

https://veterinariamexico.fmvz.unam.mx/

# The efficacy of a pharmaceutically alginate-coated phytoremedy (black garlic and turmeric) for the control of natural coccidia infestation in turkeys

## Yazmin Alcalá-Canto<sup>1</sup>

- © 0000-0001-7809-392X
  - Héctor Sumano<sup>2</sup>
- © 0000-0002-8802-5274 **Lilia Gutiérrez<sup>2, \*</sup>**
- **0000-0002-4823-0388**

<sup>1</sup>Universidad Nacional Autónoma de México. Facultad de Medicina Veterinaria y Zootecnia. Departamento de Parasitología. Mexico City, México.

<sup>2</sup> Universidad Nacional Autónoma de México. Facultad de Medicina Veterinaria y Zootecnia. Departamento de Fisiología y Farmacología. Mexico City, México.

> \*Corresponding author Email address: liliago@unam.mx

# **Abstract**

In searching for herbal alternatives for treating coccidiosis in turkeys, this trial aimed to evaluate whether minced black garlic (Allium sativa) or turmeric powder (Curcuma longa) or their combination, included in dried alginate beads, controlling clinical coccidiosis in turkey male poults. A total of 150 twelve-day-old male turkeys were randomly distributed into 15 pens of 10 turkeys per pen. Groups were: CTR = control untreated turkeys; GAR = turkeys fed 4 % of their diet with minced garlic included in dried alginate beads; CUR = treated fed 4 % powder curcuma, also prepared as dried alginate beads; GA = containing only alginate), and GC= turkeys fed 8 % of their diet with the mixture of minced black garlic plus powder curcuma (4 %), entrapped in dried alginate beads. Treatments were incorporated as feed-dressing, considering their mean feed intake. Results reveal that turkeys in group GC showed a significant decrease in oocysts per gram of feces during the first six weeks of age compared to values found in the CTR group and the other treatments. These results identify that in turkey poults, there is a statistically significant anticoccidial activity of the combination of minced garlic plus powder curcuma, included in alginate beads and administered as 4 % of their feed intake. Additionally, better productive variables were recorded for this combination compared to the other three groups (P < 0.05). The use of vehicles for the digestive tract of turkeys may improve the effectiveness of plant extracts for the control of coccidiosis.

Keywords: Alginate; Turmeric; Garlic; Turkeys; Eimeria spp.

Submitted: 2023-08-29 Accepted: 2024-06-24 Published: 2024-10-02

Additional information and declarations can be found on page 12

© Copyright 2024 Yazmin Alcalá-Canto et al.





Cite this as:

Alcalá-Canto Y, Sumano H, Gutierrez L. The efficacy of a pharmaceutically alginate-coated phytoremedy (black garlic and turmeric) for the control of natural coccidia infestation in turkeys. Veterinaria México OA. 2024;11. doi: 10.22201/fmvz.24486760e.2024.1259.

# **Study contribution**

Coccidiosis in turkeys poults is a great therapeutic problem, although its presentation is not as serious as in poultry production, it does represent a great loss in productive variables. Given the new international guidelines to reduce the use of coccidiostats, phytoelements are becoming a necessity for coccidia treatments in turkeys.

#### Introduction

Turkey meat (Meleagris gallopavo) is a product of constant and stable demand in North America, Europe, and some countries in Latin America. After chicken meat, it is the second-largest contributor to poultry meat production worldwide. (1) However, consumption per capita varies among countries. It has been accepted that higher consumption of turkey meat is directly linked to socioeconomic status. Thus, consumption is higher in some countries with high economic situations than in developing ones.

However, on some holidays and festivities, its consumption increases sharply in many Western countries. Nevertheless, rising living standards, the rapid pace of urbanization, the growing popularity of Western diets in Asia, and increased attention to lower fat intake have led to a significant increase in the production and consumption of turkey meat worldwide in recent years. For example, Mexico produced 157 thousand tons, and Germany produced 117 thousand tons in 2018. However, these countries were the largest importers of turkey meat that year. The Mexican national poultry census grew 2.1 % in one year (2021-2022), closing at 541 million animals as follows: 163.3 million laying hens, 310 million broiler chickens per cycle, and 459 thousand turkeys per cycle. (2)

The incidence of coccidiosis in turkeys is high. However, lesions caused by these protozoa are less severe than in broiler chickens and laying hens. The majority of coccidia infections show subclinical features. An enteric process caused by lesions induced by the parasite's replication in the gastrointestinal epithelium becomes evident in more clinically apparent cases. Coccidiosis reduces feed intake, diminishes digestibility, and causes poor absorption of nutrients. Rapid weight loss, shedding, and ruffled feathers become evident, affecting weight gain and productive variables. (3)

Severe diarrhea or at least wet stools with mucus may be present, and increased susceptibility to bacterial and viral diseases follows. Turkeys of all ages are susceptible to coccidial infection, but they develop a reasonable immune response when the outbreak occurs from 6-8 weeks of age onwards. Later, morbidity rises in weeks 8–9, but mortality is usually not very high.(4,5) A total of 40 field specimens were collected in a previous study $^{(6)}$  from diverse turkey flocks across the Midwestern United States with the aim of assessing the prevalence and diversity of Eimeria species using a species-specific PCR method.

This study yielded positive results for at least one type of Eimeria spp., with the majority exhibiting the presence of either two (20 out of 40) or three (14 out of 40) distinct species in total. The prevalence of Eimeria species across farms was predominantly characterized by high rates of occurrence for E. meleagrimitis (97.5 %), E. adenoides (95 %), and E. gallopavonis (40 %). Notably, nearly half

of the samples that tested positive for both E. adenoeides and E. meleagrimitis (17 out of 40) also showed the presence of additional species.

Manifestations of pathogenicity predominantly characterized by a decrease in body weight gain resulting from infection with Eimeria species in turkeys are affected by factors such as the freshness of the inoculum, sporulation quality, and the age of the turkeys. (7) Likewise, mortality of poults infected with E. adenoides is usually low even after artificial inoculation of lethal doses for poults less than five weeks of age. Mortality is 100 % after the administration of 2×10<sup>5</sup> infectious oocysts of this species to 3-week-old poults; yet older birds survived after receiving  $3 \times 10^5$  oocysts. (5)

Coccidiosis poses a complex challenge for turkeys, presenting distinctive clinical indications. Turkeys affected by this condition often display symptoms like diarrhea, leading to dehydration and lethargy. Eimeria, particularly species like E. adenoides, hinders nutrient absorption, causing weight loss and a decline in overall health. The birds may also show decreased efficiency in feed conversion, and in severe instances, mortality can occur, especially among young or stressed individuals. (7)

Eimeria species linked to clinical cases do not present cross-immunity between them. Only four of the seven coccidia species in turkeys are considered pathogenic, i.e., E. adenoides, E. dispersa, E. gallopavonis y E. meleagrimitis. As in broiler chickens, diagnosis is established by localizing the gastrointestinal tract's affected section based on the oocyst morphology, the incubation period, its pathogenicity, details of each coccidial life cycle, and absence of cross-species immunity, and host specificity. (7-9) Coccidiosis in turkeys is usually controlled by ionophore drugs such as lasalocid and monensin. Diclazuril has been used during outbreaks. lonophore derivatives are administered for up to 12 weeks, and resistance to these treatments has already been reported. (10-12)

An alternative that is being pondered in the world for turkey production is botanical products, mainly essential oils, colorants, and phenolic compounds. (13) The mechanism of action of many herbal remedies has yet to be fully characterized. However, evaluations in various species, including pigs, dogs, chickens, and humans, have found that some of these substances may exert effects through one or more mechanisms, such as 1) disruption of the pathogens' cell membranes, 2) physicochemical modification of the cell surface and thus affecting the virulence of pathogens, 3) stimulating the immune system, specifically through the activation of lymphocytes, macrophages, and large granular-natural killer lymphocytes, 4) protecting the intestinal mucosa from pathogen colonization, and 5) promoting competitive exclusion in the intestinal lumen. (14, 15)

Among the products potentially beneficial for their anticoccidial activity are curcumin, which has been shown to induce apoptosis by means of the presence of precipitates on the sporozoite surface that affect its morphology, viability, and adhesion ability, (16) and black garlic and its derivatives as they inhibit oocysts sporulation. They have been linked to anti-inflammatory and antioxidant properties, and the anticoccidial potential of garlic is linked to its immunostimulatory activity. Garlic and its derivatives inhibit the sporulation of oocysts in vitro. The supplementation of garlic in coccidiosis-infected broilers improves weight gain and feed efficiency. It reduces fecal oocysts output, lesion score, and clinical signs postinfection. (17)

Based on previous findings, (18-20) supplementation with either garlic or turmeric-derived products demonstrates anticoccidial effects comparable to those of

standard anticoccidial drugs. This conclusion is supported by observations of reduced mortality rates, decreased gut lesion scores, and lower fecal oocyst shedding in infected chickens compared to the untreated infected group. These results, particularly the significant reduction in oocyst output, suggest garlic or turmeric can inhibit or hinder the invasion, replication, and development of *Eimeria* parasites within the gut tissues of avian species, potentially acting on intracellular stages such as the second schizogony stage and the sexual stage within the intestinal lumen. (21)

This impairment or inhibition of intracellular parasite development by garlic mirrors the mechanisms of action observed with most conventional anticoccidial drugs. After the use of garlic, for example, oocysts were not detected in poultry after the 5<sup>th</sup> to 7<sup>th</sup> d of infection. (21) One of the key mechanisms through which turmeric or curcuma and garlic exert their anticoccidial effects is by interfering with the invasion, replication, and development of Eimeria parasites within the gut tissues of chickens. (22-24)

These natural compounds have been found to inhibit or impair the intracellular stages of coccidia, particularly during the second schizogony stage of the asexual cycle, as well as during the sexual stage within the intestinal lumen. By targeting these critical stages of the parasite's life cycle, curcuma and garlic help disrupt the development and maturation of Eimeria, (24) ultimately reducing the severity of infection and limiting the spread of coccidiosis within poultry flocks. Curcuma and garlic contain bioactive compounds such as curcumin and allicin, which possess immunomodulatory properties.

These compounds can enhance the immune response in poultry, including boosting antibody production, promoting phagocytosis, and increasing the activity of immune cells such as macrophages and lymphocytes. By strengthening the immune system, curcuma and garlic help birds better resist Eimeria infections and recover more quickly from disease challenges. (23) The bioactive compounds found in curcuma and garlic can positively influence the gut microbiota composition and diversity in poultry. By promoting a balanced gut microbiome, turmeric extracts and garlic help have been proved to maintain intestinal health and function, reducing the risk of opportunistic infections and improving nutrient utilization in poultry. A healthy gut microbiota is essential for optimal digestion, absorption, and overall performance in poultry production. (25)

However, there are no data on its use in the production of turkeys. Another potentially helpful botanic product is sodium alginate. (26-28) This material has broad applications in veterinary medicine, including as a pharmaceutical vehicle to increase the stability of active principles and as a polymer to allow sustained release. In poultry farming, alginates have been evaluated as probiotics and a pharmaceutical vehicle for antibacterial drugs because their gel-forming properties can provide a modified release pattern of active ingredients. (29–31) It is compatible with the poultry's digestive tract, and it may be helpful for the control of coccidiosis in turkeys. Thus, this trial aimed to test the anticoccidial efficacy in turkeys of pharmaceutical preparations made with minced black garlic, powdered curcumin, or both coated with sodium alginate.

## Materials and methods

## Ethical statement

The study design and animal handling complied with the Mexican regulations for using experimental animals as established by the Universidad Nacional Autónoma de México (UNAM) in its internal committee for the care and use of animals (CICUA-Protocol #0673).

# Experimental design and management of turkeys

A total of 150 twelve-day-old male turkeys untreated with an antiparasitic drug were randomly allocated, in groups of 15 animals, to each of five treatments, with two replicates per treatment, as follows: 1) CTR: control group, no treatment; 2) GAR: fed the same diet as CTR plus dried alginate beads containing minced black garlic (4%); 3) CUR: incorporating powder curcuma in dried alginate beads (4%); 4) GC: containing both minced garlic plus powder curcuma (8 %), also entrapped in dried alginate beads; and 5) GA: containing only alginate. Under this scheme, each treatment had 30 animals in total. They were kept under the same temperature, ventilation, feeders, diet, drinkers, and health care activities for 42 days. Turkeys were kept outdoors in an "all-in, all-out" system, and were provided with an indoor area of 4 poults/m<sup>2</sup>. A feed with a built-in scale was used to weigh the amount of feed given to the poults each day, and food waste weight was recorded at the end of the day.

Their body weight was recorded before they were fed with a commercial brand (Pavo Ganador®, Api-Aba, Mexico, without coccidiostats) for the starters phase (from hatching to 8 weeks old) (22 % crude protein [CP] and 3 015 kcal/kg ME); the growing phase (9 to 18 weeks old) (20 % CP, 3 180 kcal/kg ME) and the finishing phase (18 weeks old up to market weight) (19 % CP and 3 100 kcal/kg ME). The rations were based on yellow corn (Zea mays), 45 % soybean paste, and sunflower oil, supplemented with amino acids, vitamins, and minerals. Feed did not contain anticoccidial drugs. The treatments included the herbal remedies in alginate beads as dressing in an amount of 4 % of their feed intake, as follows: control group (CTR) received no treatment; group GAR: fed the same diet as CTR group plus dried alginate beads containing minced black garlic (4 %); group CUR incorporating powder curcuma in dried alginate beads (4 %); group GC, containing both minced garlic plus powder curcuma (8 %), also entrapped in dried alginate beads and group GA, containing only alginate. Treatments were incorporated into daily feed intake as dressing for six weeks. Turkeys were monitored to ensure that all animals were eating. Feeders that were appropriate for the number of turkeys in the pen were used to prevent larger or dominant poults from monopolizing the amount of feed. Daily evaluation of product and feed waste was carried out.

Fifty grams of sodium alginate (Silverquim®, Mexico) are added to one liter of water until wholly suspended. Then, 100 g of powder curcuma (Entera®, Entera Pharma, Mexico) or 100 g of freeze-dried black garlic powder (RV-Organic®, RV-Organic, Mexico) are added and stirred for a further 60 minutes. The suspension is poured drop by drop employing a multiple injection system into a stirred solution of 0.2 % calcium chloride (SILVERQUIM®, Mexico), where alginate beads are formed instantly.



Figure 1. The aspect of the dried alginate beads recently manufactured and still in their wet phase. (A) containing powder curcuma (*Curcuma longa*) and minced black garlic (*Allium sativa*), and (B) containing only minced black garlic.



Figure 2. An aspect of the dried beads of alginate containing powder curcuma and minced garlic as were administered to turkey poults as feed dressing at a dose of 4 % of their total daily intake.

When the batch is finally processed, the beads are rinsed with bidistilled water and left to dry at room temperature for three days. Loss of active ingredients was determined with UV visible spectroscopy and calculated as < 10 % for curcuma and < 8 % for black garlic. (32, 33) Hence, an approximate 85 % inclusion rate of each herb in each dried alginate bead was established. Figure 1 shows the alginate beads in their wet phase, and Figure 2 shows the alginate beads once dried, as they were fed to the animals.

Eimeria oocysts per gram of feces (OPG) were counted by McMaster's coproparasitological analysis. (34) Feces were collected from the floor on days 0, 7, 14, 21, 28, 35, and 42. Approximately ten fresh fecal droppings were collected from each pen and combined into one sample per pen. The pens were sampled in a zig-zag fashion to randomize the sample collection, and samples that were dried or contaminated with litter or food were not collected. Species were determined by incubating isolated oocysts in Petri dishes in 5 mL of potassium dichromate for 48 h at 25-29 °C and 80 % relative humidity. At the end of the incubation period, the oocysts were identified according to the essential taxonomic keys. (35) One hundred oocysts were counted to determine the percentage of each species found.

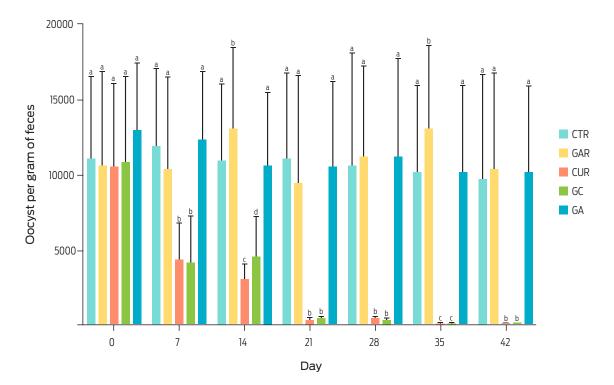
The following parameters were recorded weekly per pen: weight gain (WG), feed intake (FI), and feed conversion ratio (FCR):

$$FCR = \frac{FI}{WG}$$

For statistical analysis, the ANOVA and Kruskal-Wallis tests were used to find differences between pens and treatments, and the Kruskal-Wallis and Tukey's tests were used to find differences between production performance using GraphPad Prism version 8.0.0 for Mac, and GraphPad Software, San Diego, California USA (www.graphpad.com).

#### Results

No statistical differences were found between repetitions of the same treatment (P < 0.5000). Figure 3 shows the decrease in oocysts in the groups, results recorded for the turkeys in group CUR and GC significantly reduced OPG compared to the untreated controls turkeys (CTR and GA) and those that were fed only GAR. There is no statistical difference in groups CTR and GA the alginate alone did not present an anticoccidial effect. It behaved statistically like the control group. End-of-day alginate beads and feed waste in the CUR and GC groups were confirmed to be almost null throughout the evaluation. In contrast, alginate bead wastage in the GAR group averaged 18 % daily. This was taken as an indication that, even though the alginate masks the taste, turkeys rejected the taste of garlic. Table 1 shows that the ingestion of Curcuma longa significantly reduces the shedding of the most pathogenic species of Eimeria, i.e., E. adenoides, E. gallopavonis, and E. meleagrimitis.



Phytoelements vs natural coccidia infestation in turkeys

Figure 3. Comparison of the mean  $\pm$  1 SD for each group (CTR: control group; GAR: fed the same diet as CTR group plus dried alginate beads containing minced black garlic (4 %); CUR: incorporating powder curcuma in dried alginate beads (4 %); GC: containing both minced garlic plus powder curcuma (8 %), also entrapped in dried alginate beads and GA: containing only alginate) and the two replicates counts of oocyst per gram of feces in turkeys given or not curcuma, minced garlic, or their mixture. (n = 30).

 $^{a, b, c, d}$  different literals on the bar mean statistical differences between groups by day of evaluation P < 0.0500.

Table 1. Eimeria species percentage identified in turkeys given or not curcuma, minced garlic, their mixture or alginate alone (n = 30)

Species	CTR	GAR	CUR	GC	GA
E. gallopavonis	20.64 %	22.29 %	8.94 %	14.25 %	10.99 %
E. subrotunda	6.09 %	16.96 %	23.59 %	11.79 %	6.13 %
E. innocua	17.32 %	6.13 %	10.99 %	2.51 %	8.94 %
E. meleagrimitis	27.00 %	25.93 %	5.68 %	30.38 %	20.64 %
E. dispersa	3.05 %	4.33 %	24.30 %	4.82 %	11.79 %
E. meleagridis	8.73 %	9.50 %	21.68 %	6.98 %	17.21 %
E. adenoides	17.18 %	14.86 %	4.82 %	29.27 %	24.30 %

CTR: control untreated turkeys.

GAR: minced garlic in alginate beads.

CUR: Curcuma powder in alginate beads.

GC: a mixture of 4 % GAR plus 4 % CUR.

GA: group with alginate only.

Table 2. Productive performance in control untreated turkey poults and in turkey poults given or not curcuma, minced garlic or their mixture (n = 30)

Group	Weight gain (kg)	Feed consumption (kg)	Feed conversion index
CTR	$3.55 \pm 0.2^{a}$	$12.34 \pm 0.8^{a}$	$3.47 \pm 0.2^{a}$
GAR	$3.20 \pm 0.2^{a}$	$10.88 \pm 0.9^{a}$	$3.40 \pm 0.1^{a}$
CUR	$3.38 \pm 0.3^{a}$	11.80 ± 1.0 <sup>a</sup>	$3.49 \pm 0.2^{a}$
GC	$4.89 \pm 0.2^{b}$	11.04 ± 1.0 <sup>b</sup>	$2.25 \pm 0.2^{b}$
GA	$4.69 \pm 0.3^{b}$	$11.09 \pm 0.9^{b}$	$2.28 \pm 0.1^{b}$

 $^{a, \, b}$  Different letters in the same column indicate a significant difference (P < 0.05)

CTR: control untreated turkeys.

GAR: minced garlic in alginate beads.

CUR: *Curcuma* powder in alginate beads.

GC: a mixture of 4 % GAR plus 4 % CUR.

GA: group with alginate only.

No statistical differences were found between repetitions of the same treatment (P < 0.5) in productive parameters; the arithmetic means of the productive parameters recorded are presented in Table 2. Weight gains and feed intake were similar between CTR, GAR, and CUR treatments; consequently, feed conversion ratios were also statistically indistinguishable among treatments, except for the GC and GA groups, as turkeys in this group gained more weight in a statistically significant manner (P < 0.05).

#### **Discussion**

Coccidiosis in turkeys (*Meleagris gallopavo*) is a prevalent veterinary disease with a significant economic impact. (7, 12) It has been pointed out that the severity of this disease is increasing in parallel to the intensification of turkey production world-wide. (5, 8) With the emergence of pathogen resistance and the new international trends to reduce the use of drugs in livestock production and complying with consumers' demands, a reduction in the use of anticoccidial drugs has been attempted in some countries. It is relevant to note that overreliance on synthetic anticoccidials can lead to the development of drug-resistant strains of *Eimeria*, compromising the effectiveness of these medications over time. (10, 11)

To address this concern, many producers are exploring alternative approaches, including the use of organic anticoccidials, probiotics, vaccination programs, and improved management practices to reduce reliance on chemical interventions and promote long-term sustainability in poultry production. On the other hand, the use of anticoccidials that have been proven to be effective such as ionophores is limited in turkeys due to their toxicity in this species. Herbal remedies and their phytogenic bioactive principles are viable alternatives for controlling coccidiosis. However, rather than simply adding herbal remedies to the poultry feed, it is crucial to develop preparations whose pharmaceutical design allows optimal contact with the active ingredients available in herbal remedies and can endure longer shelf-life.

In recent years, advances in pharmaceutical technology have led to the development of economically viable drug/herbal preparations for poultry. For instance, oral drug formulations can be designed to modify a drug release and increase its bioavailability (42) or extend an active principle's contact time with a given pathogen. (43) Recent studies have found that chickens have a better-developed flavor ability than suspected. Therefore this feature will significantly impact their feeding behavior more than previously appreciated. (44, 45) There are only a few studies in this regard on turkeys.

However, in this trial, it became evident that the group dosed with garlic failed to eat all their food, suggesting a rejection of garlic, even as alginate-coated beads. In this context, alginate was included as a pharmaceutical excipient in the preparation assessed in this trial. Alginates are valuable vehicles for many pharmaceutical applications and possess antimicrobial and antiviral protection properties. They are biodegradable, biocompatible, and lack toxicity. Besides, alginates are reasonably inexpensive and can be used as a gelling vehicle to thicken, stabilize, and emulsify various drug preparations. Multiple studies have shown that it can coat or encapsulate active principles and natural substances for better drug delivery after oral administration. (46-49)

Consequently, in the case of the herbal constituents utilized in this trial, it is safe to assume that the alginate employed acted as stabilizing agent, modifying the release of the active principles of the minced black garlic and turmeric powder. This pharmaceutical maneuver presumably enhanced the anticoccidial action of the active principles derived from the formula. (26) It is also crucial to consider some manufacturing procedures if this herbal formulation is planned to be used in large production centers.

The active principles of garlic are rapidly degraded by oxidation, and this makes it necessary to add other elements to the described combination, i.e., antioxidants, such as butylated hydroxytoluene, as well as other stabilizing elements and dispersant chemicals. Furthermore, stability studies of the proposed combination and quality control studies are needed to guarantee the clinical repeatability of this formula in a large-scale production scenario. Nevertheless, it is safe to stand out that the pharmaceutical association of garlic and powder Curcuma with alginate and not only the herbal resources alone were responsible for the anticoccidial effects noted in this trial.

Our results comply well with knowledge accumulated for garlic and its secondary metabolites, propyl thiosulfinate (PTS) and propyl thiosulfinate oxide (PTSO). These compounds have been shown to enhance intestinal immunity during experimental infections by Eimeria acervulina. (50, 51)

Also, in vitro assays showed that both PTSO (67 %) and PTS (33 %) possess a dose-dependent killing ability against invasive E. acervulina sporozoites. Garlic toxicity is negligible, and in dose-toxicity studies, it was found that 500 mg/kg body weight may induce a certain degree of liver damage. In contrast, lower doses have a well-defined hepatoprotective action. Hence, our findings agree with previous work that evaluated feed consumption by adding curcuma for up to 42 d in broilers from the Ross line, which gained significantly more weight during the productive cycle. (52)

Another work reported that adding 0.02 %, 0.03 %, and 0.04 % of powder Curcuma in feed caused significant weight gain increments, whether measured in

the initiation or the growth period. (53) Thus, apart from the anticoccidial efficacy observed with the herbal formulation, the added curcuma powder exhibited a nutritional role that became apparent through the productive parameters obtained. Curcuma powder is rich in minerals, vitamins, protein, and carbohydrates and can reduce oxidative stress in intestinal cells, limiting cell damage. (14, 54) The turmeric plant is used in traditional medicine in humans. It is considered a phytoremedy for various diseases, i.e., respiratory, cardiovascular, hepatobiliary, and irritable bowel disease. Curcuma powder is non-mutagenic, non-carcinogenic, non-hepatotoxic, and does not possess known adverse effects. (55)

However, researchers' main difficulty in taking advantage of all these properties is determining and enhancing the intestinal absorption and contact time of its active principles, modulating its biotransformation, and controlling its rapid gastrointestinal clearance.

However, it is crucial to consider that even natural compounds have been shown to generate resistance in the Eimeria genus, and further studies should be carried out to characterize this problem for the presented herbal formulation. However, the herbal constituents utilized in this trial have been described by the absence of coccidial resistance development. (56, 57) Further studies may also reveal if this herbal formula has immunomodulatory action and improves gut integrity, as suggested elsewhere. (58, 59) The feed conversion index was higher for the groups receiving garlic and turmeric. For curcuma, this can be ascribed to its well-known production enhancement properties and to its analgesic, anti-inflammatory, antioxidant, antibacterial, and immunomodulatory effects in poultry production.

However, it is here noted that, to date, no publications have been published in formal literature in this regard, except Al-Shuwaili et al., (60) who found that supplementation of 4 % garlic extract in 28-week-old turkeys improved daily weight gains compared to the untreated group. (59) Finally, as pharmaceutical optimization of herbal remedies may maintain its efficacy for a more extended period, (60) it is necessary to carry out further studies to establish the optimal pharmacokinetic/ pharmacodynamic ratios of the garlic, curcuma, alginate preparation here tested, i.e., minced garlic has been linked to a time-dependent ratio for optimal anticoccidial effect. (32)

## Conclusions

This study shows that turkey poults that received minced garlic plus curcuma powder prepared as dried alginate beads at a rate of 4 % of their daily diet and for 42 d and administered as a feed-dressing showed a significant decrease in the excretion of oocysts per gram of feces, revealing a potentially useful anticoccidial activity. Treatment of turkey poults during this period did not reduce feed intake, weight gain, or feed conversion rate. Conversely, a clear trend to improve these parameters when using these plants included in dried alginate beads were identified.

# **Data availability**

The original datasets used in this research and if applicable, supporting information files, are deposited and available for download at the SciELO Dataverse repository doi: 10.48331/scielodata.VIGWBB.

# **Funding statement**

This research was funded by PAPIIT IT200322, Universidad Nacional Autónoma de México. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

#### Conflicts of interest

The authors confirm that they do not have any conflicts of interest.

## **Author contributions**

Writing-original draft: Lilia Gutiérrez, Héctor Sumano, Yazmin Alcala-Canto.

Conceptualization: Yazmin Alcala-Canto, Lilia Gutiérrez.

Investigation: Lilia Gutiérrez, Yazmin Alcala-Canto.

Writing-review and editing: Lilia Gutiérrez, Héctor Sumano, Yazmin Alcala-Canto.

## References

- 1. Food and Agriculture Organization of the United Nations. Gateway to poultry production and products [html]. https://www.fao.org/poultry-production-products/ en/
- 2. Unión Nacional de Avicultores. Compendio de Indicadores Económicos del Sector Avícola 2022 [html]. https://una.org.mx/indicadores-economicos/
- 3. Taylor MA, Coop RL, Wall RL. Veterinary Parasitology. 4<sup>th</sup> ed. Hoboken, NJ: USA. Wiley-Blackwell; 2015. doi: 10.1002/9781119073680.
- 4. Fatoba AJ, Adeleke MA. Diagnosis and control of chicken coccidiosis: a recent update. Journal of Parasitical Diseases. 2018;42(4):483-493. doi: 10.1007/s12639-018-1048-1.
- 5. Chapman HD. Coccidiosis in the turkey. Avian Pathology. 2008;37(3):205–223. doi: 10.1080/03079450802050689.
- 6. Duff AF, Briggs WN, Bielke JC, McGovern KE, Trombetta M, Abdullah H, Bielke LR, Chasser KM. PCR identification and prevalence of Eimeria species in commercial turkey flocks of the Midwestern United States. Poultry Science. 2022(Sep);101(9):101995. doi:10.1016/j.psj.2022.101995.
- 7. Vrba V, Pakandl M. Coccidia of turkey: from isolation, characterisation and comparison to molecular phylogeny and molecular diagnostics. International Journal for Parasitology. 2014:44(13);985–1000. doi:10.1016/j.ijpara.2014.06.004.
- 8. Mesa-Pinesa C, Navarro-Ruiz J, López-Osorio S, Chaparro-Gutirrez JJ, Gómez-Osorio LM. Chicken coccidiosis: from the parasite lyfe cicle to control of the disease. Frontiers in Veterinary Science. 2021;8. doi: 103389/fvets.2021.787653.
- 9. Chapman HD, Rathinam T. Focused review: the role of drug combinations for the control of coccidiosis in commercially reared chickens. International Journal for Parasitology: Drugs and Drug Resistance. 2022;18:32-42. doi: 10.1016/j.ijpddr.2022.01.001.

- 10. Chapman HD. Milestones in avian coccidiosis research: a review. Poultry Science. 2014;93(3):501–511. doi:10.3382/ps.2013-03634.
- 11. Madlala T, Okpeku M, Adeleke MA. Understanding the interactions between Eimeria infection and gut microbiota, towards the control of chicken coccidiosis: a review. Parasite. 2018;28:48. doi: 10.1051/parasite/2021047.
- 12. El-Shall NA, Abd El-Hack ME, Albaqami NM, Khafaga AF, Taha AE, Swelum AA, et al. Phytochemical control of poultry coccidiosis: a review. Poultry Science. 2022;101(1):101542. doi:10.1016/j.psj.2021.101542.
- 13. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008;15(8):639–652. doi: 10.1016/j.phymed.2008.06.008.
- 14. Kim JE, Lillehoj HS, Hong YH, Kim GB, Lee SH, Lillehoj EP, et al. Dietary Capsicum and Curcuma longa oleoresins increase intestinal microbiome and necrotic enteritis in three commercial broiler breeds. Research in Veterinary Science. 2015;102:150—158. doi: 10.1016/j.rvsc.2015.07.022.
- 15. Yadav S, Teng PY, Souza Dos Santos T, Gould RL, Craig SW, Lorraine Fuller A, et al. The effects of different doses of curcumin compound on growth performance, antioxidant status, and gut health of broiler chickens challenged with *Eimeria* species. Poultry Science. 2020;99(11):5936—5945. doi:10.1016/j.psj.2020.08.046.
- 16. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: biological actions and medicinal applications. Current Science. 2004:44–53.
- 17. Adjei-Mensah B, Atuahene CC. Avian coccidiosis and anticoccidial potential of garlic (*Allium sativum* L.) in broiler production: a review. Journal of Applied Poultry Research. 2022:100314. doi: 10.1016/j.japr.2022.100314.
- 18. El-Khtam AO, Abd El Latif A, El-Hewaity MH. Efficacy of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) on *Eimeria* species in broilers. International Journal of Basic and Applied Sciences. 2014;3(3):349. doi:10.14419/ijbas.v3i3.3142.
- 19. Ali M, Chand N, Khan R U, Naz S, Gul S. Anticoccidial effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) against experimentally induced coccidiosis in broiler chickens. Journal of applied animal research. 2019;47(1):79–84. doi: 10.1080/09712119.2019.1573731.
- 20. Pourali M, Kermanshahi H. Golian A, Razmi GR, Soukhtanloo M. Antioxidant and anticoccidial effects of garlic powder and sulfur amino acids on *Eimeria*-infected and uninfected broiler chickens. Iranian Journal of Veterinary Research. 2014;15(3):227–232. doi:10.22099/IJVR.2014.2531.
- 21. Waqas M, Akhtar R, Akbar H, Lateef M, Rashid I, Ijaz M. Evaluation of anti-coccidial activity of different extraction products of *Allium sativum* (garlic) in broilers. Journal of the Hellenic Veterinary Medical Society. 2018;69(3):1055—1058. doi:10.12681/jhvms.18872.
- 22. Galli M, da Silva AS, Biazus AH, Reis JH, Boiago MM, Topazio JP, Migliorini MJ, Guarda NS, Moresco RN, Ourique AF, Santos CG, Lopes LS, Baldissera MD. Stefani LM. Feed addition of curcumin to laying hens showed anticoccidial effect, and improved egg quality and animal health. Research in Veterinary Science. 2018;118;101—106. doi: 10.1016/j.rvsc.2018.01.022.

- 23. Kim DK, Lillehoj HS, Lee SH, Jang SI, Lillehoj EP, Bravo D. Dietary *Curcuma longa* enhances resistance against *Eimeria maxima* and *Eimeria tenella* infections in chickens. Poultry Science. 2013;92:2635—2643. doi: 10.3382/ps.2013-03095.
- 24. Yadav S, Teng PY, Souza Dos Santos T, Gould RL, Craig SW, Lorraine Fuller A, Pazdro R, Kim WK. The effects of different doses of curcumin compound on growth performance, antioxidant status, and gut health of broiler chickens challenged with *Eimeria* species. Poultry Science. 2020;99;5936—5945. doi: 10.1016/j.psj.2020.08.046.
- 25. Sidiropoulou E, Skoufos I, Marugan-Hernandez V, Giannenas I, Bonos E, Aguiar-Martins K, Tzora A. *In vitro* anticoccidial study of oregano and garlic essential oils and effects on growth performance, fecal oocyst output, and intestinal microbiota *in vivo*. Frontiers in Veterinary Science. 2020;7:420. doi: 10.3389/fvets.2020.00420.
- 26. Shabkhiz MA, Pirouzifard MK, Pirsa S, Mahdavinia GR. Alginate hydrogel beads containing *Thymus daenensis* essential oils/glycyrrhizic acid loaded in β-cyclodextrin. Investigation of structural, antioxidant/antimicrobial properties and release assessment. Journal of Molecular Liquids. 2021;344:117738. doi:10.1016/j.molliq.2021.117738.
- 27. Noppakundilograt S, Piboon P, Graisuwan W, Nuisin R, Kiatkamjornwong S. Encapsulated eucalyptus oil in ionically cross-linked alginate microcapsules and its controlled release. Carbohydrate Polymers. 2015;131:23—33. doi:10.1016/j. carbpol.2015.05.054.
- 28. Gholamian S, Nourani M, Bakhshi N. Formation and characterization of calcium alginate hydrogel beads filled with cumin seeds essential oil. Food Chemistry. 2021;338:128143. doi: 10.1016/j.foodchem.2020.128143.
- 29. Zhang Y, Gong J, Yu H, Guo Q, Defelice C, Hernandez M, et al. Alginate-whey protein dry powder optimized for target delivery of essential oils to the intestine of chickens. Poultry Science. 2014;93(10):2514–25. doi:10.3382/ps.2013-03843.
- 30. Voo W-P, Ravindra P, Tey B-T, Chan E-S. Comparison of alginate and pectin-based beads for production of poultry probiotic cells. Journal of bioscience and bioengineering. 2011;111(3):294–299. doi: 10.1016/j.jbiosc.2010.11.010.
- 31. Gómez-García J, Chávez-Carbajal A, Segundo-Arizmendi N, Barón-Pichardo MG, Mendoza-Elvira SE, Hernández-Baltazar E, et al. Efficacy of *Salmonella* bacteriophage S1 delivered and released by alginate beads in a chicken model of infection. Viruses. 2021;13(10):1932. doi:10.3390/v13101932.
- 32. Khoshtinat K, Barzegar M, Sahari MA, Hamidi Z. Encapsulation of Iranian garlic oil with  $\beta$ -cyclodextrin: optimization and its characterization. Journal of Agricultural Sciences and Thechology. 2017;19(1):97–111.
- 33. Hazra K, Kumar R, Sarkar BK, Chowdary YA, Devgan M, Ramaiah M. UV-visible spectrophotometric estimation of curcumin in nanoformulation. International Journal of Pharmacognosy. 2015;2(3):127–130. doi:10.13040/IJPSR.0975-8232.IJP.2(3).127-30.
- 34. Figueroa CJA, Jasso VC, Liébano HE, Martínez LP, Rodríguez VRI, Zárate RJJ. Examen coproparasitoscópico. In: Técnicas para el Diagnóstico de Parásitos con Importancia en Salud Pública y Veterinaria. AMPAVE-CONASA, editor. DF, Mexico: AMPAVE-CONASA; 2015. pp. 517.

- 35. Clarkson MJ. The life history and pathogenicity of *Eimeria meleagrimitis* Tyzzer 1929, in the turkey poult. Parasitology. 1959;49(1–2):70–82. doi:10.1017/S0031182000026718.
- 36. Halvorson DA, van Dijk, Brown P. Ionophore toxicity in turkey breeders. Avian Diseases. 1982:26(3);634—639. doi:10.2307/1589913.
- 37. Ficken MD, Wages DP, Gonder E. Monensin toxicity in turkey breeder hens. Avian Diseases.1989:33(1);186–190. doi:10.2307/1591087.
- 38. Andreasen JR, Schleifer JH. Salinomycin toxicosis in male breeder turkeys. Avian Diseases. 1995;39(3);638–642. doi:10.2307/1591821.
- 39. Van Assen EJ. A case of salinomycin intoxication in turkeys. The Canadian Veterinary Journal. 2006;47(3):256–258.
- 40. Ekinci IB, Chlodowska A, Olejnik M. Ionophore toxicity in animals: a review of clinical and molecular aspects. 2023;24(2):1969. doi: 10.3390/ijms24021696.
- Carrillo L, Bernad MJ, Monroy-Barreto M, Coello LC, Sumano LH, Gutiérrez L. Higer bioavailability of calcium in chicken with a novel in-feed pharmaceutical formulation. Frontiers in Veterinary Sciences. 2020:17(7)343. doi:10.3389/fvets.2020.00343.
- 42. Gutierrez OL, Sumano LH, Zamora QM. Administration of enrofloxacin and capsaicin to chickens to achieve higher maximal serum concentrations. Veterinary Record. 2002;150(11):350–353. doi: 10.1136/vr.150.11.350.
- 43. Vermeulen B, de Backer P, Remon JP. Drug administration to poultry. Advanced Drug Delivery Reviews. 2002;54(6):795–803. doi:10.1016/S0169-409X(02)00069-8.
- 44. Roura E, Baldwin MW, Klasing KC. The avian taste system: potential implications in poultry nutrition. Animal Feed Science and Technology. 2013;180(1–4):1–9. doi: 10.1016/j.anifeedsci.2012.11.001.
- 45. Liu H-X, Rajapaksha P, Wang Z, Kramer NE, Marshall BJ. An update on the sense of taste in chickens: a better-developed system than previously appreciated. Journal of Nutrition & Food Sciences. 2018;8(2):686. doi: 10.4172/2155-9600.1000686.
- 46. Severino P, da Silva CF, Andrade LN, de Lima Oliveira D, Campos J, Souto EB. Alginate nanoparticles for drug delivery and targeting. Current Pharmaceutical Design. 2019;25(11):1312—1334. doi:10.2174/138161282566619042516 3424.
- 47. Gheorghita Puscaselu R, Lobiuc A, Dimian M, Covasa M. Alginate: from food industry to biomedical applications and management of metabolic disorders. Polymers (Basel). 2020(Oct);12(10):2417. doi:10.3390/polym12102417.
- 48. Gutiérrez L, Tapia G, Gutiérrez E, Sumano H. Evaluation of a tasteless enrofloxacin pharmaceutical preparation for cats. Naive Pooled-sample approach to study lts pharmacokinetics. Animals. 2021;11(8). doi:10.3390/ani11082312.
- 49. Gutiérrez L, Lechuga T, Marcos X, García—Guzmán P, Gutiérrez C, Sumano H. Comparative bioavailability of enrofloxacin in dogs when concealed in non-commercial morsels, either as a tablet or as enrofloxacin-alginate dried beads. Journal of Veterinary Pharmacology and Therapeutics. 2021;44(4):522—532. PMC7411182 doi:10.1111/jvp.12925.
- 50. Wunderlich F, Al-Quraishy S, Steinbrenner H, Sies H, Dkhil MA. Towards identifying novel anti-Eimeria agents: trace elements, vitamins, and plant-

- based natural products. Parasitology Research. 2014;113(10):3547-3556. doi: 10.1007/s00436-014-4101-8.
- 51. Huang L, Liu Z, Wang J, Fu J, Jia Y, Ji L, Wang T. Bioactivity and health effects of garlic essential oil: A review. Food Science & Nutrition. 2023;11(6):2450-2470. doi: 10.1002/fsn3.3253.
- 52. Rajput N, Naeem M, Zhang JF, Zhang L, Wang T. The effect of dietary supplementation with the natural carotenoids curcumin and lutein on broiler pigmentation and immunity. Poultry Science. 2013;92(5):1177-1185. doi: 10.3382/ps.2012-02853.
- 53. Khan RU, Naz S, Javdani M, Nikousefat Z, Selgaggi M, Tufarelli V, Laudadio V. The use of tumeric (Curcuma longa) in poultry feed. World Poultry Science. 2012;68. doi: 10/10.17/S0043933912000104.
- 54. Al-Rubaei ZM, Mohammad TU, Ali LK. Effects of local curcumin on oxidative stress and total antioxidant capacity in vivo study. Pakistan Journal of Biological Sciences. 2014;17(12):1237-1241. doi: 10.3923/pjbs.2014.1237.1241.
- 55. Balaji S, Chempakam B. Toxicity prediction of compounds from turmeric (Curcuma longa L). Food and Chemical Toxicology. 2010;48(10):2951–2959. doi: 10.1016/j.fct.2010.07.032.
- 56. Ahad S, Tanveer S, Nawchoo IA, Malik TA. Anticoccidial activity of Artemisia vestita (Anthemideae, Asteraceae) - a traditional herb growing in the Western Himalayas. Kashmir, Indian Microbial Pathogenesis 2017;104:289-295. doi: 10.1016/j.micpath.2017.01.053.
- 57. Burt SA, Tersteeg-Zijderveld MH, Jongerius-Gortemaker BG, Vervelde L, Vernooij JC. In vitro inhibition of Eimeria tenella invasion of epithelial cells by phytochemicals. Veterinary Parasitology. 2013;191(3-4):374-378. doi: 10.1016/j. vetpar.2012.09.001.
- 58. Galli GM, da Silva AS, Biazus AH, Reis JH, Boiago MM, Topazio JP, et al. Feed addition of curcumin to laying hens showed anticoccidial effect and improved egg quality and animal health. Research in Veterinary Sciences. 2018;118:101–106. doi: 10.1016/j.rvsc.2018.01.022.
- 59. Al-Shuwaili MA, Ibrhim IE, Al-Bayati MTN. Effect of dietary herbal plants supplement in turkey diet on performance and some blood biochemical parameters. Global Journal of Bio-Science and Biotecnology. 2015;4(1):153–157.
- 60. Kim DK, Lillehoj HS, Lee SH, Lillehoj EP, Bravo D. Improved resistance to Eimeria acervulina infection in chickens due to dietary supplementation with garlic metabolites. British Journal of Nutrition. 2013;109(1):76-88. doi: 10.1017/ S0007114512000530.