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Research Article

Pilot Test on the Hypoglycemic, Immunomodulatory and Productivity Effect of *Costus pictus* Liquefied in Wistar Rats and Laying Hens

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Abstract

The use of the leaf of *Costus pictus* D. Don has demonstrated a hypoglycemic effect in the rat model of streptozotocin-induced diabetes. In addition, other species of the same genus and different types of extractions show anti-inflammatory activity. Processing the leaves of this plant to administer experimentally allows a more reasonable approach to study the effects on the physiology of the organism in a comprehensive way and an adequate comparison with the human body. Previously, our group published results on the same treatment with 150-week-old hens of the Lohmann line and in the present work we report: physical-chemical analysis of the plant, its hypoglycemic effect in the rat model and on hens of the Hy Line and 50 weeks of age, as well as on egg production and peripheral blood leukocyte formula. comparing with the aqueous extract of the leaf of *Costus pictus* D. Don microencapsulated. The same trend was found regarding the hypoglycemic effect in both models and in terms of what was reported in long-lived hens *versus* young hens, both for production parameters and for the immune response. The relevance of the formulation to be administered in the laying hen model and the strategy to achieve better results in preclinical models with extracts and with respect to application times and probable unwanted effects are discussed.

Keywords: *Costus pictus*; Antioxidant; Olive Oil

Introduction

The aerial parts of *Costus pictus,* or insulin plant, are used in traditional medicine in many countries [26], as they have potent antidiabetic properties. In addition, the results obtained with methanolic extracts administered to rats with streptozotocin-induced diabetes show antidiabetic efficacy [9]. Aqueous extracts also show antibacterial activity, as well as those achieved with polar solvents [31]. Fractions with methanolic extracts with antidiabetic potential have been identified, but their efficacy has not been proven with beverages commonly used by people who treat their ailments with leaves liquefied in water, even though the extractive value is higher (around 30%) than solvent extractions [18,31,39].

The use of the leaf of *Costus pictus* D. Don has shown a hypoglycemic effect in the model of rats with streptozotocin-induced diabetes [15], in addition, other species of the same genus [8] and different types of extractions show anti-inflammatory activity. Nephroprotective and antioxidant, among others [27]. Previously, our group published results on the same treatment with 150-week-old hens from the Lohmann line [22]. Currently, the same effects of the plant on hens of the Hy Line and 50 weeks of age or the last part of phase 1 of production are unknown.

Coating bioactive substances in order to obtain their release in a controlled manner by microencapsulation and achieving structures between 0.1 and 100 µm in diameter, is a technique used to obtain a release of these bioactive substances in a controlled manner [5]. This method is widely used in the food and pharmaceutical industry, as it protects bioactive substances from oxidation and/or degradation due to heat, light, enzymes or undesirable chemical reactions. Therefore, it allows easy-to-degrade substances to be kept in optimal condition. In addition, they can mask flavors and aromas that directly impact the consumer's final product [2]. In the poultry industry, there are incipient works for the use of essential oils and antibiotics in a microencapsulated form in order to improve various aspects of poultry health and feeding [21]. In this sense, vegetable oils are currently gaining great interest in commercial development due to their availability, low price, biodegradability and low environmental impact [12].

Among the different beneficial activities of the plant and that has not been given the importance it deserves, according to our point of view, is its effect on the immune response. Given its implications for general physiology, metabolic compromises, variability in physiological sensitivity or genetic predisposition [35]. It is known that hens present Natural Antibodies (NAb) when they are healthy and without exposure to an exogenous antigen and that a possible selection that allows high levels of NAb to be obtained could contribute to a better overall resistance to diseases,[11] however, results are not usually reported to this part of the innate immune response. And this type of response can contribute to reviewing the state of the immune system of animals that are continuously exploited and the effects of medicinal plants.

This report presents the results obtained with 21 hens grouped into 7 birds per group that received the same treatment scheme with liquefied *Costus pictus* D. Don leaves, as well as aqueous extract of the plant microencapsulated with olive oil for 20 days and its control group, the weight of the egg was recorded. the blood leukocyte pattern, as well as some photochemical characteristics of the plant and bromatological analysis. Here we discuss the possible effects of *Costus pictus* D. Don in the form of liquefied leaves served in drinking water on the immune response.

Materials and Methods

Organoleptic test

The organoleptic evaluation consisted of examining the leaves of *Costus pictus* D. Don based on their color, smell, flavor, texture, among other aspects.

Bromatological analyses

The samples were collected from leaves of *Costus pictus* D. Don, established in the open air, in the experimental fields of the University Center for Biological and Environmental Sciences. Thus, from 500 g of plant material, stored in paper bags, it was dried in an oven at 60 °C, for 48 h and ground in a blade mill (IKA ALL Basic), until a homogeneous particle size was obtained. The dust obtained was stored in airtight plastic bags, and kept at room temperature in a dry and dark place until analysis. For the bromatological composition, the variables were analyzed using the AOAC or Official Methods of Analysis of Association of Analytical Chemists techniques of ash (AOAC 942.05), crude protein (AOAC 955.04), ether extract (AOAC 920.39), neutral detergent fiber (AOAC 962.09) and acid detergent fiber (AOAC 973.18), Moisture and volatile matter (AOAC 934.01), Nitrogen-Free Extract (ELN)

and Dry matter was calculated by weight difference of the crude biomass.

Animal model in rats

Eight male rats from the Wistar SEPA divided into four experimental groups were used, evaluated at the beginning of the experiment and after 20 days of treatment. Prior to the start of the glucose tolerance test, the animals underwent a 16-hour fasting period, during which they only had access to water. Subsequently, they were randomly distributed into four experimental groups (n=2 per group), according to the treatment assigned:

- **Group 1 (Negative control):** Received only water.
- **Group 2 (Treatment 3 g/kg):** Received fresh smoothie of leaves of *Costus pictus* D. Donation at the rate of 3 g/kg of body weight.
- **Group 3 (Treatment 6 g/kg):** Received fresh smoothie of *Costus pictus* D. Gift at the rate of 6 g/kg of body weight.
- **Group 4 (Positive control):** Received the hypoglycemic drug Sitagliptin as a therapeutic reference.

Preparation of plant material and dosage

The leaves of *Costus pictus* D. Don were collected, washed with running water and soap to remove residues and impurities. Freshly cut, they were liquefied after weighing and leaving them at concentrations of 3 g/kg and 6 g/kg body weight of Wistar rats. They were administered in drinking water over and over again. The solution was changed every 24 hours to ensure freshness when ingested. It should be noted that, during the trial, the animals were monitored daily to record feed intake, clinical signs and variations in behavior. Likewise, the recommendations of the Official Mexican Standard NOM-062-ZOO-1999, which establishes the technical specifications for the production, care and use of laboratory animals, were strictly followed, guaranteeing adequate animal welfare conditions throughout the experiment.

The rats were kept from two animals per box, in polycarbonate boxes of 26.6 × 48.2 × 20.3 cm, controlled environment with a temperature of 20°C to 26°C and a relative humidity of 40% to 70% and light-dark periods of 12 hours. Sawdust feed that was changed every 24 hours, constant access to fresh water through water bottles with stainless steel spout.

Microencapsulated in Olive Oil

The aqueous extract of *Costus pictus* D. Don, was prepared with 100 g of fresh leaves introduced in 1000 mL of distilled water, they were liquefied for 3 min (TPISA industrial blender, model T12L). The solid waste was filtered with a common strainer, the filtrate was centrifuged for 15 min at 10,000 rpm (Welso WTD600, China), the precipitate was removed and the supernatant was kept at 4°C until use. It was mixed with 92 mL of aqueous extract, 5 mL of extra virgin olive oil and 3 mL of Tween 80. The mixture was sonicated with an ultrasonic processor (model CV188, USA), at an amplitude of 65% (AMP1), for 10 min with cycles of 1 min of sonication, followed by 1 min of rest, until a homogeneous emulsion was achieved.

Glucose tolerance test

In order to determine the possible hypoglycemic effect in the experimental groups under the consumption of *Costus pictus* D. Don extracts, a glucose tolerance test (GTT) was performed. In accordance with international guidelines for the evaluation of compounds with glycemic regulatory activity in murine models. Fasted for 16 hours, the rats were orally given a glucose solution at a standard dose of 2 g/kg body weight, using a flexible intragastric tube to ensure precise administration. Blood samples were obtained by puncture in the caudal vein, at intervals of 0 (baseline), 30, 60, 90 and 120 minutes after glucose administration. The blood glucose concentration was determined using a glucometer (ONE TOUCH), which uses an electrochemical biosensor based on the enzyme glucose oxidase, which allows the quantification of glucose, by measuring the current generated by the oxidation of blood glucose [12]. The values obtained were expressed in milligrams per deciliter (mg/dL) and compared between the experimental groups. These values were contrasted with the glycemic parameters considered normal in rats, which range from 50 to 135 mg/dL by the International Diabetes Federation [13,36].

The animals did not show any mortality, not even signs of toxicity were manifested: tremors, convulsions, salivation, diarrhea, lethargy, sleepiness and coma during the entire observation period.

Microencapsulation

The aqueous extract of *Costus pictus* D. Don was made with 100 g of fresh leaves introduced in 1000 mL of distilled water, they were liquefied for 3 min (TAPISA, model T12L, Mexico). The solid waste

was filtered with a common strainer, the filtrate was centrifuged for 15 min at 10,000 rpm (Welso WTD600, China), the precipitate was removed and the supernatant was kept at 4°C until use. It was mixed with 92 mL of aqueous extract, 5 mL of extra virgin olive oil and 3 mL of Tween 80. The mixture was sonicated with an ultrasonic processor (model CV188, USA), at an amplitude of 65% (AMP1), for 10 min with cycles of 1 min of sonication, followed by 1 min of rest, until a homogeneous emulsion was achieved.

Model in Hy line chickens

Groups of animals

This part of the experiment was carried out within the facilities of the University Center for Biological and Agricultural Sciences (CUCBA) in the corresponding experimental hut with a controlled environment and with a duration of 4 weeks, a previous adaptation period of 2 weeks and without sacrificing any bird at the end of the work.

21 50-week-old hens from the Hy Line were grouped, all fed the recommended diet for phase 2 laying hens according to the current manual. 4 groups of 7 birds each were formed randomly, as follows:

- The control group was given tap water to drink,
- The group liquefied solution of leaves of *Costus pictus* D. Gift in drinking water concentration of 6g/kg body weight,
- Aqueous extract group at 6g/Kg body weight in the form of microemulsion.

Prior to the installation of the chickens, the experimental house was washed and disinfected. The hens were housed in individual cages (45 x 30 x 45 cm), 50 and 100 cm from the cement floor, to avoid stress and achieve animal welfare while controlling water consumption, in 500 mL volume drinkers and feed in the gutters designed for ad *libitum feeding*.

Egg weight

The eggs were weighed daily individually and recorded per hen, it was done regularly at 10 am to consider the production per 24 hours.

Determination of glucose by blood chemistry

400 µL of blood obtained from the cervical-cranial hollow of the hens were deposited in a tube (green) with anticoagulant, the sample chamber was filled in the disc with reagents for blood chemistry tests, specific for the veterinary analyzer (Skyla VB1, Taiwan) the equipment works fully automated.

Leukocyte formula

At the end of the experiment, 1 mL of blood was obtained from the cervico-cranial cavity of the hens and deposited in red tubes without anticoagulant. The leukocyte package was manually quantified under the microscope to report the leukocyte formula individually. Populations of Leukocytes, Lymphocytes, Monocytes and Eosinophils were recorded.

Statistical analysis

For egg weight, the range ANOVA test was performed and then Dunn's Method was run for the groups that showed differences in the comparison of means.

Results

The organoleptic properties of the leaves of *Costus pictus* D. Don correspond in appearance, color, smell and flavor characteristic of the same and although with a lower pH than expected in Table 1, those recorded in the present work are shown.

Regarding the agronomic characteristics obtained in the conditions within the University Center of Biological and Agricultural Sciences in Zapopan, Jalisco, Mexico, the feasibility of cultivating it in a healthy and productive way can be observed, as well as the characteristics of the plant that can reach up to 3m in this region of the world, the shape of its rhizomes and leaves, in addition to the colors and shape of the flowers (Image 1).

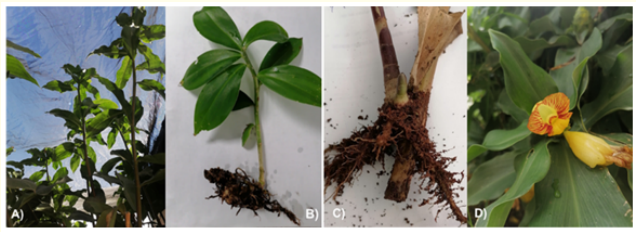


Image 1: Once transplanted, the first leaves initially emerge on average 10 to 14 days after transplanting, with an intense green coloration, a sign of a good nutritional status and active photo-synthetic capacity.

Organoleptic properties	
Appearance	Toothed oval
Color	Lemon Green
Odor	Characteristic
Taste	Characteristic sour
pH	3.45 Smoothie 3.35 Aqueous extracts

Table 1: Organoleptic properties of the leaf of *Costus pictus* D. Don.

The results of the nutritional composition by proximal analysis of the plant of our interest show the large amount of nutrients that are obtained in their digestible form and can be seen in table 2. The crude method to determine protein shows all that source of nitrogen that includes non-protein molecules such as nucleic acids where non-coding RNAs can be considered that can show metabolic activity and expression of very important genes. Although semi-quantitative, the method does not allow to evaluate its digestibility or the presence of essential amino acids.

Nutritious	Determination (Proximal Analysis)	Theoretical chemical components
Water	Humidity	Water
Structural carbohydrates	Crude Fiber	Cellulose, Hemicellulose and Lignin
Carbohidratos solubles	Nitrogen-free extract	Monosaccharides, disaccharides, trisaccharides, starches, pectins, resins, water-soluble organic acids.
Lipids	Crude fat	Waxes, fatty acids, pigments and vitamins
Proteins	Crude protein	Protein, amino acids, non-protein nitrogenous organic compounds, amines, nucleic acids and nitrogenous glycosides, chlorophylls, nitrogenous inorganic compounds, such as ammonium salts, ammonium hydroxide, ammonia, nitrites, and nitrates.
Minerals	Ashes	Compounds of Ca, Mg, K, Na, P, Fe, Mn, Cl, S, Cu, Co, Se, Si, Mo and Zn.
Vitamins		Water-soluble vitamins, B vitamins

Table 2: Nutritional composition of the proximal analysis of *Costus pictus* D Don.

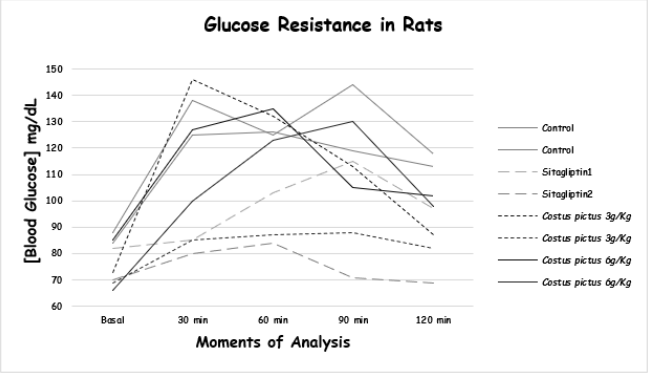
The bromatological composition (Table 3) was developed from the sheet only and the percentages of each determination are shown in the results column on the far right.

Determination	Method	Results (%)
Moisture and volatile material	AOAC 934.01	6.93
Protein (% N x 6.25)	AOAC 955.04	15.37
Ethereal Extract	AOAC 954.02	5.27
Ashes	AOAC 942.05	13.0
Crude Fiber	AOAC 962.09	16.52
ELN	By difference	42.91
Dry matter	By difference	93.07

Table 3: Bromatological composition of *Costus pictus* D. Don.

AOAC. Oficcial Methods of Analysis of Association of Official Analytical Chemists. ELN. Nitrogen-free extract.

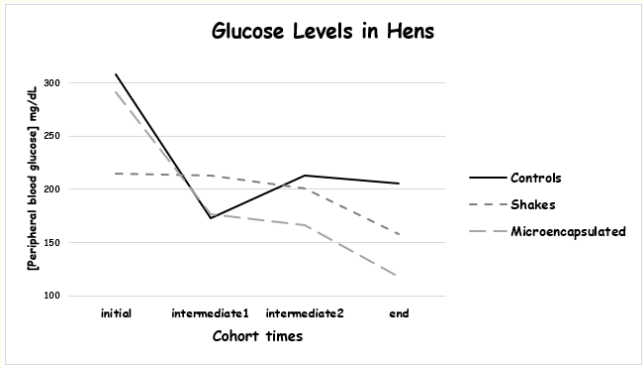
To confirm the hypoglycemic effect of *Costus pictus* D. Don in Wistar rats and compare it with the probable effect on the glycemia of laying hens, 8 rats grouped from 2 animals per treatment group were used and the glucose tolerance test with Sitagliptin was applied as a reference control, the individual results were plotted (Graph 1) and although the data do not show statistically significant differences, Perhaps due to a very low N value, the trend of the hypoglycemic effect is clearly appreciated.



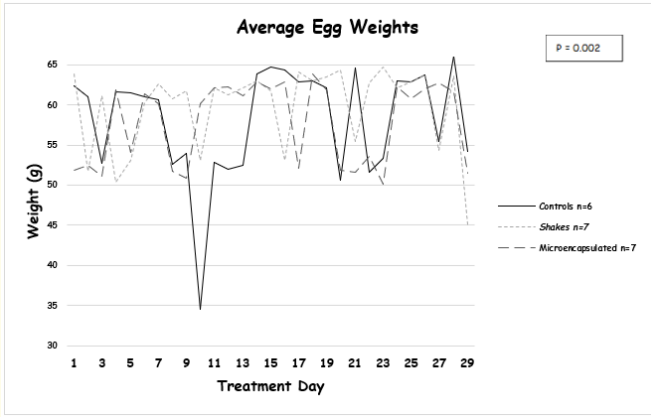
Graph 1: Effect of *Costus pictus* D. Don on the glucose tolerance test, alongside Sitagliptin, at doses of 3 and 6 g of liquefied leaf/kg of body weight administered in the drinking water of Wistar rats.

The glucose levels of hens of the Hy Line were determined within the blood chemistry parameters in the automated spectrophotometer equipment mindray medical (Shenzhen, China) and the recorded data of three treatment groups were plotted, including the control without *Costus pictus* D. Don. The other two received the liquefied leaf, from the same plants that were provided to the Wistar rats and also liquefied with the same process, as well as the aqueous extract of the leaf and microencapsulated in olive oil. The results are presented in graph 2 and although there is no significant statistical difference, the trend of hypoglycemic behavior can be seen in the groups treated with *Costus pictus* D. Don, both liquefied and in aqueous extract and microencapsulated with olive oil.

From the point of view of productivity in laying hens, it is important to consider the egg weight in an estimated period of time during treatments with *Costus pictus* D. Don. For this purpose, daily weight was evaluated, per bird and during the 30 days of treatment. In graph 3 you can see with statistically significant differences the increase in egg weight, both in the treatment with liquefied *Costus pictus* D. Don leaves and its aqueous extract and microencapsulated in olive oil.

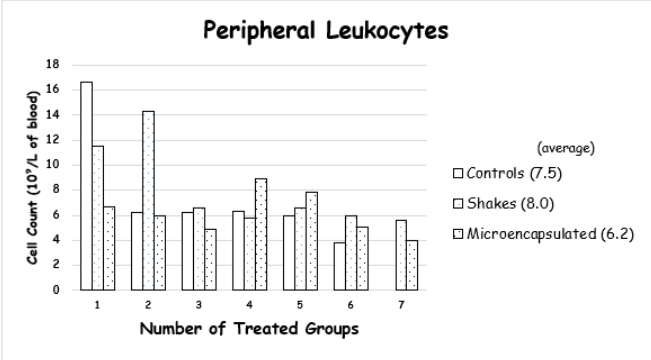


Graph 2: Peripheral blood glucose levels of Hy-Line genetic line hens treated with liquefied *Costus pictus* D. Don leaves and their aqueous extract microencapsulated with olive oil, along with their controls without treatment.



Graph 3: Average egg weights per treatment group of Hy-Line hens treated with leaves of *Costus pictus* D. Don. The product was liquefied in drinking water and used with aqueous extract and microencapsulated with olive oil, evaluated over 30 days of laying. A statistically significant difference was found, as demonstrated by ANOVA for range testing and multiple pairwise comparisons using Dunn's method.

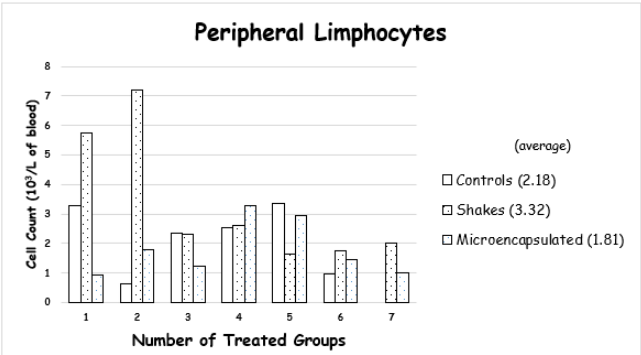
The leukocyte pattern in peripheral blood shows higher averages for any of the groups of hens of the Hy Line breed treated with the leaf of *Costus pictus* D. Don and in all cell lines counted. For leukocytes, a higher average was observed for the administration of the liquefied leaf with 8x109 cells per liter of blood, compared to the untreated controls that showed 7.5x109 and the birds treated with the microencapsulated extract with $6.2x10^9$ cells per liter of blood (Graph 4).



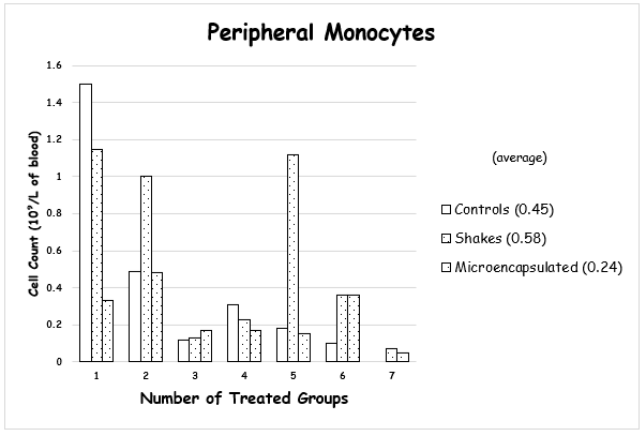
Graph 4: Total leukocyte count in peripheral blood of Hy-Line hens after receiving treatment with *Costus pictus* D. Don. The liquefied and aqueous extract of the same was microencapsulated with olive oil. The average number of cells per 7 replicates is shown in parentheses, except for group 7 where there are only 6 birds.

In the case of blood lymphocytes, once again a higher average value is observed for treatment with the liquefied leaf, than with untreated controls and the nanoencapsulated extract (Graph 5). It is important to mention that the amount of this cell population ranges from thousands ($10^{to\ 3}$ cells/L of peripheral blood) per volume of vital fluid.

The population of monocytes in venous blood, although with much lower values than previous cell lineages, continues to show a greater number of cells in hens treated with liquefied *Costus pictus* D. Don leaves compared to controls and microencapsulated extract (Graph 6).



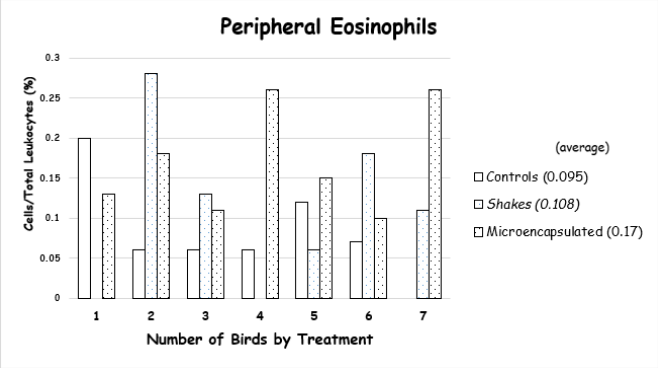
Graph 5: Total lymphocyte count in peripheral blood of Hy-Line chickens after treatment with *Costus pictus* D. Don. The liquefied and aqueous extract of the same was microencapsulated with olive oil. The average number of cells per 7 replicates is shown in parentheses, except for group 7 where there are only 6 birds.



Graph 6: Total number of monocytes in peripheral blood of Hy-Line hens after receiving treatment with *Costus pictus* D. Don. The liquefied and aqueous extract of the same was microencapsulated with olive oil. The average number of cells per 7 replicates is shown in parentheses, except for group 7 where there are only 6 birds.

If we look (graph number 7) the percentage of Eosinophils with respect to the total number of blood leukocytes, we have that the controls represent the group with the lowest number of cells, and even in this case, the extract of the leaf of *Costus pictus*

D. Don shows the highest value (0.17%) compared to the controls (0.095%) and with the liquefaction of the leaves administered in the drinking water (0.108%). In such a way, this study covers all possible populations to be quantified by means of manually developed blood counts.



Graph 7: Percentage of eosinophils in total leukocytes in peripheral blood of Hy-Line chickens after receiving treatment with *Costus pictus* D. Don. The liquefied and aqueous extract of the same was microencapsulated with olive oil. The average number of cells per 7 replicates is shown in parentheses, except for group 7 where there are only 6 birds.

Discussion

C. pictus D. Don belongs to the family Costaceae, within the order Zingiberales. With 200 species in a family of four genera. The genus *Costus* is the most numerous, as it has more than 148 recognized species. Native to our country, Mexico, it is from tropical regions in the southern part, its floristic biology has been well studied (Sharma and Malik, 2025; Sharma and Sharma, 2021), as well as its phytochemical profile, which is very diverse and with therapeutic properties validated by preclinical studies. The results obtained are associated with the content of bioactive compounds such as some alkaloids, terpenes, flavonoids, fatty acids (hexadecanoic, oleic) and specialized metabolites such as diosgenin, corosolic acid and quercetin, which are distributed in its leaves, stems, rhizomes and flowers [32,33].

As for their organoleptic properties, they seem to be very similar to those recorded by Shaikh [31] (2022), except for the pH recorded for *Costus pictus* D. Don and *Costus speciosus* J. Koenig where they show a more neutral pH compared to our data, both in the liquefied leaf and in the aqueous extract of it and prior to its encapsulation.

Given that it is a plant native to this region of the world, it is not surprising that it is easy to propagate and the economy involved in developing it on a large scale (Image 1). If we take into account that Mexico is among the countries most affected by type 2 Diabetes Mellitus and that it shows a percentage of between 15.7 and 18.4% of adults over 20 years of age, depending on sex [25], which makes *Costus pictus* D. Don a natural alternative to address the problem of hyperglycemia in the general population, as it is considered a key indicator to evaluate possible success against this disease [37]. We believe that it is necessary to disseminate more about the culture of consumption of our plant of interest.

Natural products, such as prebiotics, probiotics, synbiotics, organic acids and medicinal plants have been used as supplements in the diets of developing and producing birds, with the aim of improving health status, reducing pathogenic microorganisms, modulating a better immune response and increasing productive behavior [27].

Given that in 2010 Al-Romaiyan's [6] group demonstrated that an extract of *Costus pictus* D. Don stimulates insulin secretion, through an effect on calcium physiology in the islets of Langerhans, it is very likely that it presents a systemic effect that has not been addressed due to lack of adequate studies and models to exclude the antidiabetic effect and focus the objectives on the physiology of reproduction.

In previous studies, results were achieved using long-lived hens to see the effects of *Costus pictus* D. Don administered in the way that people with Diabetes Mellitus usually ingest it, not giving an insight into its antidiabetic effects, but about the physiology of reproduction in laying hens, since these birds do not suffer problems due to the hyperglycemia they present and their ability to mature an egg every 24-25 hours. The results showed encouraging numbers regarding egg quality and immunostimulators, with all the positive applications that they entail [22]. These results inspire us to continue, now with hens from the Hy Line and at the end of their laying phase 1. Here we present the results of the nutritional analysis of the plant (Table 3) and bromatological analysis (Table 4), where it is possible to verify the low probability of presenting

adverse effects on animal or human health, as confirmed by the results of Sarkar group´s [28].

To compare the hypoglycemic effect of *Costus pictus* D. Don in mammals [24] with respect to birds, we tested the leaves of the same liquefied plants added to the drinking water and acceptance was observed in both models from the beginning of the experiment. With the glucose tolerance test with the hypoglycemic Sitaglyptin in Wistar rats and in graph 1 we can observe that both the dose of 3 and 6g/Kg of body weight the effect is marked (Graph 1), results with the same tendency to those found by Agu[1] (2018) in a similar model and with a methanolic extract of *Costus afer*. On the other hand, in laying hens, the hypoglycemic effect can be observed as a trend without significant statistical difference (Graph 2), both with the liquefied leaf and its aqueous and microencapsulated extract. The work of Bagunoc (2023) [8] shows contrasting results to those presented here, given the differences in the model, broilers, the presentation of the plant and the amounts of *Costus pictus* D. Don administered in powder form and from 5 to 15 grams/kg of feed. In a model that is closer physiologically speaking, although with less natural administration of processed supplements added to drinking water twice a week, it provided improvements in egg quality in laying hens [17]. Our group [22] found that long-lived hens produce heavier eggs, consume less feed and drink more water when they consume *Costus pictus* D. Don in the same way as here. Everything indicates that the management of supplementation, the route of administration and the doses require more studies to establish the best strategy to treat laying hens and achieve success in production parameters.

Among the beneficial properties reported for *Costus pictus* D. Don and related species is anti-inflammatory activity [3,4], leukocyte counts [38], antiviral molecules with ethanolic extracts[30] and signal transduction important for the immune response [19]. In the present work, an immunostimulatory effect was found in all cell lineages analyzed by blood biometry and in the final part of the experiment. The relevant data can be seen as an average of cells evaluated per group of treated hens; Peripheral lymphocytes showed $0.5x10^9$ cells per liter of blood in the group of *Costus pictus* D. Don liquefied in drinking water, compared to the untreated controls and $1.8x10^9$ cells per liter compared to the group treated with aqueous extract and microencapsulated (Graph 4); peripheral lymphocytes, similarly, showed a greater number of blood cells in the liquefied leaf ($3.32x10^3$ cells/L of blood), $2.18x10^3$ in controls and even less in those treated with the microencapsulation process ($1.18x10^3$ cells/L of blood fluid), data that can be seen in Figure 5. In previous studies [22] it was found that long-lived hens of the Lohmann line developed a greater number of lymphocytes of the same type when they receive the same treatment as those used in this study; with respect to monocytes in peripheral blood, it is the group treated with the liquefied, with $0.58x10^9$ cells/L against $0.45x10^9$ cells/L in controls and $0.24x10^9$ cells/L (Graph 6); finally, the percentage of Eosinophils taking the total number of Leukocytes continues to show a greater number of cells (0.108%), 0.095% for the control group and in this case the group with the highest value was the microencapsulated with 0.17%, numbers shown in Graph 7. The case of Eosinophils as cells that participate in allergic phenomena makes sense, given that it has been reported that Quercetin, a molecule reported as a secondary metabolite in the *genus Costus* with an immunomodulatory effect [10] and that when extracting the substances from the leaves of *Costus pictus* with water D. Don most likely concentrated and favors, among other molecules, this polyphenol, which exhibits this activity. In addition, it is not surprising that most of the results associated with the immunostimulatory effect occur just by liquefying the leaf, given that it has shown a higher percentage of extractive value when compared to the rhizome of *Costus speciosus* J. Koenig [31], the latter and with a record by a different author, Koen (Keu, ginger crepe), as well as its extraction, even shows activity in favor of fertility when disorders caused by Rheumatoid Arthritis occur in rats [20].

Based on the above, the effects on the immune system can probably explain the other activities that have been reported on the genus *Costus* [23]. Nephroprotective activity (Ali., *et al*. 2022), different types of inflammatory processes and perhaps chronic headache, diarrhea, hyperlipidemia, diabetes, cancer, ringworm infections [32], hepatoprotective [7,16,23].

It is relevant to mention that when the plant is ingested without any extraction process, substances that are concentrated when solvents are used are not removed and probably, according to our results, the formulation from liquefied leaves that is administered provides a greater number of positive activities [38]. Given the types of metabolites such as steroids, alkaloids, flavonoids, triterpenes, glycosides, and saponins as in *Costus igneus* [7] and

that their extractive values indicating purity, quality, and presence of desired phytochemicals are higher than in extracts. Extractive values (expressed as a percentage) measure the amount of active components extracted from a raw plant or herbal medicine using specific solvents. This percentage indicates the purity, quality, and presence of desired phytochemicals in a sample [31].

In Animal Production Sciences it is very important to determine the benefits related to supplementation from medicinal plants in the feed and in this case, the net production in Kg of egg produced during treatment shows statistically significant differences in the two types of treated groups, with respect to the control group (Graph 3). a trend similar to our results obtained with long-lived hens [22].

Conclusions

The organoleptic properties, nutritional and bromatological analyses of *Costus pictus* D. Don allow it to be proposed as a plant with low health risks when the liquefied leaf is ingested. This preparation shows a hypoglycemic effect in both Wistar rats and hens of the Hy Line, and the aqueous and microencapsulated extract shows the same tendency. In the productivity parameters, the egg weight showed important statistical differences between the treatments and the control during the experiment. In addition, the two treatments applied for 30 days show immunostimulatory effects, mainly the ingestion of the liquefied leaves in drinking water. We believe that more studies are required to deepen more production, immunological and histology benefits to guarantee the probable safety of the intake of *Costus pictus* D. Don leaves in the drinking water of hens of the Hy Line and to establish the temporality of their application.

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