Hindawi
Journal of Chemistry
Volume 2018, Article ID 4073068, 6 pages
https://doi.org/10.1155/2018/4073068

*Research Article*

# Dietary Inclusion of a Mixed Powder of Medicinal Plant Leaves Enhances the Feed Efficiency and Immune Function in Broiler Chickens

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Received 15 October 2017; Accepted 8 May 2018; Published 27 May 2018

Academic Editor: Sevgi Kolaylı



The trial was designed to determine the effect of the mixed powder of leaves of *Anacardium occidentale* (60%), *Psidium guajava* (20%), and *Morinda citrifolia* (20%) on growth performance, nutrient digestibility, and immunoglobulin concentration in broiler chickens. A total of 80 one-day-old Ross 308 broiler chickens were randomly assigned to two dietary treatments from days 1 to 21 of age, with 8 replicates by treatments and 5 birds per replicate. Treatments consisted of a control diet (T0) and the dietary inclusion of 0.5% of mixed powder of medicinal plant leaves (T1). The inclusion of herbs decreases the feed intake (FI) and feed conversion ratio (FCR; $P < 0.05$) in week 2 and in the period studied (1–21 days) compared with T0 but did not modify ($P < 0.05$) the body weight (BW) and average gain (AG). Also, the mixed powder has no influence ($P > 0.05$) on nutrient digestibility and IgA concentration; however, the IgG concentration increased by the effect of this experimental treatment. The results allow recommending the dietary inclusion of mixed powder of medicinal plant leaves as an alternative for obtaining acceptable performance in broilers.

## 1. Introduction

Medicinal plants have been used by humans from remote times to cure or to alleviate their illnesses or ailments. Also, they were used in the animals with therapeutic ends, for their antidiarrheal, antiseptic, antimicrobial, and anti-inflammatory properties [1]. Nowadays, the application of medicinal plants in the human health and animal production is increasing, due to the great existent concern at world level for the crossed possible resistance to the antibiotics for many microorganisms as a response to indiscriminate subtherapeutic use in animals [2].

Several studies have demonstrated that phytobiotics in the diets of farm animals improved bodily development, gut integrity, nutrient absorption, antioxidant activity, and immunity, with decreasing the diarrhea syndrome [3–5]; in consequence, these natural products have been considered as an effective alternative to feed antibiotics, mainly to diminish or decrease the residual effects in meat, egg, and milk. However, many researchers question these results, especially because of the variations found in biological indicators in *in vivo* studies; it is important to note that the positive effects will depend on the animal species, the productive category, environmental conditions, and characteristics of the plant material used [4]; this means that the best results are found in young birds under stressful environmental conditions in intensive production and with the use of phytobiotics rich in nontoxic secondary metabolites, with medicinal properties that attenuate these negative factors.

Good examples of medicinal plants are *Anacardium occidentale*, *Psidium guajava*, and *Morinda citrifolia*. In this sense, the leaves of *A. occidentale* have astringent, antidiarrheal, antioxidant, and antimicrobial properties, due to the presence of polyphenols (mainly tannins) and coumarins [6]; its use in poultry and swine diets improves the growth performance and the production and quality of eggs and decreases the incidence of diarrhea, respectively [7]. Also, *P. guajava*, a plant of great application in the treatment of the diarrheic syndrome in pigs (mainly the leaves), has hypoglycemic, antioxidant, and anti-inflammatory properties due to the presence of flavonoids (mainly gammapirons) and triterpenoids [8], and also its application as a medicinal drug in the diets of mice showed an antidiabetic effect [9]. Moreover, *M. citrifolia* is rich in alkaloids in its leaves and fruits, which can be recommended mainly for more than 20 illnesses to human and animals, due to its antioxidant, antimicrobial immune-stimulant properties [10]; specifically, in birds, Sunder et al. [11] have demonstrated that daily use of this plant in the diets of laying hens increases the egg weight and shell thickness.

In these three plants, separately, their effects have been investigated in animals of zootechnical interest with good results, mainly as nutraceutical supplements [9, 11, 12]. However, little is known about the use of these plants when they are mixed and supplemented or are included as powder in diets for broiler chickens. For this study, we hypothesized that inclusion in the diet with mixed powder, with a greater incorporation of *A. occidentale* in the powder, due to its beneficial secondary metabolites and its positive evidence on the production and health of young birds [12], might stimulate the immune system and, as a consequence, increase the productive behavior in broiler. Thus, the objective of this experiment is to determine the effect of the mixed powder of leaves of plants with medicinal properties on growth performance, nutrient digestibility, and immunoglobulin concentration in broiler chickens.

## 2. Materials and Methods

All experimental procedures used in this trial have been approved by the University of Manitoba, Animal Care Protocol Management and Review Committee, and birds were handled in accordance with the Canadian Council on Animal Care guidelines.

*2.1. Animal, Housing, and Treatment.* A total of 80 one-day-old Ross 308 male broiler chickens were divided into 16 uniform groups of 5 birds balanced for body weight and were randomly assigned to two dietary treatments (8 groups per treatment) from days 1 to 21 of age (Table 1).

The chicks in electrically heated cages in a battery brooder in a light- and temperature-controlled facility were housed. Upon arrival, the temperature inside the room was adjusted to 32°C on the first day and was then reduced by 2.5°C each week. The mean value of temperature in the experimental room throughout the whole experimental period was approximately 27°C.

Table 1: Composition and nutrient content.

| Ingredients | Days 1 to 21 | |
|---|---|---|
| | T0 | T1 |
| Corn | 55.94 | 55.66 |
| Soybean meal, 44% CP | 35.00 | 34.83 |
| Canola oil | 4.00 | 3.98 |
| Calcium carbonate | 1.50 | 1.48 |
| Monocalcium phosphate | 1.70 | 1.69 |
| DL-methionine | 0.19 | 0.19 |
| L-lysine | 0.01 | 0.01 |
| L-threonine | 0.06 | 0.06 |
| Common salt | 0.30 | 0.30 |
| Vitamin-mineral premix[1] | 1.00 | 1.00 |
| Titanium dioxide | 0.30 | 0.30 |
| Mixed powder of medicinal plants[2] | — | 0.50 |
| *Calculated composition, % as fed* | | |
| Crude protein, % | 22.01 | 21.94 |
| Metabolizable energy, kcal/kg | 3086 | 3074 |
| Ca, % | 1.11 | 1.09 |
| Available P, % | 0.48 | 0.46 |
| SID[2] lysine, % | 1.10 | 1.08 |
| SID methionine, % | 0.53 | 0.50 |

[1]Provided per kilogram of diet: manganese, 55 mg; zinc, 50 mg; iron, 80 mg; copper, 5 mg; selenium, 0.1 mg; iodine, 0.36 mg; sodium, 1.6 g; retinyl acetate, 8,250 IU; cholecalciferol, 1,000 IU; DL-alpha-tocopherol, 11 IU; cyanocobalamin, 0.012 mg; phylloquinone, 1.1 mg; niacin, 53 mg; choline, 1,020 mg; folacin, 0.75 mg; biotin, 0.25 mg; riboflavin, 5.5 mg. [2]Mixed powder of medicinal plant leaves consisted in 60% of *Anacardium occidentale* 20% of *Psidium guajava*, and 20% of *Morinda citrifolia*.

They received water and feed on *ad libitum* basis throughout the experimental period. The two diets were made on the basis of standardized ileal digestible (SID) amino acids and consisted of a basal diet (T0) and dietary inclusion of 0.50 % of mixed powder of medicinal plant leaves (T1) (60% of *Anacardium occidentale*, 20% of *Psidium guajava*, and 20% of *Morinda citrifolia*), formulated to NRC [13] to meet nutrient requirements.

*2.2. Growth Performance.* In all experimental weeks, the BW (g), FI (g/bird), and viability were determined; then, based on these data, the AG (g) and FRC (kg/kg) were calculated according to the method of Aguilar et al. [14].

*2.3. Nutrient Digestibility.* Excreta from each pen during the last two days of each week were collected and frozen at −20°C until the laboratory analysis. At 21 days, all chicks within a pen by $CO_2$ asphyxiation were euthanized to collect ileal digesta, and then, the same conservation method was used. Then, excreta samples from each pen were pooled and weighed before and after being dehydrated in an oven at 60°C for 36 hours to determine excreta moisture content. Also, ileal digesta samples within a pen were pooled for apparent ileal digestibility (AID) calculation.

Diet samples were finely ground to pass through a 1 mm screen in a Thomas-Wiley mill (Thomas Scientific, Swedesboro, NJ). Also, ileal digesta samples were freeze-dried and finely ground (CBG5 Smart Grind; Applica Consumer Products Inc., Shelton, CT). Both samples were analyzed for dry matter (DM),

gross energy (GE), nitrogen, crude fat (CF), and titanium concentration.

DM and CF were analyzed according to AOAC [15]. GE was determined using the Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Nitrogen was analyzed using an N analyzer (model NS-2000, Leco Corporation, St. Joseph, MI). Samples for titanium analysis were ashed, digested according the method described by Lomer et al. [16], and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA).

The nutrient digestibility was calculated using the following equation:

$$\%\ \text{apparent nutrient digestibility} = \left\{1 - \left[\left(\frac{T_d}{T_f}\right) \times \left(\frac{N_f}{N_d}\right)\right]\right\} \times 100, \quad (1)$$

where $T_d$ = the titanium dioxide ($TiO_2$) concentration in the diet, $T_f$ = the $TiO_2$ concentration in the excreta or ileal digesta, $N_f$ = the nutrient concentration in the excreta or ileal digesta, and $N_d$ = the nutrient concentration in the diet.

The AME and AMEn contents of experimental diets were calculated using the following equations:

AME (kcal/kg) = $GE_{\text{kcal/kg of the diet}} - [GE_{\text{kcal/kg of excreta}} \times (TiO_{2\ \%\ \text{diet}}/TiO_{2\ \%\ \text{excreta}})]$; and AMEn (kcal/kg) = $GE_{\text{kcal/kg of the diet}} - [GE_{\text{kcal/kg of excreta}} \times (TiO_{2\ \%\ \text{diet}}/TiO_{2\ \%\ \text{excreta}})] - 8.22 \times \{N_{\%\ \text{diet}} - [N_{\%\ \text{excreta}} \times (TiO_{2\ \%\ \text{diet}}/TiO_{2\ \%\ \text{excreta}})]\}$, where 8.22 is the energy equivalent of uric acid $N$.

*2.4. Immunoglobulin Concentration.* At 21 days of sacrifice, 5 mL of blood samples was collected from the jugular vein of 5 birds per treatment. Blood samples were placed in 2 mL tubes, and then, sodium heparin was added at a ratio of 2 : 1 and then stored at −15°C. Sandwich IgG ELISA Quantification Set (Bethyl Laboratories, Montgomery, TX) protocols were followed to determine natural immunoglobulin concentrations. A dilution of 1 : 10,000 of serum samples was used for natural IgG determination. Serum IgA concentrations in diluted samples were calculated by a Sandwich ELISA kit (Bethyl Laboratories Inc., Montgomery, TX) using 96-well microtiter plates.

Data of the experiment were subjected to unpaired $t$-test to independent samples of SAS (SAS software release 9.3; SAS Inst., Inc., Cary, NC). $P$ values < 0.05 were taken to indicate significance.

## 3. Results

*3.1. Growth Performance.* Data on BW, FI, AG, and FCR during 21 days of broiler supplemented with mixed powder of medicinal plants are presented in Table 2. Viability was excellent in both treatments (100%; data not shown), and BW and AG did not show changes ($P > 0.05$) by the effect of the treatment with herbs. However, FI decreased and FCR improved ($P < 0.05$) with this natural product in week 2 and in the period of 1–21 days, where the chicks used 25.90% less feed than the control group, keeping no difference in the BW ($P > 0.05$).

Table 2: Effect of the mixed powder of leaves of plants with medicinal properties on growth performance in broiler.

| Age (days) | Treatments | | ±SEM | $P$ value |
|---|---|---|---|---|
| | T0 | T1 | | |
| *BW (g)* | | | | |
| 1 | 42.43 | 42.75 | 0.163 | 0.168 |
| 7 | 121.05 | 123.76 | 3.128 | 0.564 |
| 14 | 346.96 | 348.13 | 12.70 | 0.951 |
| 21 | 725.14 | 704.73 | 22.71 | 0.550 |
| *FI (g/bird)* | | | | |
| 1–7 | 110.70 | 109.53 | 1.688 | 0.547 |
| 7–14 | 406.00 | 300.58 | 7.879 | <0.001 |
| 14–21 | 509.96 | 489.98 | 15.93 | 0.407 |
| 0–21 | 1027.29 | 900.10 | 22.85 | 0.002 |
| *AG (g)* | | | | |
| 1–7 | 78.62 | 81.09 | 3.167 | 0.616 |
| 7–14 | 225.91 | 224.37 | 10.79 | 0.919 |
| 14–21 | 378.17 | 356.60 | 11.75 | 0.232 |
| 0–21 | 682.71 | 661.98 | 22.75 | 0.544 |
| *FCR (kg/kg)* | | | | |
| 1–7 | 1.42 | 1.37 | 0.030 | 0.502 |
| 7–14 | 1.81 | 1.35 | 0.066 | <0.001 |
| 14–21 | 1.35 | 1.38 | 0.006 | 0.032 |
| 1–21 | 1.51 | 1.37 | 0.021 | <0.001 |

The experiment lasted for 21 days; $n = 40$. BW: body weight; FI: feed intake; AG: average gain; FCR: feed conversion ratio.

Table 3: Effect of the mixed powder of leaves of plants with medicinal properties on nutrient digestibility in broiler.

| Items | Treatments | | ±SEM | $P$ value |
|---|---|---|---|---|
| | T0 | T1 | | |
| AMEn | 2669 | 2822 | 61.82 | 0.244 |
| NATTD | 63.28 | 61.37 | 2.541 | 0.720 |
| NAID | 81.73 | 78.93 | 1.137 | 0.231 |
| DMATTD | 61.83 | 63.05 | 2.248 | 0.797 |
| DMAID | 67.01 | 62.99 | 1.406 | 0.190 |

The experiment lasted for 21 days; $n = 40$. AMEn: nitrogen-corrected apparent metabolizable energy; NATTD: nitrogen apparent total tract digestibility; NAID: nitrogen apparent ileal digestibility; DMATTD: dry matter apparent total tract digestibility; DMAID: dry matter apparent ileal digestibility.

*3.2. Nutrient Digestibility.* Table 3 shows the effect of the mixed powder of leaves of plants with medicinal properties on AMEn, NATTD, NAID, DMATTD, and DMAID in broilers. The inclusion of the medicinal powder did not show significant changes ($P > 0.05$) for any of these indicators shown in Table 3.

*3.3. Immunoglobulin Concentrations.* The effect of the mixed powder of leaves of plants with medicinal properties on IgG and sIgA serum concentration is shown in Table 4. Herb powder increased ($P < 0.05$) the serum concentration of IgG

Table 4: Effect of the mixed powder of leaves of plants with medicinal properties on IgG and sIgA serum concentration.

| Items | Treatments | | SEM± | $P$ value |
|---|---|---|---|---|
| | T0 | T1 | | |
| IgG ($\mu$g/ml) | 326.43 | 555.14 | 11.37 | 0.001 |
| sIgA ($\mu$g/ml) | 91.70 | 126.17 | 41.34 | 0.140 |

The experiment lasted for 21 days; $n = 5$.

in 228.71 $\mu$g/ml compared with T0. Although it is possible to observe a numerical stimulation of secretory IgA (sIgA) serum concentration due to T1 (91.70 versus 126.17 $\mu$g/ml), this did not indicate statistical differences ($P > 0.05$).

## 4. Discussion

One of the aims of this experiment was to evaluate whether the inclusion of the mixed powder of medicinal plant leaves in broiler diets would influence the growth performance because their separate use has shown the regulatory effect on body weight and feed intake. Our study showed that dietary inclusion of 0.5% of mixed powder of leaves with medicinal properties improved the feed efficiency in broiler, because the FI and FCR decreased in week 2 and the experimental period (1–21 days; Table 2), without affecting the viability (data not shown) and normal digestive function of birds, which suggest that this natural product has beneficial phytochemical compounds that can improve the biological development of broiler chickens.

In this sense, Martínez et al. [7] reported that the medicinal effect of plants is due to the secondary metabolites; also, their effects will depend on the concentration and relationship of these compounds and its inclusion or supplementation on animal diets. Therefore, the herbs used in small concentrations rich in secondary metabolites such as tannins, coumarins, triterpenoids, flavonoids, and alkaloids may have influence on animal response due to their astringent, anti-inflammatory, antioxidant, and antimicrobial properties [17].

The powder was prepared to intensify the content of polyphenols, especially tannins obtained from the leaves of *A. occidentale*, which has the highest proportion in the mixture, mainly because this polyphenol has beneficial activity at intestinal level [7]. In this sense, this secondary metabolite is known for its astringent property, because they can bind to saliva lubricating proteins by hydrogen bonds [17]; therefore, an increase of this metabolite in the diet could reduce the passage of the digesta in the GIT and decrease the feed intake by a higher state of satiety in this period (Table 2). In addition, tannins have demonstrated *in vitro* antibacterial effects against strains of *Escherichia coli* and *Staphylococcus aureus*, with pathogenic bacteria being more frequent in the gastrointestinal tract (GIT) of poultry, which could reduce the population of these bacteria and disorders of the intestine [18]. However, an excess of tannins can provoke metabolic disturbances leading to an antinutritional influence [19], such as inhibiting the absorption of iron and sulphur-containing amino acids causing anaemia and depression of growth, respectively.

Moreover, the incorporation of the leaves of *M. citrifolia* and *P. guajava* into the mixed powder rich in secondary metabolites with antioxidant and anti-inflammatory effects [10, 20] could influence the better use of the nutrients with lower feed intake, mainly during the first moments of the bird's life that they are often exposed to different stressful conditions and presence of pathogenic agents, which increase the production of free radicals and postprandial inflammatory process and decrease the growth performance [21]. Taking into account our results, we can affirm that the secondary metabolites in adequate concentration have a direct effect on poultry development [18].

On the contrary, several studies have reported that medicinal plants (with emphasis on the leaves) in diets enhance the growth performance in birds. In the same way, Salami et al. [22] reported that use of medicinal herbs in the diets of broiler improved the FCR values at the end of the trial. Also, Buchanan et al. [23] stated that broiler chickens fed diets having plant extract blends had minimum FCR and had increased weight gain and breast yield.

In spite of the phytochemical components on mixed powder, the dietary inclusion of 0.5% has no change in the total and apparent ileal digestibility of both nitrogen (N) and dry matter (DM) in broilers. Our study did not indicate an adverse effect on nutrient digestibility because Savón et al. [19] have referred that the secondary metabolites as antinutritional factors affect the digestibility and therefore growth performance. Results of Hernández et al. [24] and Hassan et al. [25] showed an increase in digestibility coefficient of DM, ether extract, CP, and organic material with the dietary supplementation of Labiatae plants (*Salvia officinalis*, *Rosmarinus officinalis*, and *Thymus vulgaris*) and artichoke extracts in broilers, respectively. In our study, one reason for the nonpositive effect of the inclusion of mixed powder of medicinal plants in some productive indicators up to 21 days would be the controlled environment of the experiment and the use of the crude powder instead of the leaf extract. However, more studies are needed to corroborate this hypothesis.

At present, the scientific community is discussing the main mode of action of natural products. Some researches have reported that it is due to its anti-inflammatory properties that decrease inflammation of the small intestine and increase the absorption of nutrients [26], and other studies have shown that the inclusion of medicinal herbs reduces the proliferation of pathogenic bacteria in the GIT, which increases intestinal health and favours greater digestibility of nutrients and animal response [3, 25]. Similarly, other researchers using the same medicinal plants as our experiment have found both responses *in vitro* and *in vivo*. Although it was observed that T1 increased numerically the AMEn, maybe a higher concentration of this mixed powder of medicinal plant leaves may be necessary to modify these indicators in broilers.

The level of serum antibodies is currently an important indicator to know the effect of a new natural product on immune response in experimental animals [27]. An increased immunoglobulin concentration has been associated with a benefit in the immune status, and IgG and IgA are the main

immunoglobulins protecting against pathogenic microorganisms [28], mainly to intestinal level. Our results showed that a small amount of powder inclusion (0.5%) exerts a beneficial immune effect, through an increase in the IgG concentration and with a synthesis of appropriate immune cells, without diminishing the growth performance [29].

IgG is one of the main defence barriers during the bacterial attack in the gastrointestinal tract (GIT), and the early proliferation of this cell is essential to improve the feed efficiency in these animals [30]. Thus, they are less exposed to bacterial attack and hence, to intestinal disorders. In addition, IgG is the main active antibody against infections due to its high degree of specificity and its bactericidal action and also is most abundant in the serum; compared to IgA and IgM, IgG has a longer life and can shuttle between serum and endothelium surfaces [31, 32]. Although the IgA concentration was higher compared with T0, this did not show significant difference ($P > 0.05$) among treatments. However, an increase of this immunoglobulin in a controlled environment shows that this powder can favour immunity in young birds, perhaps with higher efficacy under stress conditions. According to Merino-Guzmán [28], sIgA inhibits the uncontrolled absorption of macromolecules or the binding of allergens to target cells of the mucosa, has inflammatory and bactericidal effects, and improves nonspecific immunological defence mechanisms.

In general, this result indicates that the dietary inclusion of the mixed powder may assist a fast response of the immune system. In this sense, Tajodini et al. [33] using artichoke (*Cynara scolymus*) powder in broiler diets found that this product significantly increased antibody serum levels, resulting in a higher activity of immune system.

## 5. Conclusions

The experimental treatment in the diet decreased the FI and FCR during week 2 and throughout the trial period (1–21 days) and increased the IgG concentration compared with the control treatment (T0), without affecting the nutrient digestibility and IgA concentration. Taking into account the results, we recommend the inclusion of 0.50% of this mixed powder of medicinal plant leaves (60% *Anacardium occidentale*, 20% *Psidium guajava*, and 20% *Morinda citrifolia*) in broiler diets.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

## Authors' Contributions

Roisbel Aroche and Yordan Martínez contributed equally to this manuscript.

## Acknowledgments

This study was funded by the China Scholarship Council (201708430008), the National Natural Science Foundation of China (31402092), the Scientific Research Fund of Hunan Provincial Education Department (17K043), the Hunan Provincial Science and Technology Department (2017NK2322 and 2016NK2103), and the Open Project Program of State Key Laboratory of Food Science and Technology, Nanchang University (SKLF-KF-201416).

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