https://doi.org/10.22319/rmcp.v16s4.6687

Article

Antibody response to Newcastle vaccination and productivity in chicks supplemented with increasing doses of an extract of *Larrea tridentata*

Juan Carlos García-López [a]

Juan Manuel Pinos-Rodríguez [b*]

Jorge Genaro Vicente-Martínez [b]

Samuel López-Aguirre [b]

Angélica Olivares-Muñoz [b]

Francisco Fabián Vanoye-Lara [b]

[a] Universidad Autónoma de San Luis Potosí. Instituto de Investigación de Zonas Desérticas, Altair 200, Fraccionamiento del Llano, C.P. 78377, San Luis Potosí, México.

[b] Universidad Veracruzana. Facultad de Medicina Veterinaria y Zootecnia, Veracruz, México.

* Corresponding author: jpinos@uv.mx

Abstract:

The objective of this study was to evaluate the effect of aqueous extract of chaparral (*Larrea tridentata*) on the productive performance, hematological profile, white cell count, organ weights, and antibody titers against Newcastle disease in broiler chicks. 600 1-d-old Cobb breed chicks were assigned to the following treatments: 0, 5, 10, 10, 15, 20, and 25 mg of an aqueous chaparral extract per kg of feed. Feed consumption, weight gain, and feed conversion rate were recorded and analyzed. In addition, leukocyte cell counts, Newcastle antibody titers were evaluated and the weight of the thymus, spleen, and Fabricius' bag was recorded. Weight gains were improved (P<0.05) with the chaparral extracts compared to the control group, while the best feed conversion rate (P<0.05) was obtained with 15 mg of the extract. Leukocyte counts and organ weights were not affected by the extract. The highest

titers against Newcastle disease (P<0.05) were found with 15 mg of chaparral extract. This study concludes that an aqueous extract of chaparral at a dose of 15 mg per kg of feed is an alternative to improve the immune response and, therefore, the weight gain in broiler chicks.

Keywords: Antibodies, Newcastle disease, Organs, Chaparral, Creosote bush, *Larrea tridentata.*

Received: 14/05/2024

Accepted: 09/10/2024

Introduction

Antibiotics have been utilized as growth promoters for decades in commercial industrial broilers with excellent production parameters. As a result, these antibiotics are the main factors of antimicrobial resistance in many pathogenic bacteria. This has been a reason for consumers in several countries to question the safety of poultry meat and eggs[(1)]. For this reason, the use of antibiotics for growth promotion has been banned in certain countries. At the same time, other research has been carried out to search for alternatives. For example, plant extracts have received attention as potential replacements for growth promoters[(2)]. The incorporation of medicinal plants into the diet has been shown to provide beneficial effects on the health and performance of poultry due to the antimicrobial activity of their phytocompounds[(3)]. The use of antimicrobial compounds obtained from natural sources has been gaining public approval[(4)].

The creosote bush or chaparral (*L. tridentata*) is a shrubby plant belonging to the Zygophyllaceae family, which dominates the desert of the southwestern United States and north-central Mexico[(5)]. Tea made from the leaves of chaparral has been used in traditional medicine to treat digestive disorders, rheumatism, venereal diseases, sores, and common cold[(6)]. Phytochemical studies carried out with chaparral indicate that it contains lignans, flavonoids, condensed tannins, triterpenes, saponins, and naphthoquinones[(5)], which possess antioxidant, anti-HIV, antimicrobial, and enzymatic inhibitory and antitumor properties[(7,8)]. Nineteen (19) flavonoid aglycones were found in the resin and wax covering the leaves of chaparral, as well as several lignans, in particular nordihydroguaiaretic acid (NDGA), a natural phytochemical lignan extracted from the shrub with properties such as antitumor, anticancer, phenolic antioxidant by inhibiting 5-lipoxygenase, arachidonic acid, and

thromboxane A2 biosynthesis[9]; glycosylated flavonoids, sapogenins, essential oils, and halogenic alkaloids have also been isolated[10].

The hematological characteristics or hemogram of the birds are important as predictors of health because a good balance between hematological cells can improve the immunological and productive response of the birds[11]. Therefore, it is pertinent to research the effects of chaparral on these indicators. The objective of the present study was to evaluate the effect of a chaparral (*Larrea tridentata*) extract in the diet on the productive performance and antibody response of broiler chickens.

Material and methods

Chaparral (*Larrea tridentata*) leaves were collected in December, during the dry season, in the municipality of Matehuala (23.6667 N and 100.583 W), in the state of San Luis Potosí. This site of the Potosi-Zacatecas Highland is flat, with calcareous clayey soil and evidence of secondary disturbance-succession from microphyllous desert scrubland to *L. tridentata* due to the abandonment of corn cultivation. The creosote bush specimens were taxonomically identified by specialists from the Isidro Palacios Herbarium of the Desert Zone Research Institute (Instituto de Investigaciones de Zonas Desérticas) of the Autonomous University of San Luis Potosí (Universidad Autónoma de San Luis Potosí). Leaves were randomly collected from 10 mature shrubs with a minimum height of 1 m and abundant foliage. Approximately 10 % of the total leaves were collected from each shrub in order to form a composite sample.

The leaves were dried under the shade to constant weight and ground in a Thomas 4 mill (Willey, Thomas Scientific, NJ, USA) to a 2-mm particle size. This material was kept away from the light at 22 ºC for later use. The extraction process used was infusion with deionized water at a ratio of 30 ml of solvent per gram of leaf, at 65 ºC for 40 min. The suspension thus obtained was filtered through Whatman grade 4 paper, concentrated in a vacuum apparatus under reduced pressure at a temperature of 38 ºC for 5 h (Gastrovac, GC2000, ICC, Barcelona, Spain), and dried by lyophilization (Labconco, 7400040, USA) until obtaining samples with 7% moisture content. The process was carried out in triplicate.

Animal procedures were reviewed and supervised by a Bioethics Committee per the Official Mexican Regulation on Technical Specifications for the Production, Maintenance, and Use of Laboratory Animals[12]. In a completely randomized design, 600 1-d-old Cobb chicks were assigned to six experimental diets containing 0, 5, 10, 15, 15, 20, and 25 mg of aqueous extract of chaparral (*L. tridentata*) per kilogram of feed. The powdered plant extract was

mixed with the diet. The chicks were housed in brooder battery cages (Petersime Inc., Gettysburg, OH, USA) in a temperature-controlled room (25 °C) under 24-h light. All the chicks were immunized at 7 d of age, via ocular route, with a drop of active Newcastle virus strain La Sota (Newcastle, BioZoo, Zapopan, Jalisco, MX).

The chicks were fed free range with fresh, clean water and concentrate feed formulated to meet the NRC requirements for broiler chicks[13]. The basal diet consisted of 566.33 g/kg yellow corn, 360.28 g/kg soybean paste, 28.35 g/kg vegetable oil, 17.94 g/kg monobasic/dibasic phosphate, 15.82 g/kg calcium carbonate, 4 g/kg sodium chloride, 2.38 g/kg DL-methionine, 1.75 g/kg L-lysine, 1.07 g/kg Choline, 5 g/kg vitamins and minerals, and 0.30 g/kg L-threonine. The chemical composition of the diet was 21.73 % CP, 5.3 % fat, 2.91 % crude fiber, 6.66 % ash, 1 % calcium, 0.75 % phosphorus, 1.33 % lysine, 0.9 % methionine, and 0.86 % threonine.

The experiment lasted 21 d. Feed consumption and initial and final weights were recorded. The weight gains per day and the total and final weight were estimated based on this information. The total weight gain and total feed consumption were used to calculate the feed conversion. On d 22, 20 birds from each treatment had a blood sample collected by cardiac puncture and placed in vacuum tubes with anticoagulant (EDTA) for analysis.

A hematological analysis was performed on a blood smear by differential leukocyte count including lymphocytes, heterophils, eosinophils, monocytes, and basophils. The leukocyte count was performed using Natt and Herrick's solution at a ratio of 1:1 200. The diluted blood was allowed to mix for 2 min before being discharged into a hemacytometer chamber. The white blood cell count was performed under a 40X objective of the microscope[14]. The immune response against Newcastle disease was assessed by immunoadsorbent assay (ELISA) using the Newcastle disease antibody test kit (IDEXX Laboratories Inc. Westbrook, MEO4092). The serum samples were diluted and dispensed into the plates with negative control and positive control in duplicate following the manufacturer's incubation, washing, and dispensing recommendations. The plates were read spectrophotometrically at 650 nm absorbance (Multiskan FC, Thermo Fisher Scientific Inc., Waltham, MA, USA). The chicks were then euthanized, and the final weight of their lymphoid organs such as thymus, spleen, and Fabricius' bag was measured.

The data were analyzed as a completely randomized design to evaluate the six doses of the aqueous chaparral extract. For this purpose, the chicks were randomly placed in 10 brooders (60 chicks per brooder) of six levels each (60 levels with 10 chicks per level), so that there were 10 replicates (levels) per treatment. The analysis of variance was performed using the MIXED procedure of SAS and the differences between the means using Tukey's test[15]. The

treatments (fixed) were the inclusion dose of *L. tridentata* extracts and the (randomized) replicate.

Results and discussion

The average daily weight gain, total weight gain, and final weight were higher ($P<0.05$) in the chicks that consumed the aqueous chaparral extracts compared to the control. The feed conversion rate was lower ($P<0.05$), i.e., the best was in the chicks consuming feed with 15 mg of the aqueous extract (Table 1). The different doses of aqueous chaparral extract did not modify the leukocyte profile or the weight of the spleen, thymus, and Fabricius' bag. The leukocyte parameters were within the reference values reported for Cobb chicks[16]. However, the chicks that consumed feed with aqueous chaparral extract at a dose of 15 mg/kg had the best antibody titers ($P<0.05$) against Newcastle disease compared to those that did not receive the extract (Table 2).

Table 1: Productive development of chickens supplemented with aqueous extracts of *L. tridentata*

	***L. tridentata* extract, mg/kg**						
	0	**5**	**10**	**15**	**20**	**25**	**SEM**
Initial weight, g	42.02	42.14	42.04	42.08	41.96	41.78	1.14
Final weight, g	675.0^{b}	732.6^{a}	748.4^{a}	786.4^{a}	703.0^{b}	726.8^{a}	32.52
Total weight gain, g	633.0^{b}	690.5^{a}	706.3^{a}	744.3^{a}	661.0^{b}	685.0^{a}	47.82
Daily weight gain, g	30.14^{b}	32.88^{a}	33.63^{a}	35.45^{a}	31.48^{b}	32.62^{a}	1.19
Total consumption, g	1130.2^{a}	1020.4^{a}	966.0^{b}	990.6^{b}	1039.0^{a}	1016.2^{a}	66.24
Feed conversion	1.78^{a}	1.48^{a}	1.37^{b}	1.33^{b}	1.57^{a}	1.48^{a}	0.12

SEM= standard error of the mean.

^{ab} Means with different letters in the same row are significantly different ($P<0.05$).

Table 2: Lymphoid organ weights and Newcastle antibody titers (NAT) in chickens supplemented with *L. tridentata*

	Extracto *L. tridentata*, mg/kg						
	0	**5**	**10**	**15**	**20**	**25**	**EEM**
Thymus, g	1.37	1.30	1.31	1.37	1.39	1.36	0.174
Spleen, g	0.27	0.31	0.26	0.27	0.26	0.28	0.051
Fabricius' bag, g	1.49	1.49	1.48	1.48	1.49	1.50	0.121
NAT, Log10	1.91[b]	2.09[b]	2.30[a]	2.62[a]	2.26[a]	2.24[a]	0.34

SEM= standard error of the mean.

[ab] Means with different letters in the same row are significantly different ($P<0.05$).

Unlike the present study, which deals with the extract of chaparral leaves only, a previous research evaluated a 20 mg/kg dose of an aqueous whole-plant extract of chaparral leaves and stems in broiler chicks and also showed significant improvements in daily and total weight gain, a decrease in crop, gizzard, and liver weights, and a reduction in liver enzymes[17], concluding that chaparral contains secondary metabolites with antioxidant activity in cell cultures[18], as well as antiparasitic[19] and antimicrobial activity against *Salmonella* isolated from poultry facilities[20], so that this plant functions adequately, without side effects, as an alternative growth promoter for poultry. According to the present study, the 20 mg dose, unlike the 25 mg one, reduced weight gain although the feed intake remained the same. The previous research had reported that the 20 mg dose induced a feed intake reduction and concluded that a high dose produced an undesirable effect in terms of low palatability or subacute toxicity[17]; however, this was not confirmed by the results of the present work.

Reports related to these bioactive compounds in the immune response are more limited. In a study with mint, echinacea, thyme, and propolis on the performance, immune response, and hematological indices of broilers, they found higher productivity, leukocyte profile, and immune response due to the effect of the mixture, rather than individually, of the various bioactive compounds contained in these plants and in propolis[20]. There is a natural immune response that generates leukotriene A4, which is mediated by lipoxygenases (LOX), leukotrienes that belong to the arachidonic acid cycle, which are responsible for regulating inflammation and immune response against infections and tissue injury. Nordihydroguaiaretic acid (NDGA), also known as masoprocol, is a phenolic lignan extracted mainly from plants of the genus *Larrea.* NDGA has an affinity to regulate the effects of lipoxygenases, acting as an antioxidant to inhibit the presence of iron that allows the unwanted effects of LOX, whereby it reduces harmful arachidonic acid-derived metabolites[21].

In this study, the extract did not affect the white blood cell count, possibly because only one extract was used, unlike other studies where several plants were utilized to synergistically stimulate the immune system function in humans and poultry[20,22]. However, *L. tridentata* extract enhanced antibody titers to the ND vaccine, indicating that a better immunomodulatory response was induced. Quantification of immunoglobulin IgA titers in chickens would have helped assess this immune response to Newcastle disease vaccination[23]. The pathogenesis of most viral infections, including the Newcastle virus, involves apoptosis due to virus proliferation in infected cells as well as cellular injury in infected and uninfected cells due to reactive oxygen species (ROS) produced by neutrophils and macrophages infiltrating infected organs[24]. NDGA from *L. tridentata* shown in virus-infected chorion cells not only efficiently inhibited virus proliferation but also had a potent effect as an antioxidant. The importance of not affecting the weight of the thymus, spleen, and Fabricius' bag lies in the fact that the Newcastle disease causes atrophy and severe destruction in these lymphoid organs, inducing immunosuppression, so the chaparral extract may induce a preventive action by enhancing the immune response and inhibiting viral replication in chickens[25,26].

Conclusions and implications

From the results of the present study, it can be concluded that the aqueous extract of chaparral at a dose of 15 mg/kg of feed improves the performance and immune response of broiler chicks against Newcastle disease. In general, the secondary compounds contained in chaparral leaves may improve the animals' health and productive performance; nevertheless, further knowledge regarding the diversity and concentration of the active components benefitting them is required.

Acknowledgments

The authors are grateful to the Autonomous University of San Luis Potosí (Universidad Autónoma de San Luis Potosí) and the University of Veracruz (Universidad Veracruzana) for the support provided for the first author's sabbatical stay.

Conflict of interest

None of the authors has any conflict of interest.

Literature cited:

1. Bagal VL, Khatta VK, Tewatia BS, Sangwan SK, Raut SS. Relative efficacy of organic acids and antibiotics as growth promoters in broiler chicken. Vet World 2016;9(4):377-382. https://doi.org/10.14202/vetworld.2016.377-382.

2. Khan RU, Fatima A, Naz S, Ragni M, Tarricone S, Tufarelli V. Perspective, opportunities and challenges in using Fennel (*Foeniculum vulgare*) in poultry health and production as an eco-friendly alternative to antibiotics: a review. Antibiotics 2022;11(2):278. https://doi.org/10.3390/antibiotics11020278.

3. Khan RU, Khan A, Naz S, Ullah Q, Laudadio V, Tufarelli V, *et al*. Potential applications of *Moringa oleifera* in poultry health and production as alternative to antibiotics: a review. Antibiotics 2021;10(12):1540. https://doi.org/10.3390/antibiotics10121540.

4. López-Aguirre S, García-López JC, Pinos-Rodríguez JM, Yáñez-Estrada L, López-Hernández Y. Chemical characterization and oral toxicity of an aqueous extract of *Larrea tridentata.* J Nat Prod 2016;2(2):79-82.

5. Reyes-Melo KY, Galván-Rodrigo AA, Martínez-Olivo IE, Núñez-Mojica G, Ávalos-Alanís FG, García A, *et al*. *Larrea tridentata* and its biological activities. Curr Top Med Chem 2021;21(26):2352-2364. https://doi.org/10.2174/1568026621666210727170908.

6. Posadzki P, Watson LK, Ernst E. Adverse effects of herbal medicines: an overview of systematic reviews. Clin Med 2013;13(1):7-12. https://doi.org/10.7861/clinmedicine.13-1-7.

7. Gnabre J, Bates R, Huang RC. Creosote bush lignans for human disease treatment and prevention: Perspectives on combination therapy. J Tradit Complement Med 2015;12;5(3):119-126. https://doi.org/10.1016/j.jtcme.2014.11.024.

8. Guzmán-Beltrán S, Rubio-Badillo MÁ, Juárez E, Hernández-Sánchez F, Torres M. Nordihydroguaiaretic acid (NDGA) and α-mangostin inhibit the growth of *Mycobacterium tuberculosis* by inducing autophagy. Int Immunopharmacol 2016;31:149-57. https://doi.org/10.1016/j.intimp.2015.12.027.

9. Zuniga KB, Chan JM, Ryan CJ, Kenfield SA. Diet and lifestyle considerations for patients with prostate cancer. Urol Oncol 2020;38(3):105-117. https://doi.org/10.1016/j.urolonc.2019.06.018.

10. Morales-Ubaldo AL, Rivero-Perez N, Valladares-Carranza B, Madariaga-Navarrete A, Higuera-Piedrahita RI, Delgadillo-Ruiz L, *et al*. Phytochemical compounds and pharmacological properties of *Larrea tridentata*. Molecules 2022;27(17):5393. https://doi.org/10.3390/molecules27175393.

11. Gutiérrez Castro LL, Corredor Matus JR. Parámetros sanguíneos y respuesta inmune en pollos de engorde alimentados con probióticos. Veterinaria y Zootecnia 2017;11(2):81-92. https://doi.org/10.17151/vetzo.2017.11.2.7.

12. NOM-062-Z00-1999. Especificaciones técnicas para la producción, cuidado y uso de animales de laboratorio. Artículo 2.2. México, 2014.

13. NRC. Nutrient requirements of Poultry 8th Rev. Ed. Washington DC. USA. National Academy Press; 1994.

14. Campbell TW. Avian hematology and cytology. Ames, Iowa State University Press; 1995:3-19.

15. SAS Institute. SAS User´s Guide: Statistics, SAS Institute Inc., Cary, NC. USA. 1991.

16. Talebi A, Asri-Rezaei S, Rozeh-Chai R, Sahraei R. Comparative studies on haematological values of broiler strains (Ross, Cobb, Arbor-acres and Arian). Int J Poultry Sci 2005;4:573-579. doi: 10.3923/ijps.2005.573.579.

17. García-López JC, Lee-Rangel HA, López S, Vicente J, Pardio-Sedas V, Estrada-Coates AT, *et al*. Efectos de *Larrea tridentata* en el crecimiento, peso de los órganos y enzimas hepáticas en pollos. Agrociencia 2018;52:1-8.

18. Skouta R, Morán-Santibañez K, Valenzuela CA, Vasquez AH, Fenelon K. Assessing the antioxidant properties of *Larrea tridentata* extracts as a potential molecular therapy against oxidative stress. Molecules 2018;23:1826.

19. Bashya B, Li L, Bains T, Debnath A, La Barbera DV. *Larrea tridentata*: A novel source for anti-parasitic agents active against *Entamoeba histolytica*, *Giardia lamblia* and *Naegleria fowleri*. PLoS Negl Trop Dis 2017;11:e0005832.

20. López-Aguirre S, Pinos-Rodríguez JM, Álvarez-Fuentes G, García-López JC, Méndez-Corte H. Uso potencial de extractos de creosote bush (*Larrea tridentata*) como desinfectante sobre *Salmonella typhimurium* en instalaciones avícolas. ITEA-Inf Tec Econ Agrar 2020;116(4):294-305.

21. Manda G, Rojo AI, Martínez-Klimova E, Pedraza-Chaverri J, Cuadrado A. Nordihydroguaiaretic acid: From herbal medicine to clinical development for cancer and chronic diseases. Front Pharmacol 2020;11:151. https://doi.org/10.3389/fphar.2020.00151.

22. Abbas AO, Alaqil AA, El-Beltagi HS, Abd El-Atty HK, Kamel NN. Modulating laying hens productivity and immune performance in response to oxidative stress induced by *E. coli* challenge using dietary propolis supplementation. Antioxidants 2020;21;9(9):893. https://doi.org/10.3390/antiox9090893.

23. Zhao K, Rong G, Hao Y, Yu L, Kang H, Wang X, *et al*. IgA response and protection following nasal vaccination of chickens with Newcastle disease virus DNA vaccine nanoencapsulated with $Ag@SiO_2$ hollow nanoparticles. Sci Rep 2016:25720. https://doi.org/10.1038/srep25720.

24. Rehman ZU, Meng C, Sun Y, Safdar A, Pasha RH, Munir M, *et al*. Oxidative stress in poultry: lessons from the viral infections. Oxid Med Cell Longev 2018:5123147. doi: 10.1155/2018/5123147.

25. Ezema WS, Eze DC, Shoyinka SVO, Okoye JOA. Atrophy of the lymphoid organs and suppression of antibody response caused by velogenic Newcastle disease virus infection in chickens. Trop Anim Health Prod 2016;48:1703-1709. doi:10.1007/s11250-016-1147-x.

26. Uchide N, Ohyama K, Bessho T, Toyoda H. Inhibition of influenza-virus-induced apoptosis in chorion cells of human fetal membranes by nordihydroguaiaretic acid. Intervirology 2005;48(5):336-340. doi:10.1159/000085103.