Effect of added different levels of Graviola Leavs meal (Annona muricata L) on biochemical blood traits and bacteriological account in broilers chicken

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Abstract

The study was carried out at the Animal Production Department Faculty of agriculture, university of Kufa to investigate the effect of replacing (GLM) Graviola leaves meal (Annona muricata) fruit with antibiotics and antioxidant in the broiler diets. The experiment was conducted at the poultry farm of the Animal Production Department, College of Agriculture, university of Kufa. Starter diets (23.82% C.P and 3029.7 kcal ME/kg) feed were gave to the birds from week 1 to week 3 of age, and finisher diets which contained 20.94% C.P. and 3204.5 kcal ME/kg were gave from week 4 to week 5 of age. Three hundred sixty one day old Ross 308 chicks were randomly divided into six groups with three replicate (20 bird /replicate) per group, Each group was subjected to one of the following treatments (T1) positive control contained antibiotics and antioxidant, (T2) negative control without antibiotics and antioxidant, (T3) T2 + 0.1% Graviola leavs powder, (T4) T2 + 0.2% GLP, (T5) T2+0.3% GLP, (T6) T2+ 0.4% GLP. Using Randomized completely Block Design (RCBD). Biochemical blood traits, benefits and harmful bacteria in small intestine. Result showed that there were significant decreasing (P<0.05) in all treatments contained (GLM) in cholesterol, triglycerids, uric acid, albumen, globulin, and LDL, VLDL, ALPase, glucose, phosphorus and calicium in blood serum compromised with control treatment.

Keyword: Broiler, Graviola, Biochemical blood traits, Bacteria, Ross 308, antibiotics, Phytobiotics, Salmonella.

1. Introduction

Plants have used the basis of the traditional medicine system around the world for thousands of years, and continue to provide humanity with new treatments, and from the group of medicinal plants is the craviola tree or the so-called cream tree Annona muricata, soursop or graviola. It is known to contain a large group of anti-cancer substances such as compound Acetogenins. They are strong inhibitors of the enzymes NADH (nicotiamide adenine dinucleotide) found in the plasma membranes of cancer cells. Craviola fruits are of economic value, and are therefore widely cultivated and used as an edible food as well as for their medicinal benefits against harmful microbes. Anti leishmanial, wound healing: It also contains tannins, steroids, and glycosides, known for their pharmacological importance and their role as antioxidants. As for the craviola fruit, it has a distinctive aroma and its pulp is creamy and sweet juice and is used in making jam and ice cream. Graviola leaves are the most beneficial parts of this tree. They have Acetogenins that contain Compounds, namely Albolatacin and Alacemisin. Acetogenin acts as a defense against insects it is often used to kill insects and pests that die by consuming small amounts of these leaves. Scientific research conducted by the National Cancer Institute in America indicates that Craviola leaf powder can effectively attack and destroy cancer cells. Moreover, it is also used in the treatment of many other diseases [1], [2], reported excellent amounts of enzymes (pectinase, catalase, and peroxidase), protein and amino acids (clutamic acid, aspartic acid, serine, eycin, alanine, citrulline, cysteine, arginine, lysine), minerals, vitamins and sugar. Therefore, the aim of the present study was the possibility of knowing the active compounds and nutritional value of the powder of graviola leaves and using them as feed additives in different levels in order to evaluate their biological and pharmacological effectiveness by substituting them in diets free from antibiotics and industrial antioxidants in the blood biochemical characteristics and bacteriological account in broilers.
2. Materials and Methods

2.1. Experimental animals and experimental design

The research study was carried out at the Poultry farm of the Animal Production, College of Agriculture, Kufa University. The field experiment was carried out on 2/12/2018 until 6/1/2019. A total of 360 day-old unsex broiler chickens of the Ross 308-average initial weights 38 gm/bird equipped by one of the commercial hatcheries in the province of Babylon. The pens were thoroughly washed and disinfected. A few hours to arrival, all equipment were put in place (feeders, drinkers, bulbs, heat source, etc) and heated to a suitable temperature (about 35°C). The experiment was a Randomized Completely Block Design (RCBD) Experiment made up of six dietary inclusion (T1): positive control (contain synthesis antibiotics and antioxidant), (T2): negative control (without antibiotics and antioxidant), (T3): T2+ 0.1% Graviola leaves powder (Annona Muricata), (T4): T2+0.2% Graviola Leaves Powder, (T5): T2+0.3% Graviola Leaves Powder, (T6): T2+0.4% Graviola Leaves Powder to form Diet 1, 2, 3, 4, 5 and 6 respectively (Table 1). On arrival, the birds were weighed and allocated randomly into the six dietary treatment groups consisting of 60 birds per treatment and three replicates per diet; with each replicate made up of 20 birds. The experiment lasted for 5 weeks during which time they were managed intensively using the standard code of procedure as recommended for [3]. The birds were fed ad libitum with the experimental diets throughout the period of the experiment. Routine management operations such as daily removal of left-over (uneaten) feed, washing of drinkers, provision of clean drinking water and cleaning of the environment were carried out. The birds were also given vaccination against the common poultry diseases but without standard medication and as recommended for this region by veterinary hospital.

2.2. Parameters determined

The following parameters were determined according to of The following parameters were determined as follows:

2.2.1. Chemical analysis

The proximate chemical analysis of the annona muricata leaf meal (AMLM) Table 1, while the fiber composition of AMLM was determined using the procedure the procedures of AOAC [4].

| Table 1. Approximate chemical analysis of annona muricata leaf meal. |
|--------------------------|------------------|
| nutrient                | %                |
| Crude Protein           | 50.24            |
| Moisture                | 10.00            |
| Ash                     | 3.70             |
| Crude Fiber             | 2.40             |
| Ether Extract           | 2.20             |
| Nitrogen Free Extract   | 57.20            |

The components of the bioactive compounds Table 2: Alkaloids were determined HPLC system by using the procedure of [4,5]. Phenols and Flavonoids according to [6], by using system FLC (Fast liquid Chromatographic) for preparation of analysis column and reading the result by HPLC system on wave lengths 280 nm. Acetogenin determined according to [7] with HPLC on wave lengths 220 nm.

| Table 2. Bioactive Compounds in Graviola Leaves meal |
|-------------------------------|------------------|
| Bioactive Compounds           | Name             | Concentrate(ppm) |
| Alkaloid&Isoquinon ppm        | Coclaurine       | 8.8              |
|                               | Coreximine       | 10.3             |
|                               | Atherosperminine | 8.4              |
|                               | Reticuline       | 32.2             |
|                               | Stepharine       | 10.1             |
|                               | Anomurine        | 16.3             |
|                               | Asimilarbine     | 10.8             |
|                               | Anomuricine      | 9.6              |
| Phenols &Flavonoids ppm       | Tannic acid      | 2.68             |
|                               | Callic acid      | 3.47             |
3. Experimental diets

Six isocaloric and isonitrogenous experimental diets were made ready to meet the requirements of ross broiler management [3] for starter table 3 and finisher periods table 4 as follow:

1. Diet (T1) positive Control, containing antibiotics and antioxidant.
2. Diet (T2) negative Control without antibiotics and antioxidant.
3. Diet (T3): T2 + contains 2 g/kg (0.2%) of Graviola Leaves Powder.
4. Diet (T4): T2 + contains 4 g/kg (0.4%) of Graviola Leaves Powder.
5. Diet (T5): T2 + contains 6 g/kg (0.6%) of Graviola Leaves Powder.
6. Diet (T6): T2 + contains 8 g/kg (0.8%) of Graviola Leaves Powder.

Table 3. Starter Diets for period (1-21 d) for all treatments.

| Ingredients                  | T1**** + Control | T2***** - Control | T3    | T4    | T5    | T6    |
|------------------------------|------------------|-------------------|-------|-------|-------|-------|
| Corn                         | 55.00            | 55.00             | 54.90 | 54.80 | 54.70 | 54.60 |
| Soybean meal (48%CP)         | 38.00            | 38.00             | 38.00 | 38.00 | 38.00 | 38.00 |
| Graviola Leaves powder       | —                | —                 | 0.2   | 0.4   | 0.6   | 0.8   |
| Antibiotic & Antioxidant     | +++              | —                 | —     | —     | —     | —     |
| Premix*                     | 2.50             | 2.50              | 2.50  | 2.50  | 2.50  | 2.50  |
| Corn oil                    | 2.90             | 2.90              | 2.90  | 2.90  | 2.90  | 2.90  |
| Di Calcium Phosphate***      | 1.20             | 1.20              | 1.20  | 1.20  | 1.20  | 1.20  |
| L. Lysine***                 | 0.10             | 0.10              | 0.10  | 0.10  | 0.10  | 0.10  |
| DL. Methionine****           | 0.30             | 0.30              | 0.30  | 0.30  | 0.30  | 0.30  |
| Total                        | 100.00           | 100.00            | 100.00| 100.00| 100.00| 100.00| 100.00| 100.00|

Calculated chemical analysis

| ME, Kcal/Kg      | 3025.9 | 3025.9 | 3025.9 | 3025.9 | 3025.9 | 3025.9 |
| Crude Protein    | 23.54  | 23.54  | 23.57  | 23.60  | 23.62  | 23.64  |
| Ingredients                        | Treatments        | T1 Control | T2 Control | T3 | T4 | T5 | T6 |
|-----------------------------------|-------------------|------------|------------|----|----|----|----|
| Corn                              |                   | 59.72      | 59.72      | 59.62 | 59.52 | 59.42 | 59.32 |
| Soybean meal(48%CP)               |                   | 31.70      | 31.70      | 31.70 | 31.70 | 31.70 | 31.70 |
| Graviola leaves powder            |                   | —          | —          | 0.20 | 0.40 | 0.60 | 0.80 |
| Antibiotic &Antioxidant premix*   |                   | —          | —          | —    | —    | —    | —    |
| Corn oil                          |                   | 4.80       | 4.80       | 4.80 | 4.80 | 4.80 | 4.80 |
| Di Calcium Phosphate**            |                   | 1.20       | 1.20       | 1.20 | 1.20 | 1.20 | 1.20 |
| L. Lysine***                      |                   | —          | —          | —    | —    | —    | —    |
| DL. Methionine****                |                   | 0.08       | 0.08       | 0.08 | 0.08 | 0.08 | 0.08 |
| Total                             |                   | 100.00     | 100.00     | 100.00 | 100.00 | 100.00 | 100.00 |

Calculated chemical analysis

|                        |                   | ME, Kcal/Kg | Crude Protein | Total Calcium |
|------------------------|-------------------|-------------|---------------|--------------|
|                        |                   | 3214.0      | 20.5          | 1.00         |
|                        |                   | 3214.0      | 20.5          | 1.00         |
|                        |                   | 3217.0      | 20.5          | 1.00         |
|                        |                   | 3220.0      | 20.5          | 1.00         |
|                        |                   | 3223.0      | 20.5          | 1.00         |
|                        |                   | 3226.0      | 20.5          | 1.00         |

* Premix M-25 and its ingredients per 1 kg are: Vitamin A 400,000 IU, Vitamin D3 160,000 IU, Vitamin E 1600 IU, Vitamin K 80 mg, Vitamin B1 80 mg, Vitamin B2 240 mg, Calcium Pantothenate CAL- PA 5200 mg, niacin 1400 mg, vitamin 1200 B6 mg, biotin 2 mg, folic acid 40 mg, vitamin B12 0.4 mg. Inorganic dicalciu phosphate 120,000 mg, phytase (4,000 mg, oil 20,000 mg, choline 20,000 mg). Premix components with enzymes (%): protein (20%), energy represented as Kcal / kg (3000 kcal), digested lysine (5.71), digested methionine (8.2), table salt (5.92).

** Calcium phosphate of Turkish origin contains: 22% inorganic calcium and 18% inorganic phosphorus

*** L-Lysine HCl: 98.5% purity

**** DL-Methionine: DL methionine Turkish origin 99% purity.

***** Commercial premix contains ethoxyquin antioxidant 125 gm / ton, antibiotic neomycin 125 gm / ton.

****** The second diet is free of ethoxyquin as an antioxidant and free of neomycin as antibiotic.

Table 4. Finisher Diets at Period(22-35d) for all treatments
| Available Phosphorus | 0.53 | 0.53 | 0.53 | 0.53 | 0.53 | 0.53 |
|----------------------|------|------|------|------|------|------|
| Crude Fiber          | 3.38 | 3.73 | 4.08 | 4.43 | 4.76 | 4.76 |
| Lysine               | 1.09 | 1.09 | 1.09 | 1.09 | 1.09 | 1.09 |
| Methionine + Cysteine| 0.86 | 0.86 | 0.86 | 0.86 | 0.86 | 0.86 |
| Calorie/Protein Ratio| 156.95 | 156.95 | 156.86 | 156.78 | 156.70 | 156.62 |

* Premix M-25 and its ingredients per 1 kg are: Vitamin A 400,000 IU, Vitamin D3 160,000 IU, Vitamin E 1600 IU, Vitamin K 80 mg, Vitamin B1 80 mg, Vitamin B2 240 mg, Calcium Pantothenate CAL-PA 5200 mg, niacin 1400 mg, vitamin 1200 B6 mg, biotin 2 mg, folic acid 40 mg, vitamin B12 0.4 mg. Inorganic dicalcium phosphate 120,000 mg, phytase (4,000 mg, oil 20,000 mg, calcium carbonate 422,000 mg, choline 20,000 mg Premix components with enzymes (%): protein (20%), energy represented as Kcal / kg (3000 kcal), digested lysine (5.71), digested methionine (8.2), table salt (5.92).

** Calcium phosphate of Turkish origin contains: 22% inorganic calcium and 18% inorganic phosphorus

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**** DL-Methionine: DL methionine Turkish origin 99% purity.

***** Commercial premix contains ethoxyquin antioxidant 125 gm / ton, antibiotic neomycin 125 gm / ton.

****** The second diet is free of ethoxyquin as an antioxidant and free of neomycin as antibiotic.

3.1. Statistical analysis

Data collected were statistically analyzed Using Randomized completely Block Design (RCBD), and SAS [8] Package. Where means were significant (p< 0.05), (p< 0.01) they were separated using the Duncan’s Multiple Range Test [9].

4. Results and Discussion

Table 5 the statistical analysis has biochemical characteristics of blood serum for all treatments, and it shows the presence of significant differences (P≤0.05) in all the characteristics. The cholesterol level decreased in most of the birds that consumed feeds added to graviola leaf powder, reaching 91.7, 107.7, 112.3 mg / dl of T6, T5, and T4 treatments, respectively, against 139.0 in T2 negative control the same direction, the triglyceride values were reflected in the values of triglycerides, which reached 51.3 mg / dl in treatment T6 compared to 102.0 in treatment T2, and the reason for the decrease in blood serum fats of birds consuming diets added to graviola leaves powder may be due to the presence of flavonoids and coumarin, which reduce blood cholesterol levels such as The compound coumarins and Sesquiterpene, which are antioxidants, worked to suppress free radicals and remove them and also reduce the level of free fatty acids in the plasma, thus reducing the level of triglycerides [10]. Or perhaps the flavonoids because of their vitamin properties, they work to lower cholesterol, or perhaps the reason for the decrease in blood fats is the presence of tannins in the powder of the graviola leaves because tannins inhibit the digestion of fats as a result of reducing the activity of the enzyme lipase secreted inside the bird's body [11]. As the lowest concentration was 3.0 mg / dl in treatment T6 versus the highest concentration of 5.0 in treatment T2, the decrease in uric acid may be due to the presence of the active substances in the graviola leaves powder because it increased the production of urine [12]. Also, there was a significant decrease in the values of albumin and globulin concentration (P≤0.05) with an increase in the percentage of addition of graviola leaves powder in the diets, while the differences were not significant in the concentration of total protein in blood serum despite its low value in the addition factors, and it reached the lowest value of 3.26 g / dl. In treatment T6 versus 4.36 in treatment T2, or perhaps the presence of quercetin in the analyzes of our current study has formed a beneficial factor for the health of the body as it supports the work of the stomach and spleen as it helps with digestion and is useful in preventing atherosclerosis as well as controlling the level of stress by inhibiting the enzyme producing cortisol [13]. As for the low levels of blood proteins, it may be due to the presence of the coumarin compound in the graviola leaves powder, as it is used in the treatment of dropsy due to protein accumulation and delayed fusion of the wound under the skin by reducing the edema resulting from thermal damage by reducing the level of protein in the tissues [14, 15]. The explanation of the mechanism of treatment is due to the action of coumarin by its association with plasma proteins, followed by activation of macrophage white cells [14]. Or maybe the graviola leaves powder contains the rutin compound, as the rutin inhibits protein disulfide isomerase or PDI, which is one of the most important proteins that platelets and endothelial cells secrete during the formation of clots inside blood vessels, and research and studies have proven that inhibiting this protein Flavonoid "rutin" has had a major role in reducing and preventing thrombosis [16].
Table 5. Biochemical blood traits in blood serum of chicks at age 5 wks.

| Treatments | Cholesterol mg/dl | Triglycerides mg/dl | Uric acid mg/dl | Total protein gm/dl | Albumine gm/dl | Gluobuline gm/dl |
|------------|-------------------|---------------------|-----------------|---------------------|----------------|------------------|
| T1         | 134.7 ± 0.057     | 91.3 ± 1.45         | 4.7 ± 0.03      | 4.16 ± 0.57         | 1.42 ± 0.03    | 2.74 ± 0.02      |
| T2         | 139 ± 0.08        | 102 ± 0.57          | 5.0 ± 0.11      | 4.36 ± 0.57         | 1.45 ± 0.08    | 2.91 ± 0.01      |
| T3         | 126.7 ± 0.05      | 81.3 ± 0.88         | 4.1 ± 0.03      | 3.76 ± 0.57         | 1.40 ± 0.03    | 2.36 ± 0.05      |
| T4         | 112.3 ± 0.06      | 76.3 ± 1.54         | 3.7 ± 0.03      | 3.6 ± 0.57          | 1.35 ± 0.05    | 2.25 ± 0.02      |
| T5         | 107.7 ± 0.03      | 63.3 ± 0.88         | 3.6 ± 0.00      | 3.36 ± 0.57         | 0.03 ± 1.23    | 2.13 ± 0.02      |
| T6         | 91.7 ± 0.00       | 51.3 ± 0.88         | 3.0 ± 0.03      | 3.26 ± 0.57         | 1.01 ± 0.03    | 2.19 ± 0.05      |

Significant Level: * * * N.S * *

*(1) Treatments 1, 2, 3, 4, 5, 6 are a positive control and a negative control, and 0.1 and 0.2 0.3 and 0.4% of graviola leaves powder.
Non-significant: N.S: means that there were no significant differences between the averages.
* The different letters within the same column indicate significant differences between the averages at the probability level (P < 0.05).

Table 6, it shows the averages of high and low density lipoproteins values and the concentrations of glucose, phosphorous and calcium. It was also observed that there was a significant decrease (P<0.05) in the LDL values and a significant increase in the HDL values with an increase in the percentage of graviola leaves powder. To the different diets, as treatment T6 recorded the lowest values of 13.1 mg/dl compared to the highest values in treatment T2, which amounted to 26.1 mg/dl of low-density lipoproteins. LDL resulting from free radicals [16], and the same trend was reflected in the values of the concentrations of sugar, phosphorous, calcium, and the basic phosphatase enzyme, while there was a significant increase in the average of high-density lipoproteins with the increase in the percentage of addition of graviola leaves powder to the diets. 111.7 mg/dl versus 72.7-treatment (T2 negative control), The reason may be due to the low level of the basal phosphatase enzyme due to the presence of the active substances in the powder of the graviola leaves, which led to the enhancement of the antioxidant action, which contributed to the preservation of the plasma membrane of the liver cells from damage resulting from oxidation [17,18], or perhaps the decrease in the activity of the phosphatase enzyme. Basophilia reflects the decreasing demand for these enzymes resulting from their large use in the metabolism and manufacture of proteins in the liver. With regard to the low level of glucose in the blood serum of birds. For birds, it may be due to the presence of alkaloids that stimulate beta cells in the pancreas as well as to enhance or stimulate the secretion of the largest amount of insulin in response to high blood glucose levels, increase the metabolism of glucose through the glycolysis pathway, as well as increase the entry of glucose into the plasma membrane [19]. Or perhaps the reason for the decrease in glucose is due to the flavonoids in the powder of graviola leaves, which can play an effective role in inhibiting the process of formation of glucose from non-carbohydrate sources (Gluconeogenesis) by inhibiting the enzyme G-6-P in the liver [20]. These decreases can be attributed to the effect of the active substances present in the graviola leaves powder, which generally resulted in a decrease in the values of blood fats and blood proteins, to be reflected in a decrease in the values of low lipoproteins. Hence, the powder of graviola leaves has benefits not only medicinal but also healthy in the nature of the produced meat. From chicken meat, so we can consider it a food and medicinal item at the same time.
played a major role in improving the healthy environment of the intestines of birds, and they are not deposited in the bird's
this resulted in a reduction in the secretion of bacterial toxins [21]. The polyphenolic compounds, alkaloids and flavonoids
reducing the number of harmful bacteria compared to the antibiotic in the comparison treatment, and in the final outcome,
the number of harmful bacteria colonies decreased with the increase in the percentage of addition of graviola leaf
powder, as treatment T6 recorded less. The rates are 85.0 versus 205.0 in the T1 positive control. The reason for this may be
while the number of harmful bacteria colonies decreased with the increase in the percentage of addition of graviola leaf
powder, as it reached 185.0 beneficial bacterial colonies compared to 65.0 in the treatment T2 (the negative comparison),
observed. In parallel with the increase in the percentage of addition of graviola leaf powder, the treatment T6 recorded the
best values, as it reached 185.0 beneficial bacterial colonies compared to 65.0 in the treatment T2 (the negative comparison),
were observed between all the different treatments, and a clear increase in the number of beneficial bacteria colonies was
 observed. Table 7 shows. Statistical analysis of the colonization averages of beneficial bacteria ([Lactobacillus Subtilis] and harmful
(Esherichia Coli, Salmonella SPP, Shegilla SPP) in the coils of different treatment birds, and significant differences (P≤0.05)
were observed between all the different treatments, and a clear increase in the number of beneficial bacteria colonies was
observed. In parallel with the increase in the percentage of addition of graviola leaf powder, the treatment T6 recorded the
best values, as it reached 185.0 beneficial bacterial colonies compared to 65.0 in the treatment T2 (the negative comparison),
while the number of harmful bacteria colonies decreased with the increase in the percentage of addition of graviola leaf
powder, as treatment T6 recorded less. The rates are 85.0 versus 205.0 in the T1 positive control. The reason for this may be
due to the fact that the pharmacologically active substances in the graviola leaves powder showed a remarkable activity in
reducing the number of harmful bacteria compared to the antibiotic in the comparison treatment, and in the final outcome,
this resulted in a reduction in the secretion of bacterial toxins [21]. The polyphenolic compounds, alkaloids and flavonoids
played a major role in improving the healthy environment of the intestines of birds, and they are not deposited in the bird’s
body, causing harm to the consumer of these meats, as in the antibiotics [22], these results agreement with [23,24].

Table 7. Numbers of beneficial and harmful bacteria colonies in the ileum for all treatments.

| Treatments | Initial BW,g | Final BW,g | Age (5) wk |
|------------|--------------|------------|------------|
|            | One day old  | 35day old  | beneficial bacteria CFU/ml | harmful bacteria CFU/ml |
| T1         | a            | b          | f          | a           |
| T2         | a            | b          | e          | b           |
| T3         | a            | b          | d          | c           |
| T4         | a            | b          | c          | a           |
|   | T5          | T6          |   |
|---|-------------|-------------|---|
|   | 38.0        | 1969.0 ± 59.9 | 148.3 ± 44 | 125.0 ± 28 |
| a |             | a           | e |
| b | 38.0        | 1948.6 ± 8.9  | 185.0 ± 28 | 85.0 ± 28   |

Significant level
*

(1): Treatments 1, 2, 3, 4, 5, and 6: positive control and negative control and 0.1, 0.2, 0.3, 0.4% of graviola leaf powder.

* The different letters within the same column indicate significant differences between the averages at a probability level (P < 0.05).

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