



Preliminary Data on the Effect of *Costus pictus* D. Don on productive, reproductive and hematological parameters in laying hens Lohmann

Rodríguez-Flores JR¹, Sánchez-Hernández CI², Garcia-Delgado L¹,
Rodríguez-Yañez Y⁴, Minjarez B⁴, Reynoso-Orozco R⁵ and
Mireles-Flores S^{3*}

¹Department of Veterinary Medicine, University Center for Biological and Agricultural Sciences, Camino Ramón Padilla Sánchez No. 2100, Nextipac, Zapopan, Jalisco, México

²Intern in Veterinary Medicine and Animal Science, University Center for Biological and Agricultural Sciences, Camino Ramón Padilla Sánchez No. 2100, Nextipac, Zapopan, Jalisco, México

³Department of Animal Production, University Center for Biological and Agricultural Sciences, Camino Ramón Padilla Sánchez No. 2100, Nextipac, Zapopan, Jalisco, México

⁴Department of Agricultural Production, University Center for Biological and Agricultural Sciences, Camino Ramón Padilla Sánchez No. 2100, Nextipac, Zapopan, Jalisco, México

⁵Department of Cellular and Molecular, Biology University Center for Biological and Agricultural Sciences, Camino Ramón Padilla Sánchez No. 2100, Nextipac, Zapopan, Jalisco, México

***Corresponding Author:** Mireles-Flores S, Department of Animal Production, University, Center for Biological and Agricultural Sciences, Camino Ramón Padilla Sánchez No. 2100, Nextipac, Zapopan, Jalisco, México.

DOI: 10.31080/ASVS.2025.08.1032

Received: March 26, 2026

Published: June 12, 2026

© All rights are reserved by **Mireles-Flores S., et al.**

Abstract

For decades, the state of Jalisco, Mexico has been the leading producer of table eggs in Mexico, maintaining a share exceeding 50% of the national volume. The use of plant leaf, stem, and root extracts in poultry production, which can enhance egg quality and reduce the use of synthetic additives and their negative effects, are considered relevant in the industry. Medicinal plant leaves such as *Costus pictus* D. Don, or "insulin plant," can also be used as a feed additive to support the biological function of birds, and given its known activity on calcium physiology, it is proposed as an alternative to improve, among other aspects, egg quality. The effect of the leaves of *Costus pictus* D. Don was blended into the drinking water of 150-week-old Lohmann hens, just as it is consumed by people seeking its antidiabetic benefits, in two treatment groups. Egg quality and hematological parameters (humoral and cellular fractions) were measured. The preliminary results obtained in this study allow us to propose *Costus pictus* D. Don as a highly recommended additive to improve production, egg quality and leukocyte response in Lohmann hens during their productive development.

Keywords: *Costus pictus*; Egg Quality; Leukocytes; Laying Hens

Introduction

Over the past ten years, global egg production has achieved impressive growth. According to FAO data, total egg production rose from 61.7 million tons in 2008 to 76.7 million tons in 2018—a remarkable 24% increase over the decade [31]. With nearly 172 million laying hens in Mexico, 3.1 million tons of table eggs were produced in 2022, positioning the country as the sixth-largest producer in the world [39]. The state of Jalisco reaffirms its position as the leading producer of table eggs in Mexico, maintaining a market share exceeding 50% of the national volume, according to recent data from the UNA (National Poultry Farmers’ Union) [35].

The various factors associated with egg quality attributes are studied in relation to different housing systems for laying hens, and it is known that alternative systems do not have any negative impact on egg quality [12]. Furthermore, extracts from plant leaves, stems, and roots are currently utilized in poultry production as growth promoters and additives. Among other things, this is a result of the negative impact generated by synthetic substances over the decades [2].

There is a growing trend promoting the natural growth and health of poultry through the use of natural feed additives [22]. The leaves of medicinal plants can also be utilized as feed additives to support the biological functions of birds—serving as sources of vitamins and trace elements—acting as growth promoters, absorption enhancers, antimicrobial agents, and metabolic modifiers [18].

Costus pictus D. Don—also known as the insulin plant—is native to Mexico, and several of its attributes are recognized for their antidiabetic effects, among other health benefits [33]. Al-Romaiyan’s group [1] has demonstrated its effect on insulin secretion within the pancreatic islets of Langerhans, both *in vivo* and *in vitro*, by increasing Ca⁺⁺ concentrations in the hormone-producing beta cells. Given the widespread systemic influence of Ca⁺⁺ physiology [8,14,19]—as well as its role in the secretory granules of the hen oviduct [27,41]—it is highly feasible to investigate the effects of *Costus pictus* in models that do not suffer from diabetes, such as laying hens [5,37]. Furthermore, this approach allows for the proposal of these birds as a model for studying reproduction, given the established relationship between nutrition, energy expenditure, and reproductive capacity [11,26,36].

This paper presents preliminary results from a study conducted on Lohmann laying hens, which were administered *Costus pictus* D. Don—blended into their drinking water—and which, despite the hens’ advanced age, demonstrated a positive effect on egg quality and immune response. The probable effect on Ca⁺⁺ physiology is discussed, specifically regarding both peripheral lymphocytes and the yolk index and shell quality.

Materials and Methods

This is an experimental study in which the potential of liquefied *Costus pictus* D. Don—administered via drinking water—was evaluated regarding physiological, egg quality, and hematological parameters in 150-week-old Lohmann-line hens.

The experiment was conducted at the facilities of the University Center for Biological and Agricultural Sciences (CUCBA) of the University of Guadalajara, located at coordinates 20°25’30” to 20°57’00” North latitude and 103°19’30” to 103°39’20” West longitude, at an elevation of 1,548 meters above sea level. The mean annual temperature is 22°C, with a maximum of 36.1°C and a minimum of 11°C. It has an average precipitation of 906.1 millimeters [10]. The experimental trial was carried out in the Experimental Station of the same University Center.

Guaranteed Analysis	
Minimum Protein	16.00%
Minimum Fat	3.00%
Maximum Crude Fiber	4.00%
Maximum Ash	12.70%
Maximum Moisture	14.50%
ELN	52.30%
Feed Ingredients	
Ground cereals	59.40%
Oilseed meals	25%
Soybean oil	3.20%
Calcium carbonate	9.00%
Calcium phosphate	0.375%
Lysine	0.745%
Methionine	0.365%
Threonine	0.575%
Sal	0.225%
Mycotoxin Binder	0.730%
Tryptophan	0.175%

Vitamins: A	125,000 UI/kg
D3	25,000 UI/kg
E	312 UI/kg
K	20 mg/kg
B2, B3, B5, B12	62,5 mg/kg 200mcg/kg
Choline	1700 mg/kg
Iron	680 mg/kg
Iodine	8,75 mg/kg
Selenium	3,75 mg/kg
Zinc	500 mg/kg
Manganese	937mg/kg
Copper proteinates	125 mg/kg
Copper sulfate	0.02%
Spice essences	2%
Oil essences	2%
Yeast (<i>Saccharomyces cerevisiae</i>)	2x10 ⁸ ufc/g (5Kg/ton)
Quantitative Formula	
Raw Materials (Cereal Grains and Oilseed Meals)	91%
Minerals	4%
Vitamins	4%
Additives	2%
Physical Characteristics	
Odor	Cookie
Color	Coffee
Flavor	Cookie
Texture	Small ball
Appearance	Small cylinders

Table 1: Nutritional Characteristics.

From a production flock, 20 birds—150 weeks old and in table-egg production—of the Lohmann genetic line were selected. With an average weight of 1.700 kg, they were randomly distributed into two lots of 10 birds each (n=10). The control group received the diet recommended for laying hens according to the current year’s manual (Table 1). The second group received a liquidized solution of *Costus pictus* D. Don—at a dosage of 6 g/kg of body weight—via their drinking water. The birds were housed individually in laying-hen cages and provided with controlled portions of their respective diets.

Egg weight and feed intake were recorded over a period of 180 days, while water intake was recorded for 100 days. The experiment spanned 25 weeks of plant administration, and six birds were euthanized in strict compliance with the guidelines for humane slaughter set forth in [30]—*Methods for Euthanizing Domestic and Wild Animals*—at the conclusion of the trial, and in accordance with the parameters established by the CUCBA Bioethics Committee. The euthanasia and subsequent hematological analyses were performed on six hens selected based on the egg-laying performance they exhibited up until the end of the recording period.

Playback parameters

Egg production yield was measured daily, and the weight of each egg—indicating the day of lay—was recorded. Subsequently, the mean weight of the eggs laid by each group during the day was determined; this figure was then averaged weekly or monthly, as appropriate, for use in the relevant statistical analysis.

Blood was collected from the cutaneous ulnar vein to assess hematological parameters, followed by the subsequent sacrifice of six hens—one representing each laying behavior observed over a 150-day period: one that laid no eggs (or fewer than 10%) and also presented a tumor external to the oviduct; a 42-week-old hen; one that laid consistently but experienced a decline in production toward the end of the study; one that laid eggs consistently throughout the study; one that received treatment with *Costus pictus* D. Don; and one that laid eggs continuously—producing eggs with an average weight greater than that of the other birds—and which also presented a tumor located within the oviduct.

Drinking system

Fresh leaves of *Costus pictus* D. Don were harvested daily from plants cultivated at the CUCBA Botanical Garden and blended with tap water. Laying hens were provided with water daily in 400 mL crescent-shaped drinkers, positioned at a height between the bird’s back and eye level, containing the *Costus pictus* D. Don solution—calculated at a ratio of 6 mg per kg of the hen’s body weight per liter of water. A logbook record was maintained of the daily water consumption (in mL) for both groups.

Food consumption

Feed was added to trough-type feeders—specifically, 1 kg of feed per five hens per trough—and the residual feed was weighed to calculate the average consumption per hen.

Egg quality parameters

Egg quality analysis

The internal and external quality characteristics of the eggs were evaluated using specific measurements. External characteristics included egg weight (g) (EW) and shell thickness (mm). Regarding internal characteristics, values were recorded for yolk diameter, yolk height and color (YC), albumen height (mm), and Haugh Units (HU).

The assessment of internal and external egg quality was specifically conducted using the DET 6500 digital egg tester (Japan). Haugh Units are calculated based on the egg's weight and albumen height; the higher the score, the better the egg's quality. Yolk color and yolk index—the latter calculated from the yolk's height and diameter—are also taken into account (noting the day the egg was laid to avoid skewing the index), as are shell strength and thickness.

Monitoring of hematological parameters

3 mL blood samples were obtained from both groups via cutaneous cubital vein puncture using a Vacutainer.

Hematological tests

Two hematological tests were performed: one prior to treatment with *Costus pictus* D. Don, and the second upon completion of the experiment. These tests determined the counts of red blood cells, white blood cells, platelets, and neutrophils. Additionally, the plasma was analyzed to assess its color, protein content, and the presence of parasites.

Statistical analysis

All statistical analyses were performed using SigmaPlot 11. Comparisons were conducted using the Mann-Whitney rank sum test or the Kruskal-Wallis test, followed by Tukey's test for multiple mean comparisons. A p-value of 0.05 was considered statistically significant.

Results

Decrease in average egg weight

Given that the hens used in the present study were over 150 weeks of age, the decision was made to monitor the decline in the weight of the eggs laid by the birds—which were collected daily for

recording—over a period of 135 days. Data were collected regarding the egg production of 10 hens per group: those that received a treatment consisting of 6 g/kg of body weight administered via their drinking water for 25 days, and their corresponding controls, which received no such additive in their water (Figure 1). As can be observed in the graph, initially, no significant changes in egg weight were apparent; subsequently—following the addition of the blended *Costus pictus* D. Don leaves—a more pronounced decline in egg weight was observed in the control group. Statistical analysis using an independent samples t-test indicated normality ($P = 0.431$) and yielded a P-value of 0.001, which is statistically significant.

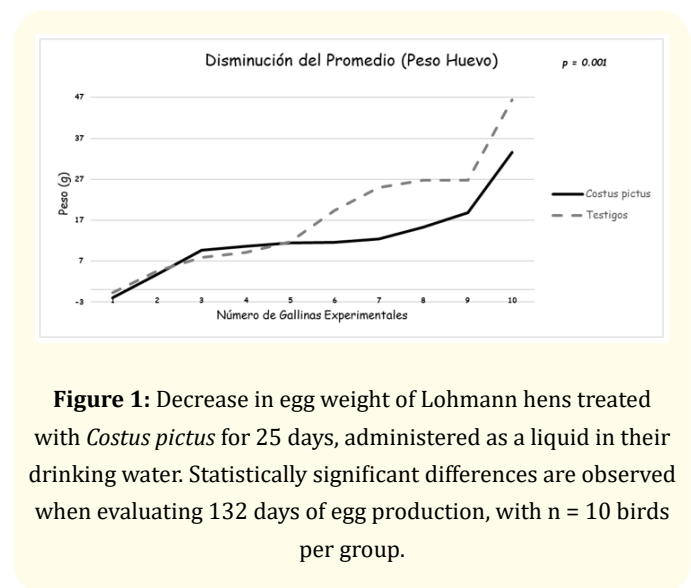


Figure 1: Decrease in egg weight of Lohmann hens treated with *Costus pictus* for 25 days, administered as a liquid in their drinking water. Statistically significant differences are observed when evaluating 132 days of egg production, with $n = 10$ birds per group.

Food consumption

The feed consumption recorded during the studied laying period shows no statistically significant difference, although it does exhibit a tendency for the control group to consume more feed when measuring the average consumption per five hens within the 5-cage modules, per administered portion (Figure 2).

Water consumption

Similarly, water consumption per hen was recorded when 400 mL of water per day was administered—either mixed with blended *Costus pictus* D. Don or, for the control group, as plain tap water.

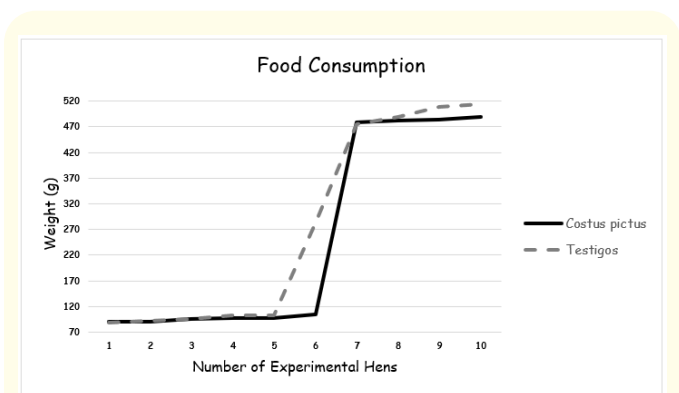


Figure 2: Feed intake of Lohmann hens over 150 days, treated with *Costus pictus* D. Don during the final laying phase (150 weeks of age).

The trend is quite distinct regarding the treated hens, as they consumed approximately 20 to 30 mL more water than the control birds (Figure 3).

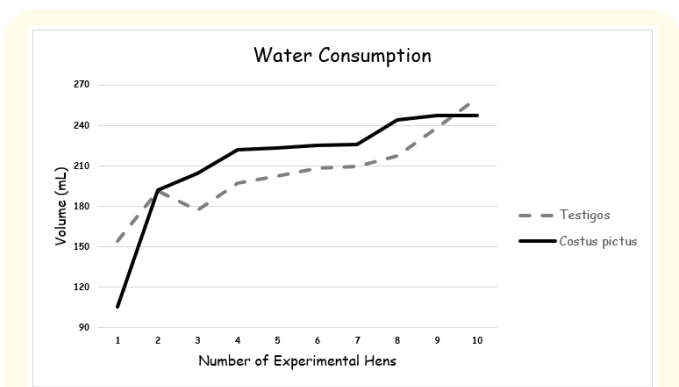


Figure 3: Water consumption of Lohmann hens treated with *Costus pictus* blended into their drinking water during the final laying phase (150 weeks of age).

Peripheral Lymphocyte-to-Basophil ratio

In general, the birds exhibited an increase in total leukocytes, among other values that also showed a tendency toward immunostimulation following treatment with *Costus pictus* D. Don blended into their drinking water. In particular, lymphocytes showed a marked increase in cell levels within the peripheral blood of hens studied over a 135-day period—35 days of which involved treatment with *Costus pictus* D. Don blended into their drinking water. When compared to hens that laid practically no eggs during the study—one of which presented a tumor outside the oviduct

upon necropsy (Bar 1, Figure 4)—the treated hens displayed nearly double the number of lymphocytes. This count was even higher than that of a young hen at the peak of production (35 weeks of lay; Bar 2), higher still than that of a hen whose production declined toward the end of the experiment despite receiving *Costus pictus* D. Don (Bar 3), and higher than that of a hen that consistently maintained its production and exhibited similar blood lymphocyte counts (Bar 4). Curiously, these values were similar to those of a hen that consistently laid eggs—producing eggs of a higher weight (Bar 6) than the rest of the studied flock—and which developed a tumor inside the oviduct (Figure 4). Regarding basophils—another leukocyte population that showed a marked increase relative to other hens with differing egg-laying characteristics—a substantial increase was observed in the hen that consistently produced eggs of a weight exceeding the flock average and which harbored a tumor inside the oviduct (Bar 6, Figure 4); notably, its basophil levels were similar to those of the young hen in full production. Conversely, the hens characterized by low egg production also exhibited a complete absence of basophils (Bars 1 and 4).

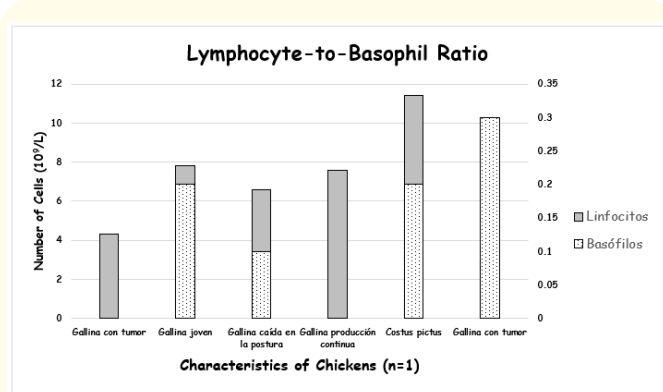


Figure 4: Lymphocyte-to-Basophil ratio in the peripheral blood of Lohmann hens after 150 weeks of age. The hen with a tumor represented by the first bar presented the neoplasm outside the oviduct and laid practically no eggs. The hen with a tumor represented by the last bar presented the neoplasm inside the oviduct and laid eggs almost continuously, producing the heaviest eggs recorded during the study. The hen represented by the second bar corresponds to a 45-week-old hen. The third bar represents a bird that showed a decline in egg production during the final month of the experiment; the hen corresponding to the fourth bar maintained continuous egg production throughout the entire duration of the study. The fifth—and highest—bar displays the peripheral blood lymphocyte count for a hen that received treatment with *Costus pictus* D. Don in her drinking water for 25 days.

Eggshell breaking strength

Regarding production parameters, those related to egg quality were studied; these were evaluated using automated equipment (DET 6500, Japan) on four dates during the administration of the treatment with *Costus pictus* D. Don. Figure 5 shows that the group treated with the addition of the plant to their drinking water exhibited greater eggshell strength than the hens that did not receive this addition, with statistically significant differences as determined by Student’s t-test (Figure 5).

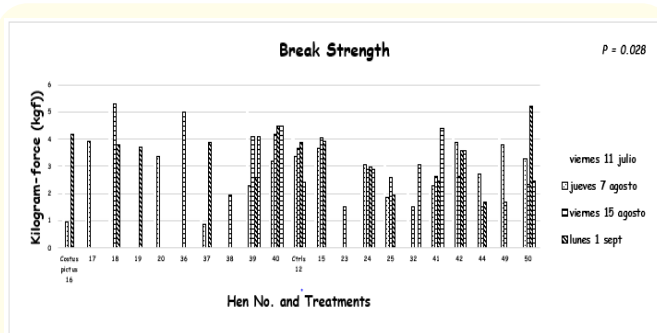


Figure 5: The graph shows the eggshell breaking strength of Lohmann hens treated with *Costus pictus* D. Don leaves blended into their drinking water (n = 10) and their controls (n = 11). Statistical analysis was performed using Student’s t-test, with a p-value of 0.028.

Shell thickness

Shell thickness was found to be increased (Graph 6) in Lohmann hens treated with *Costus pictus* D. Don across four cohorts—before, during, and after treatment—exhibiting statistically significant differences as determined by Student’s t-test, with a p-value of 0.001.

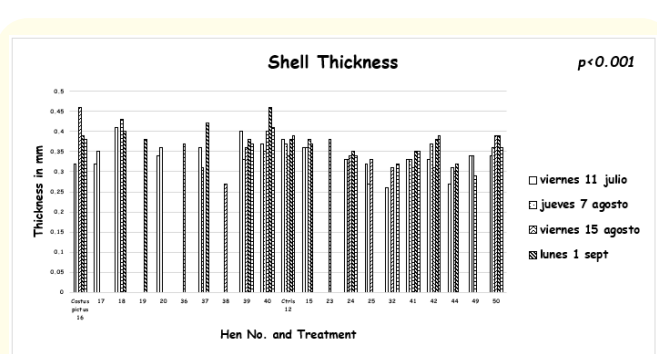


Figure 6: The graph shows the eggshell thickness of Lohmann hens treated with *Costus pictus* D. Don leaves blended into their drinking water (n = 10) and their controls (n = 11). Statistical analysis was performed using Student’s t-test, with a p-value of 0.028.

Discussion

Egg quality

Egg weight

The poultry industry is vital to a country’s economy [40]. The animal protein provided by poultry—specifically chicken and eggs—is widely consumed by humans. Poultry constituted the largest source of protein worldwide in 2019 [20].

The results obtained in the present study demonstrate an improvement in the net egg mass produced (Graph 1) in Lohmann-line hens when *Costus pictus* D. Don—liquefied and added to their drinking water—was administered; this occurred without altering feed intake (Graph 2), while resulting in an approximate 10% increase in water consumption (Graph 3). Furthermore, an increase in blood lymphocytes and basophils was observed in the treated group (Graph 4).

Water

Water is, *per se*, the most important nutrient consumed by domestic poultry, both in terms of quantity and due to their susceptibility to its absence; indeed, the survival of birds in the absence of water is very limited, as they can survive without any other nutrient for longer periods than they can without water [34].

Any nutrient that triggers an increase in mineral excretion by the kidneys also leads to an increase in water consumption. A higher protein content in the diet elevates water intake as well as the water-to-feed ratio. An increase in dietary salt—as well as ingredients rich in potassium, such as soybean and molasses—causes an increase in water consumption [6], similar to the addition of *Costus pictus* D. Don (Figure 3).

Calcium metabolism

Costus pictus D. Don exerts its effect primarily on Ca++ metabolism rather than on insulin synthesis within the pancreatic beta cells [1]; among other molecules demonstrating activity regarding the metabolism of this ion—which is indispensable to cellular physiology—is quercetin [33]. It has been demonstrated that a 0.06% dietary supplement of quercetin improves calcium and phosphorus metabolism by regulating the Wnt signaling pathway in broiler chickens [8,20]. The natural administration of phytopharmaceuticals—such as those derived from plants like *Costus pictus* D. Don, and specifically in the case of quercetin [33]—appears to provide similar benefits in laying hens, particularly when assessing egg quality and effects on the peripheral immune system, as evidenced by the results of the present study. Quercetin,

a type of flavonoid, is abundant in various teas, fruits, and leaves, and possesses antioxidant, anti-inflammatory, anti-allergic, antibacterial, and antiviral properties [21,32,42].

Leukocytes

In the present study—and generally speaking—the hens exhibited immunosuppression upon reaching 150 weeks of egg production (data not shown), given that all of them displayed values lower than those observed in the young hen ($18.4 \times 10^9/L$), as well as lower levels of heterophils or segmented neutrophils ($12.2 \times 10^9/L$), which constitute the primary barrier against bacteria and fungi [4]. Furthermore, the two hens that developed intra- and extra-oviductal tumors presented a lower number of platelets in the bloodstream (data not shown) [24], as well as lower levels of basophils (bars 1 and 4, Figure 4).

Lymphocytes

The most striking result is that observed for the blood lymphocyte population, given that the hens treated with *Costus pictus* D. Don also exhibited the highest counts compared to the other groups (Figure 4). This effect is likely associated with the flavonoid quercetin—reported in plants belonging to the genus *Costus*—and its influence on calcium and phosphorus metabolism [20,33]; as previously mentioned, this is also linked to its effect on insulin release from the islets of Langerhans in the pancreas, as observed in studies investigating the antidiabetic effects of *Costus pictus* D. Don in mice and humans [33].

Along the same lines, if we consider the implications of calcium metabolism for the development and activity of lymphocytes [20,23,25], then a more comprehensive perspective on the positive systemic effects of using *Costus* can be developed [33].

In addition to the many similarities with the mammalian immune system, the avian immune system possesses several unique features. The avian immune system consists of both an innate and an adaptive system, both of which include cellular and humoral components. Avian heterophils are functionally homologous to mammalian neutrophil granulocytes, as they exhibit strong phagocytic activity and are the first cells involved in inflammatory responses [13].

Regarding the results obtained in this study—specifically concerning leukocytes—an immunostimulatory effect was

observed (data not shown) attributable to *Costus pictus* D. Don. This effect is likely associated with an increase in intracellular calcium ion (Ca^{++}) levels, given that this constitutes one of the key triggering signals for the development of the B-cell response to antigens. The various Ca^{++} signals—which are finely controlled by multiple factors—participate in the regulation of gene expression, B-cell development, and their effector functions [3]. Similarly—and given that calcium signaling serves as a universal signal transduction mechanism in both animal and plant cells—calcium signaling is essential for the activation and reactivation of mammalian T lymphocytes; it is, therefore, critical for a functional immune response [9], even in subjects of advanced age, such as the hens examined in this study. Based on the foregoing, we may speculate regarding the immunostimulatory effects of *Costus pictus* D. Don (Figure 4)—effects that are particularly noteworthy given the plant's natural route of administration via drinking water, which the subjects accepted without any apparent difficulty.

Essential for good health is a fully functional immune system, which, however, can be threatened by stressors and other adverse environmental stimuli. A fully functional immune system is essential not only for the health and well-being of chickens but also for high productivity and safe animal products [16]. Although the number of immune cells allows for their association with stress status in laying hens, it is the Heterophil-to-Lymphocyte ratio that is used as a marker for this parameter; perhaps, in the Lohmann hen model, this could be established as a reliable test if further research is conducted with this specific focus [7]. Regarding basophils, chickens possess a higher number of basophils than mammals, and these cells play a central role in early inflammation and immediate hypersensitivity [28]. Once again, *Costus pictus* D. Don appears to stimulate the proliferation of these blood leukocytes (Figure 4). Furthermore, it has been demonstrated that effects on Ca^{++} flux and IL-3 act as potent initiating agents for basophils, often enabling or enhancing their responses to other stimuli [15].

In addition to what has already been mentioned, it is important to note that calcium is one of the most critical and essential components of the human body, as it constitutes the majority of the human skeletal system (98%). In the case of hens, it is known that shell formation within the oviduct occurs in two distinct phases. Furthermore, 5.5 grams of calcium carbonate account for approximately 95% of the dry eggshell weight. Strategies aimed

at enhancing calcium utilization in hens' diets include—among others—intestinal acidity (particularly for the absorption of CaCO_3), estrogens, Vitamin D, soluble fiber/prebiotics, probiotics, and synbiotics [36]. As is evident, natural products represent a highly suitable alternative—particularly when applied within the most adaptable contexts of nutritional management—as demonstrated in the present study. It should not be overlooked that our results indicate a comprehensive impact on systemic calcium metabolism, manifesting in physiological effects (Figure 1), immunological effects (Figure 4), and likely effects on specific tissues (Figure 5 and 6). Given that the shell gland represents the most prominent tissue involved in calcium utilization for shell formation [17], it is plausible to posit that the observed improvements in shell strength (Figure 5) and thickness (Figure 6) serve as evidence that this gland acts as a target—perhaps a specific one—for the phytochemical activity of *Costus pictus* D. Don [33] when administered via drinking water.

Finally, the results presented here—though preliminary—suggest the initiation of a line of research encompassing both basic science and industrial productivity. This is warranted by the advantages of utilizing an indigenous Mexican plant [33]—specifically *Costus pictus* D. Don—and by its potential applications, which have already been demonstrated in numerous studies. Consequently, there is promising potential for achieving highly positive results, both within the field of natural products with therapeutic applications and in terms of productivity in extensive veterinary operations.

Conclusions

Both productivity and the immune system undergo systemic deterioration as Lohmann-line hens age under captive conditions. Preliminarily, the administration of liquefied *Costus pictus* D. Don via drinking water enhances egg quality parameters—specifically increasing production and shell quality—without altering feed intake, though it does increase water consumption. Furthermore, the results obtained in the present study suggest an immunostimulatory effect on immune cells, such as leukocytes—and, in particular, lymphocytes and basophils.

Bibliography

1. Al-Romaiyan A., *et al.* "Costus pictus extracts stimulate insulin secretion from mouse and human islets of Langerhans in vitro". *Cellular Physiology and Biochemistry* 26.6 (2016): 1051-1058.
2. Azodo L C., *et al.* "Response of Starter Broiler Birds Administered with Costus afer Extract". *Asian Journal of Biological Sciences* 18.3 (2025): 633-637.
3. Baba Y and Kurosaki T. "Role of Calcium Signaling in B Cell Activation and Biology". *Current Topics in Microbiology and Immunology* 393 (2016): 143-174.
4. Barbieri Petrelli G., *et al.* "El neutrófilo y su importancia en la enfermedad periodontal". *Avances en Periodoncia e Implantología Oral* 17.1 (2005): 11-16.
5. Braun EJ and Sweazea KL. "Glucose regulation in birds". *Comparative Biochemistry and Physiology Part B* 151.1 (2008): 1-9.
6. Chango M. "Boletín El Sitio Avícola". Conferencia durante el XXIV Congreso Latinoamericano de Avicultura, Guayaquil, Ecuador (2015).
7. Das, H.; Lacin, E. The effect of diferent photoperiods and stocking densities on fattening performance, Carcass and some stress parameters in broilers". *Israel Journal of Veterinary Medicine* 69 (2014): 211-220.
8. De A. "Wnt/Ca2+ signaling pathway: a brief overview". *Acta Biochimica et Biophysica Sinica (Shanghai)* 43.10 (2011): 745-56.
9. Ernst IM., *et al.* "Adenine Dinucleotide Second Messengers and T-lymphocyte Calcium Signaling". *Frontiers in Immunology* 4 (2013): 259.
10. García, E. "Modificaciones al sistema de clasificación climática de Kopen". 5ª Edición (2004).
11. México: Instituto de geografía, UNAM.
12. Gaskins AJ., *et al.* "EARTH Study Team. Dietary patterns and outcomes of assisted reproduction". *American Journal of Obstetrics and Gynecology* 220.6 (2019): 567.e1-567.e18.
13. Gautron J., *et al.* "Review: Production factors affecting the quality of chicken table eggs and egg products in Europe". *Animal* 16 (2022): 100425.
14. Genovese KJ., *et al.* "The avian heterophil". *Developmental and Comparative Immunology* 41 (2013): 334-340.
15. Gilon P., *et al.* "Calcium signaling in pancreatic β -cells in health and in Type 2 diabetes". *Cell Calcium* 56.5 (2014): 340-361.

16. Gray N., *et al.* "Differential Upregulation and Functional Activity of S1PR1 in Human Peripheral Blood Basophils of Atopic Patients". *International Journal of Molecular Sciences* 23.24(2022): 16117.
17. Hofmann T., *et al.* "Impact of Housing Environment on the Immune System in Chickens: A Review". *Animals (Basel)* 10.7 (2020): 1138.
18. Hrabia A. "Chapter 35 - Reproduction in the female". Editor (s): Colin G. Scanes, Sami Dridi, *Sturkie's Avian Physiology (Seventh Edition)*, Academic Press (2022): 941-986.
19. Igugo RU. "An assessment of the use of varying levels of Moringa oleifera leaf meal as a substitute for vitamin+mineral premix in finisher broiler diet". *Journal of Experimental Research* 2 (2014): 88-92.
20. Jacobson DA and Shyng SL. "Ion Channels of the Islets in Type 2 Diabetes". *Journal of Molecular Biology* 432.5 (2020): 1326-1346.
21. Jahejo AR., *et al.* "Immunomodulatory and growth promoting effects of basil (*Ocimum basilicum*) and ascorbic acid in heat stressed broiler chickens". *Pakistan Journal of Zoology* 51 (2019): 801.
22. Jung CH., *et al.* "Quercetin reduces high-fat diet induced fat accumulation in the liver by regulating lipid metabolism genes". *Phytotherapy Research* 27 (2013): 139-143.
23. Kalilu R., *et al.* "Performance and Carcass Characteristics of Isa Brown Cockerels Fed Graded Levels of Air Dried Neem Leaf and Bark Meal". *Brazilian Journal of Science* 3.7 (2024): 115-125.
24. King LB and Freedman BD. "B-lymphocyte calcium influx". *Immunology Review* 231.1 (2009): 265-277.
25. Liao K., *et al.* "The role of platelets in the regulation of tumor growth and metastasis: the mechanisms and targeted therapy". *MedComm* 4.5 (2023): e350.
26. Luan S and Wang C. "Calcium Signaling Mechanisms Across Kingdoms". *Annual Review of Cell and Developmental Biology* 37 (2021): 311-340.
27. Maitin-Shepard M., *et al.* "Food, nutrition, and fertility: from soil to fork". *American Journal of Clinical Nutrition* 119.2 (2024): 578-589.
28. Makita T., *et al.* "Calcium Detection in Secretion Granules of Avian Oviduct by Scanning Electron Microscopy (SEM) and Energy-dispersive X-Ray Microanalysis (EDX) 1". *Journal of Histochemistry & Cytochemistry* 31 (1983): 219-221.
29. NOM-033-SAG/ZOO-2014.
30. Maxwell MH and Robertson GW. "The avian basophilic leukocyte: A review". *World's Poultry Science Journal* 51 (1995): 307-325.
31. Organización Mundial del Huevo. "La producción mundial de huevos sigue creciendo - febrero". *World Egg Organisation* (2021).
32. Rezk AM., *et al.* "Quercetin and lithium chloride potentiate the protective effects of carvedilol against renal ischemia-reperfusion injury in high-fructose, high-fat diet-fed Swiss albino mice independent of renal lipid signaling". *Chembio Interact* 333 (2021): 109307.
33. Rodríguez-Yáñez Y., *et al.* "Papel de *Costus* spp. en la regulación de la hiperglucemia". *TIP Revista Especializada En Ciencias Químico-Biológicas* 28 (2026).
34. Ruíz EM and Tabares JG. "La sanidad del agua de bebida en la avicultura". *Selecciones Avícolas* (2012): 1-14.
35. Sánchez SL. "AviNews: Jalisco sigue como líder nacional en huevo en México". GCMA (2025).
36. Scahan P. "An Egg Shell: A Nutritional Profile and Health Benefits". *Research in Pharmacy* 13 (2023): 1-7.
37. Sweazea KL. "Revisiting glucose regulation in birds - A negative model of diabetes complications". *Comparative Biochemistry and Physiology Part B: Biochemistry & Molecular Biology* 262 (2022): 110778.
38. Takada K., *et al.* "Female reproductive tract-organ axes". *Frontiers in Immunology* 14 (2023): 1110001.
39. United States Department of Agriculture. "Egg-grading manual (Agricultural Handbook No. 75)". Agricultural Marketing Service (2000).
40. Wang B., *et al.* "Quercetin Regulates Calcium and Phosphorus Metabolism Through the Wnt Signaling Pathway in Broilers". *Frontiers in Veterinary Science* 8 (2022): 786519.
41. Waqas MY., *et al.* "Characterization of the ultrastructure in the uterovaginal junction of the hen". *Poultry Science* 95.9 (2019): 2112-2119.
42. Zhang R., *et al.* "Antidiabetic activity of isoquercetin in diabetic KK -A (y) mice". *Nutrition and Metabolism* 8 (2011): 85.