https://doi.org/10.22319/rmcp.v16i2.6540

Technical note

Larrea tridentata as a feed additive to improve liver development and functionality in broilers

Fabián Francisco Vanoye-Lara [a]

Juan Manuel Pinos-Rodríguez [a*]

Juan Carlos García-López [b]

Samuel López-Aguirre [a]

Jorge Genaro Vicente-Martínez [a]

Argel Flores-Primo [a]

[a] Universidad Veracruzana. Facultad de Medicina Veterinaria y Zootecnia. Miguel Ángel de Quevedo S/N, esq. Yáñez, Col. Unidad Veracruzana 91710. Veracruz, México.

[b] Universidad Autónoma de San Luis Potosí, Facultad de Agronomía y Veterinaria, San Luis Potosí, México.

*Corresponding author: jpinos@uv.mx

Abstract:

The effect of adding the diet with dried and ground leaves of *Larrea tridentata* (LT) as a natural feed additive was evaluated in broilers on productive development, weight and lesions of digestive organs, and serum concentration of liver enzymes. To do this, 200 one-day-old chicks were used and fed with commercial feed with LT in doses of 0, 5, 10, and 15 g kg^{-1} of feed. The productive evaluation lasted seven weeks and recorded live weight and feed intake. At the end, the animals were slaughtered, blood was collected, and the weight of the crop, gizzard, cecum, and duodenum was recorded. Broilers that consumed feed with 5 and 10 g LT showed improvement (P<0.05) in weight gain and feed conversion and a reduction (P<0.05) in serum concentrations of alanine amino transferase, alkaline phosphatase, and aspartate amino transferase compared to broilers that did not consume LT. The weight of the digestive organs was not affected by LT and no apparent macroscopic lesions were observed. The LT dose that had the greatest beneficial effect on the productive variables was 10 g kg^{-1}. Therefore, it is concluded that adding 10 g of LT leaves per kilogram of feed improves the productive response in broilers, possibly as a response to the fact that nordihydroguaiaretic acid reduces liver enzymes, exerting a hepatoprotective effect.

Keywords: Nordihydroguaiaretic acid, Albumin, Liver enzymes, Globulin.

Received: 28/08/2023

Accepted: 10/04/2025

The social demand to produce cleaner animal food with a low carbon footprint and better ethics is increasing, and therefore, there is a constant search for natural alternative forms to chemical ones for the livestock industry. As feed additives, bioactive plant compounds have a variety of effects on animals, from those that promote health and productivity to those that are toxic and lethal. Therefore, biocompounds are viable natural alternatives to some chemicals, drugs, and growth promoters routinely used in the animal feed industry; however, it is true that more documentation, research and standardization are required to determine their optimal level of production efficiency and economic profitability(1).

Larrea tridentata (LT) or creosote bush is a perennial shrub widely distributed in the deserts of northern Mexico and the southern United States of America. Several metabolites have been identified in it, such as phenolic lignans, of which the most studied is nordihydroguaiaretic acid (NDGA), with functionalities such as bactericidal, insecticidal, fungicidal, nematicidal, antiprotozoal, antiviral, antioxidant, antitumor, and neuroprotective(2,3); nevertheless, reports indicate that the oxidation of catechol from NDGA to quinones can have undesirable cellular effects due to alteration of proteins and cellular DNA(4).

Documentary information on the use of creosote bush in animals of zootechnical interest is scarce, but some studies indicate its potential to improve productive and health variables. For example, extracts of LT leaves added to the diet of broilers and lambs improved production parameters and provided some protective activity in the liver by decreasing the concentration of liver enzymes(5,6). None of these animal studies reported toxicity effects from LT consumption.

Therefore, it is necessary to continue researching the use of creosote bush, especially the dose with the best activity as a feed additive since its addition to food in high doses can limit the consumption of the complete diet(7). This work aimed to evaluate the use of different doses of *Larrea tridentata* as a natural feed additive in broiler feed, quantifying the productive parameters, organ weight, and concentration of proteins and liver enzymes in plasma.

Larrea tridentata (LT) specimens were collected in the municipality of Villa Hidalgo, state of San Luis Potosí, Mexico, where the climate is semi-arid, temperate and cold, with a minimum temperature of 8.4 °C and a maximum temperature of 32 °C. The trial with the broilers and necropsies were conducted at the Institute of Research of Desert Areas of the Autonomous University of San Luis Potosí. The procedures were reviewed and approved by the Institute's Bioethics Committee, located in the central area of the state of San Luis Potosí, with the same environmental and climatic conditions from which the LT specimens were collected. The hematological analyses were carried out at the Faculty of Veterinary Medicine and Zootechnics of the Universidad Veracruzana.

A total of 200 one-d-old Cobb 500 chicks with an average live weight of 54.7 ± 2.8 g, vaccinated against Marek, were used. The chicks were placed in four battery-powered electric brooders with integrated thermostat to control the temperature at 37 °C and humidity (Petersime NV, Zulte, Belgium). Each brooder had five levels, that is, a total of 20 levels in the four brooders. Each level (10 chicks) was randomly assigned to one of four experimental diets or treatments (0, 5, 10, and 15 g kg^{-1} of ground *L. tridentata* leaves).

Experimental diets (Table 1) and water *ad libitum* were offered during the experiment. From d 1 to d 7 of age, chicks consumed starter feed and from d 8 to d 49 of age, finisher feed. The experimental diets were prepared in a feed-manufacturing plant in the city of San Luis Potosí expressly for the present study. Once the diets were prepared, in the same feed-manufacturing plant, they were homogeneously mixed (vertical mixer 500, Azteca, Mexico) with the dried and ground leaves of LT according to each treatment.

Table 1: Ingredients and nutrients of the starter and finisher diets for broilers

Ingredient (g kg^{-1})	**Starter**	**Finisher**
Yellow corn	563.11	682.25
Soybean meal 48 % CP	360.28	256.98
Vegetal oil	28.35	20.19
Dicalcium phosphate	17.94	14.64
Calcium carbonate 38 %	15.82	13.92
Salt	4.0	3.53
DL-Methionine 99 %	2.38	1.67
L-Lysine HCL 99 %	1.75	0.57
Choline hydrochloride 60 %	1.07	1.0
Vitamin premix[a]	2.5	2.5
Mineral premix[b]	2.5	2.5
L-Threonine 98.5 %	0.3	0.25
Nutrient (%)[c]		
Metabolizable energy, Mcal kg^{-1}	2.8	3.2
Crude protein	21.73	18.59
Fat	5.3	4.73
Crude fiber	2.91	2.79
Ash	6.66	5.66
Calcium	1.0	0.85
Phosphorus	0.75	0.64
Lysine	1.33	1.01
Methionine	0.9	0.76
Threonine	0.86	0.71

[a] Per kilogram: 0.8 mg thiamine, 2.2 mg riboflavin, 10.0 mg pantothenic acid, 11 mg niacin, 3 mg pyridoxine, 0.25 mg folic acid, 0.1 mg biotin, 0.004 mg vitamin B12, 1500 IU retinyl palmitate, 300 ICU cholecalciferol, 5.0 IU α-tocopheryl acetate, and 0.5 mg menaquinone.

[b] Per kilogram: 0.1 mg selenium, 4 mg copper, 35 mg zinc, 30 mg manganese, 60 mg iron, and 0.35 mg iodine.

[c] Provided by the feed-manufacturing plant.

Daily, on a digital scale (Uline H-9983, Mexico), the feed offered and rejected was weighed and the weekly intake was averaged. The chicks were weighed weekly for 7 wk on a digital scale (Ohaus H-5854, Switzerland). Feed conversion was calculated with weekly intake and weight. At the end of the production trial, the broilers were slaughtered in accordance with the Official Mexican Standard NOM-062-ZOO-1999[8]. An intracardiac blood sample was collected and centrifuged at 2,800 rpm for 15 min to obtain the serum. Blood samples were transported and kept at 4 °C in a portable car refrigerator (Biobase CR-18, Mexico). In the laboratory, the serum was determined for the concentration of alanine amino transferase (ALT), alkaline phosphatase (ALP), and aspartate amino transferase (AST), as well as for total proteins, albumin and globulin, with a Technicon RA 50 Chemistry System (Bayer, Dublin, Germany) and the Spinreact reagent kit (Barcelona, Spain)[9-12]. The weight of the crop, gizzard, cecum, and duodenum was recorded (Ohaus H-5854, Switzerland).

The statistical design used was a completely randomized one with four treatments (diets) and five replications (level) each. In the mixed-component statistical model (MIXED procedure), treatment was considered a random component and replication a fixed component. The comparison of means test was performed by Tukey's test with a significance level of 0.05. Statistical analyses were performed according to the SAS manual[13].

Chicks that consumed the experimental diets with 5 and 10 g of LT had higher (P<0.05) final weights, daily weight gains, and total gains than those fed 0 and 15 g. For its part, feed intake was similar (P>0.05) between treatments, and therefore, broilers that consumed diets with 5 and 10 g LT (Table 2) had the best feed conversions (P<0.05).

Table 2: Live weight, weight gain, and feed conversion of broilers fed with a concentrate feed with different levels of *Larrea tridentata* leaves

	Larrea tridentata **(g kg^{-1} concentrate feed)**				
	0	**5**	**10**	**15**	**SEM**
Initial weight, g	56.3	54.6	53.5	54.4	0.03
Final weight, g	2178.4 ^{b}	2467.9 ^{a}	2490.5 ^{a}	2248.6 ^{b}	1.45
Total gain, g	2122.1 ^{b}	2413.3 ^{a}	2437.0 ^{a}	2194.2 ^{b}	5.35
Daily weight gain, g	43.3 ^{b}	49.2 ^{a}	49.7 ^{a}	44.7 ^{b}	0.32
Feed intake, g	5100.7 ^{a}	4910.1 ^{a}	5010.8 ^{a}	5097.62 ^{a}	4.45
Feed conversion	2.4 ^{a}	2.0 ^{b}	2.0 ^{b}	2.3 ^{a}	0.02

SEM= standard error of the mean;
^{ab} Means with different letters in a row are different (P<0.05).

The weight of the gizzard, duodenum, cecum, bursa of Fabricius, liver, and crop was similar (P>0.05) in chicks fed for 49 d with the experimental diets with 0, 5, 10, and 15 g LT (Table 3).

Table 3: Organ weight of broilers fed with a concentrate feed with different levels of leaves of *Larrea tridentata*

	Larrea tridentata **(g kg^{-1} concentrate feed)**				
Organ weight (g)	**0**	**5**	**10**	**15**	**SEM**
Gizzard	17.5	19.3	21.2	18.4	0.13
Duodenum	12.3	11.9	13.7	12.9	0.56
Cecum	7.2	8.2	7.7	8.3	0.78
Burs of Fabricius	2.6	2.8	2.5	2.9	0.89
Liver	53.5	59.3	58.2	57.7	0.56
Crop	5.3	4.9	4.7	5.5	0.23

SEM= standard error of the mean.

Hematological analyses in 49-d-old broilers indicated that total protein, albumin, and globulin were not modified (P>0.05) by the effect of LT in the diet. Nonetheless, broilers that consumed the diets with 5, 10, and 15 g LT had lower plasma concentrations (P<0.05) of alanine amino transferase (ALT), alkaline phosphatase (ALP), and aspartate amino transferase (AST) compared to broilers that did not consume LT.

In a study in broilers at doses of 20 g LT leaves, body weight and weight gain were also higher compared to the control group, although they noted a significant reduction in feed intake(5) possibly due to NDGA oxidation and low LT palatability(14,15). A proximate analysis carried out in LT indicated that the aerial part of the shrub contains, on a dry basis, 12 % of crude protein, 58 % of neutral detergent fiber, and 10 % of ash, so its incorporation as a feed additive for poultry does not modify the consumption of the whole diet in poultry(6).

The benefits in weight gain and reduction of liver enzymes with the addition of LT leaves in the diet can be directly attributed to the antioxidant capacity of NDGA catechol rings(16,17). Under natural conditions, the antioxidant defenses of broilers maintain the generation of reactive oxygen species in balance, but various stressors, such as the environment or high nutritional demand, induce oxidative stress and metabolic disorders, especially in broilers with high productive performance(18). Under these production conditions, exogenous antioxidant compounds, such as those of LT, would contribute to endogenous antioxidant defense mechanisms. Therefore, diets rich in antioxidants, together with endogenous antioxidants, are essential for the maximum productivity of production animals, such as broilers, as they increase the body's ability to reduce reactive oxygen species, limit oxidative stress, prevent enteric infections, and improve productive performance in poultry(19).

The reduction of liver enzymes due to the effect of LT NDGA found in the present study has been previously corroborated; in a mouse model with induced obesity, Chan *et al*[20] found that NDGA, at a dose of 2.5 g kg^{-1} diet, reduced the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and liver triglycerides in response to the activation of PPARα, a regulator of fatty acid oxidation, and the mRNA of Cpt1c and Cpt2, genes involved in fatty acid oxidation; they also indicated the reduction of liver stress, the expression of CASP3, an apoptosis signaling protein, and the hepatic expression of antioxidant enzymes and GPX4 and PRDX3 proteins was improved; these effects could explain the reduction in the concentration of transaminases found in this study.

Because broilers that received a dose of 10 g kg^{-1} of *Larrea tridentata* leaves in the feed showed a better response in productive performance and decreased liver enzyme levels without altering organ development, it could be considered the best dose-response relationship.

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