) associated with the therapeutic effects of the plant[15] with cardiotonic effect; its leaves and flowers contain flavonoids, phenolic acids, anthocyanins, alkaloids, saponins, and tannins, as well as steroids[16,17,18]. Its antioxidant effect may be comparable to the effect of ascorbic acid[18]. The objective of this study is to evaluate the effects of the inclusion of *B. daigremontiana* as a dietary additive on the productive performance and blood concentration of liver enzymes and troponin T in broiler chickens.

Whole plants of *B. daigremontiana* were collected at the Desert Research Institute (Instituto de Investigación de Zonas Desérticas) of Universidad Autónoma de San Luis Potosí, Mexico. A certified taxonomist from the Institute identified one reference specimen. The plants were cleaned and dried in a Felisa 40L FE-291D forced air oven (Fabricantes Feligneo, S. A. de C.V. Mexico) at 75 °C to constant weight, dried and ground to 2 mm particle size (Thomas Wiley Mill, Thomas Scientific LLC, Swedesboro, NJ, USA). The dried and ground material was stored in an amber glass container at the medium's temperature (22 °C) until further use.

The research protocol and the procedures with the chickens were reviewed and supervised by a bioethics committee per the Mexican Official Standards for Technical Specifications for

the production, maintenance, and use of laboratory animals. 120 1-d-old male Cobb chicks, average weight 50 ± 5 g, vaccinated against Marek's disease, were housed in battery cages (Petersime Inc., Gettysburg, OH) with a capacity of 10 chicks per cage, in a house with a constant temperature (30 °C) and continuous light regulated by electronic timer. The chicks were randomly divided into two treatments: control (n= 60) and with *B. daigremontiana* (n= 60). Each treatment had six cages with 10 chicks each. Chicks in the starter stage (1 to 7 d of age) were offered a commercial starter powder concentrate with 23 % crude protein, while chicks aged 8 to 49 d were fed a commercial concentrate for fattening with 20 % crude protein (Table 1).

Table 1: Nutritional composition (% DM) of the commercial feed offered to broilers

Nutrient	Starter feed	Feed for fattening
Protein, %	23	20
Energy, Mcal/kg	3.2	3.2
Ether extract, %	3	3
Crude fiber, %	3	3
Nitrogen-free extract	51	51
Ashes	3	3

DM= dry matter, Mcal= mega calories, kg= kilogram.

The dried and ground plant was added at a proportion of 20 g/t feed. Chickens had free access to the concentrate and fresh water during the experimental stage. Every week until day 49, the live weight and feed consumption were recorded, and the feed conversion (FC) was calculated according to North and Bell[19], using the following formula: FC=meal intake/total weight gain.

On d 49, a blood sample was collected from each chick's brachial/ulnar vein and placed in heparinized tubes to determine the content of troponin T and liver enzymes. Subsequently, the chicks were slaughtered per the official standard applicable in Mexico[20]. To quantify the alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), and alkaline phosphatase (ALP, EC 3.1.3.1), a Mindray BA-88A semi-automated chemistry analyzer was used, following the process described by Burtis and Bruns[21]. Troponin T was quantified with an AQT90 FLEX immunoassay analyzer (Radiometer Medical ApS, Denmark) according to the indications proposed by Collinson *et al*[22].

For the statistical analysis, data were analyzed as a completely randomized design with 2 treatments and 6 replicates (cage with 10 chicks) each. For this purpose, a mixed model of the MIXED procedure of SAS[23] —which considers a random component (cage) and a fixed component (treatment)— was used. Means between treatments were regarded as different at $P<0.05$.

No differences in initial weight and feed consumption were observed between treatments, although the chickens fed with *B. daigremontiana* had a higher final weight and daily weight gain ($P<0.05$) and better feed conversion values than the chickens that did not receive the plant. Blood concentrations of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and troponin T were lower ($P<0.05$) in chickens that received *B. daigremontiana* compared to the control (Table 2). No macroscopic damage to the organs was found at the time of slaughter.

Table 2: Effect of *Bryophyllum daigremontiana* addition on the productive performance and serum concentrations of liver enzymes and troponin T in broiler chickens.

	Control	**Treatment**	**SEM**	***P* Value**
Productive performance:				
Initial weight, g	51.3	49.8	0.1	0.10
Final weight, g	2038.9	2472.6*	2.4	0.03
Average daily weight gain, g	40.5	49.8*	0.3	0.02
Feed consumption, g	5479.4	5237.9	3.1	0.12
Feed conversion	2.7*	2.1	0.1	0.04
Serum concentrations:				
Aspartate aminotransferase, IU L^{-1}	352.6 *	210.8	12.4	0.03
Alanine aminotransferase, IU L^{-1}	5.2*	4.1	0.19	0.05
Alkaline phosphatase, IU L^{-1}	84.9*	63.9	1.3	0.04
Troponin T, µg/ml	0.61*	0.47	0.01	0.01

IU L^{-1}= international units per liter, µg/ml = micrograms per milliliter, SEM= standard error of the mean; * significant difference ($P<0.05$).

These positive results on the productive performance, liver enzymes, and troponin T seem to be due to the synergistic effect of the biocompounds present in plants of the genus *Bryophyllum*, which have several pharmacological properties, including antioxidant, anti-inflammatory, hepatic, and cardioprotective activity[24]. Antioxidants contained in *B. daigremontiana*, like phenols, flavonoids, catalase, and quercetin[24,25,26], neutralize free radicals such as superoxide anions, nitric oxide, and peroxynitrites, inhibit enzymes such as xanthine oxidase, lipoxygenase and NADPH oxidase, preventing cell death, and may increase the production of endogenous antioxidants[27], which would explain the decrease in the concentration of liver enzymes and troponin T. Although no research on the use of *B. daigremontiana* in broilers was found in the literature, there is previous evidence reporting toxicity in 2-wk-old chickens when they consumed between 0.8 and 1.2 % of their live weight of *K. daigremontiana* leaves[28]. However, the inclusion of 5 % of a phytopreparation of *B. daigremontiana* in diets of laying hens did not modify the productive parameters, egg quality, serum concentration of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase[26]. The differences between the results of the experiments could be due to the physiological stage of the birds and the percentage of inclusion in the diet, suggesting that

bufadienolides in high amounts can cause toxicity especially in young animals[29]. In rats with induced liver damage, the juice of leaves of this plant reduced the damage to hepatocytes, as well as the serum concentration of aspartate aminotransferase and alanine aminotransferase[30].

The addition of *B. daigremontiana* to reduce troponin B concentrations is a very encouraging strategy for cardioprotection, given that this protein is a first-choice biomarker of cardiac damage[31]. In practice, this can positively impact the fattening of fast-growing broilers, among which there is a high incidence of cardiac dysfunctions due to increased body mass and blood requirement and reduced cardiac capacity[32].

In conclusion, the addition of *B. daigremontiana* could be a viable natural option as a non-antibiotic growth promoter, as it improves the productive performance, which is indicative of a better health status of the animal. However, a better understanding of the biocompounds contained in this plant, as well as their biochemical and physiological effects on animals, is still required.

Conflict of interest

The authors declare that they have no conflict of interest.

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