

Effect of *Ficus nota* (Blanco) Merr on the Hematobiochemical Profile and Growth Performance in Broiler Chickens

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Abstract

Ficus nota Blanco Merr, known for its positive effects on human health in traditional medicine owing to its phytobiotics, has not been adequately studied for its impact on farm animals, particularly in poultry. This research sought to address this knowledge gap by investigating its influence on growth performance, hematological and serum biochemical profiles of broiler chickens. A total of 168 10-day-old commercial Cobb 500 broiler chicks, randomly assigned into four treatment groups each replicated three times, were subjected to a 31-day experimental period. *Ficus nota* fruits were subjected to fermentation, yielding a fermented juice, subsequently administered to the chicks via their drinking water. The treatments included a control group receiving plain water and three experimental groups with varying concentrations of fermented fruit juice (FFJ): 40 ml FFJ/L, 60 ml FFJ/L, and 80 ml FFJ/L. Substantial differences ($p < 0.05$) were observed in red blood cell count, hemoglobin concentration, and hematocrit. Conversely, platelet and white blood cell counts did not show significant variations ($p > 0.05$) among the treatment groups. Except for reduced cholesterol levels in treated groups, there were no significant changes ($p > 0.05$) in other blood serum biochemical indices. Furthermore, the treated groups exhibited notable weight gain and enhanced feed conversion ratio, underscoring the potential impact of the treatments.

Keywords: Feed conversion ratio, Fermentation, *Ficus nota*, Phytobiotics, Hematological Indices, Serum biochemical profile

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INTRODUCTION

Poultry farming, an integral part of global food production, has witnessed a significant rise in the utilization of antibiotics. Antibiotics became routine additives in poultry feed as necessary tools for disease prevention and growth promotion. However, the persistent, widespread use of antibiotics in poultry farming creates a pressing concern with profound implications for public health, animal welfare, and the environment. One of the primary concerns is the development of antibiotic resistance in bacteria, a phenomenon intensified by the continuous exposure of pathogens to these drugs. Antibiotic-resistant strains can easily proliferate and pose a serious threat to both animal and human health (Hosain et al., 2021).

In response to the concerns surrounding antibiotic use and to the limitations imposed in numerous countries regarding the utilization of antibiotic growth promoters in the process of meat production, there is an increasing focus on exploring alternatives. Phytobiotics, sourced from plants and encompassing compounds such as polyphenols, flavonoids, alkaloids, and phytoestrogens, have emerged as potential alternatives to synthetic supplements and antibiotics in livestock health and nutrition (Hashemi and Davoodi, 2011; Ognik et al., 2016; Zaikina et al., 2022).

Phytobiotic feed additives (PFAs) have shown promising effects on poultry output, as indicated by studies conducted by Abudabos et al. (2018) and Gheisar et al. (2015a). These alternatives have demonstrated potential in improving growth performance, nutrient absorption (Abdelli et al., 2021), and improving the antioxidant and anti-inflammatory status of animals (Kiczorowska et al., 2019).

Ficus nota (Blanco) Merr is an indigenous plant in the Philippines known for its bioactive compounds. Phytochemical screening results revealed that both leaves and fruits contained secondary metabolites such as alkaloids, anthraquinones, tannins, flavonoids, steroids, and saponins (Mapatac, 2015). In vitro studies in mice indicated potential health benefits such as antioxidant, anti-inflammatory, and immune-boosting effects (Ahmed et al., 2015). Furthermore, *Ficus nota* holds significance in traditional human medicine. Its uses include decoction of the roots and bark for treatment of urinary tract infections, hypertension, and diabetes and drinking three times per day of the water extracted from the standing tree for fever, and muscle pain relief (Lanting and Palaypayon, 2002).

Despite these promising advantages, there is a notable absence of specific studies on the impact of phytobiotics derived from *Ficus nota* on farm animals, particularly in poultry. This creates a significant knowledge gap concerning its potential health and growth benefits in livestock and poultry production. Hence, the study addressed this gap by assessing the broiler's growth response and hematological and biochemical parameters, providing valuable insights into its potential utilization as a natural feed additive.

MATERIAL and METHOD

Experimental Design and Treatment

The study was laid out in a Completely Randomized Design (CRD). A total of 168 10-day-old commercial Cobb broiler chicks were subjected to a 31-day experimental period. The chicks were randomly assigned into four treatment groups each replicated three times, with 14 chicks per replicate. The treatments included a control group receiving plain water and three experimental groups with varying concentrations of fermented fruit juice (FFJ): 40 ml FFJ/L, 60 ml FFJ/L, and 80 ml FFJ/L.

Phytochemical Analysis of *Ficus nota* Fruit

Fruit and leaf samples were taken to the Nueva Vizcaya provincial office of the Department of Environment and Natural Resources (DENR) for verification of botanical identity. Fresh fully matured *Ficus nota* fruits were subsequently collected, thoroughly washed, and subjected to phytochemical screening. The phytochemical screening process followed the procedures outlined by Guevarra (2005).

It involved utilizing thin-layer chromatography (TLC) plates and various reagents to identify secondary metabolites in plant extracts. TLC plates were developed in an Ethyl Acetate-Chloroform mixture, and spots were visualized using UV light and hot plate. Vanillin-sulfuric acid reagent was used to identify phenols, sterols, triterpenes, and essential oils. Methanolic potassium hydroxide was used for anthraquinones, coumarins, and anthrones, while potassium ferricyanide-ferric chloride reagent was used for phenolic compounds and tannins. Alkaloids were detected using Antimony (III) chloride.

Phytochemical analysis of *Ficus nota* has revealed the presence of various bioactive compounds including phenols, flavonoids, tannins, saponins, coumarins, and steroids, among others (Table 1).

Table 1. Compounds isolated from *Ficus nota* fruits

Plant constituents	<i>Ficus</i> fruit	Plant constituent	<i>Ficus</i> fruit
Essential oils	-	Coumarins	+
Triterpenes	-	Anthrones	+
Sterols	-	Tannins	+
Phenols	+	Flavonoids	+
Fatty acids	-	Alkaloids	-
Sugars	-	Steroids	+
Anthraquinones	+	Amino acids	-

(+) Abundant; (-) Absent

Fermented Fruit Juice Preparation

Fresh fully matured *Ficus* fruits were collected for fermentation. The preparation of the fermented *Ficus nota* fruit juice followed the procedures outlined by Huervana (2016). Thinly sliced fruits were mixed with molasses in a 1:1 ratio. The resulting mixture was anaerobically fermented in an earth jar and left undisturbed for 7 days at room temperature. Fermented juice extracts were stored in clean plastic containers and kept refrigerated for up to 3 days to slow down continuous fermentation and preserve the juice until used.

Dietary Treatment

While the experimental treatments were introduced at 10 days old, the chicks were fed a commercial chick booster diet until they reached 14 days of age. Subsequently, from days 15 to 41, they were fed *ad libitum* with formulated isocaloric and isonitrogenous diet.

Distilled water was used as drinking water for the experimental birds. The use of distilled water ensures a consistent and pure water supply, minimizing the potential impact of impurities or contaminants on the test material and the birds' health and performance. Except for variations in the drinking water treatments, all other aspects of management were standardized across experimental groups. This ensures that observed differences in study outcomes primarily result from treatment variations rather than disparities in overall management, enhancing the validity and reliability of results for an accurate assessment of treatment impact on bird growth and performance.

Hematological and Serum Biochemical Analysis

On the 42nd day of the experiment, representative samples were randomly selected for blood collection. Blood samples were collected through the wing vein into EDTA bottles for hematological evaluation of the red blood cells (RBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), hematocrit (HCT) and white blood cells (WBC), neutrophil and lymphocytes, white blood cells (WBC), lymphocytes and granulocytes. The samples were analyzed using a hematology analyzer.

Whole blood samples were centrifuged (2500g for 10 minutes at 4 °C) and the resulting sera were stored at -20°C for serum biochemical analysis using a semi-automated biochemical analyzer. Blood parameters, indicative of renal function (BUN and creatinine), hepatic function (ALT and total protein) as well as cholesterol and triglyceride levels were determined.

Growth Performance Data

The initial weight of the chicks was taken during randomization on day 10, with subsequent weekly weight measurements throughout the experiment. Additionally, precise measurements of feed and water were systematically recorded to ensure uniform provision of feeds and water. Performance data collected included gain in weight, feed intake and feed conversion ratio.

Statistical Analysis

The collected data were subjected to statistical analysis using IBM SPSS 23 software with the analysis of variance (ANOVA) conducted in a Completely Randomized Design (CRD). Differences among treatment means were assessed at a significance level of 0.05. A post-hoc test, using Tukey's test was used to determine which treatments significantly differ from one another.

RESULTS and DISCUSSION

Hematological Indices

The findings presented in Table 2 demonstrate that incorporating fermented *Ficus nota* fruit juice into the drinking water of the broiler chickens resulted in significantly higher ($p < 0.05$) erythrocyte counts ($3.16 \pm 0.03 - 3.34 \pm 0.05 \times 10^{12}$ L), hemoglobin levels ($10.51 \pm 0.53 - 10.63 \pm 0.86$ g/dL), mean corpuscular hemoglobin ($46.70 \pm 0.60 - 48.10 \pm 0.70$ pg), mean corpuscular hemoglobin concentration ($43.07 \pm 2.22 - 43.43 \pm 1.98$ g/dL), and hematocrit values ($23.87 \pm 0.37 - 25.37 \pm 0.83\%$). However, no significant variations ($p > 0.05$) were observed among treatment means for mean corpuscular volume, platelets, white blood cells, lymphocytes, and granulocytes.

The results of the current study demonstrate increased red blood cell counts, hemoglobin levels, mean corpuscular hemoglobin concentration (MCHC), hematocrit, and mean corpuscular hemoglobin (MCH) percentages in treatments involving fermented *Ficus nota* fruit juice. This indicates the potential beneficial effects of the juice on red blood cell production and maintenance.

These findings align with several studies that have shown significant increases in erythrocyte counts and hemoglobin levels with the incorporation of phytobiotics such as cinnamic aldehyde, thymol, and carvacrol (Reis et al., 2018), cinnamon oil (Krauze et al., 2020), as well as organic acids and flavonoids (Gilani et al., 2018) into the diets of broiler chickens. Similarly, broilers fed with pawpaw leaf and seed meal (Oloruntola, 2019), turmeric powder (*Curcuma longa*), and cayenne pepper (*Capsicum frutescens*) (Adegoke et al., 2018) exhibited elevated hemoglobin and erythrocyte values.

Table 2. Hematological indices (mean±SD) of broiler chickens supplemented with fermented *Ficus nota* fruit juice

Parameters	T1 (Control)	T2 (40 ml)	T3 (60 ml)	T4 (80 ml)	CV (%)
RBC (10 ¹² L)	2.89±0.09 ^c	3.16±0.03 ^b	3.34±0.05 ^a	3.32±0.05 ^a	6.25
Hb (g/dL)	8.10±0.26 ^b	10.59±0.54 ^a	10.63±0.86 ^a	10.51±0.53 ^a	12.35
MCH (pg)	42.23±0.86 ^b	46.70±0.60 ^a	48.10±0.70 ^a	47.07±1.65 ^a	5.45
MCHC (g/dL)	38.03±6.63 ^b	43.10±1.13 ^a	43.07±2.22 ^a	43.43±1.98 ^a	8.89
HCT (%)	20.20±0.81 ^b	23.87±0.37 ^a	25.00±1.73 ^a	25.37±0.83 ^a	9.82
MCV (fl)	105.00±0.62	109.93±8.55	110.33±3.51	112.00±2.98	4.55
Platelet (10 ⁹ L)	18.00±3.00	18.33±2.08	21.33±4.50	25.33±3.21	20.14
WBC (10 ⁹ L)	142.83±3.75	139.73±5.03	143.80±6.92	143.40±4.37	3.31
Lymphocytes (10 ⁹ L)	103.50±3.31	102.53±4.59	102.83±3.20	102.57±0.93	2.75
Granulocytes (10 ⁹ L)	24.10±1.11	22.30±3.23	27.57±2.67	24.60±2.45	11.81

*Values with different superscripts differ significantly at the 5% level

The mean corpuscular volume (MCV) did not show significant differences among treatment groups, consistent with the results reported by Basit et al. (2023); Oghenebrorhie, and Oghenesuvwe (2016), who also found no significant differences in MCV among broilers fed with *Persicaria odorata* leaf meal and *Moringa oleifera* leaf meal, respectively.

While platelets are primarily known for their role in hemostasis or clot formation, it is important to note that they also participate in several other physiological processes such as wound healing and remodeling, tissue repair, and antimicrobial host defense (Leslie, 2010). Moreover, as stated by Huang and Chang (2012) platelets contribute to immune responses. White blood cells, which include lymphocytes and granulocytes, are crucial components of the immune system and play vital roles in defending the body against infections and diseases. Typically, when an infection or foreign organism is present in the body, white blood cell counts tend to increase as a response to combat the infection.

The results of the present study, which show a lack of significant differences in platelet and white blood cell counts among the treatment groups, suggest that the experimental birds in all treatments did not exhibit any signs of infection and were generally healthy. The absence of significant variations in lymphocyte and granulocyte counts further supports the conclusion that the experimental birds were in a healthy state and did not display any abnormalities in their immune responses.

Serum Biochemical Indices

Table 3 presents the results of serum biochemical responses indicating liver and kidney functions, as well as lipid profiles in broilers following supplementation with fermented *Ficus nota* fruit juice.

Table 3. Serum biochemical indices (mean±SD) of broiler chickens supplemented with fermented *Ficus nota* fruit juice

Treatments	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Creatinine (mg/dL)	BUN (mg/dL)	ALT (μL)	Total protein (g/L)
T1 (Control)	241.18±10.47 ^b	126.98±9.39	0.57±0.04	3.64±0.26	11.29±4.39	1.51±0.08
T2 (40 ml)	181.21±12.82 ^a	125.66±4.24	0.52±0.04	3.68±0.15	10.56±1.43	1.52±0.05
T3 (60 ml)	170.74±8.19 ^a	122.35±11.38	0.56±0.03	3.60±0.07	11.11±4.03	1.49±0.03
T4 (80 ml)	176.18±3.40 ^a	125.84±2.29	0.54±0.03	3.65±0.16	10.26±2.04	1.51±0.05
CV	15.99	6.52	3.12	6.56	15.81	3.33

*Values with different superscripts differ significantly at the 5% level

Statistical analysis revealed a notable variation ($p < 0.05$) in the cholesterol levels between the treated groups and the control group. Cholesterol levels ranged from 170.74±8.19 to 181.21±12.82 mg/dL in the *Ficus nota* treated groups, which were significantly lower compared to the cholesterol concentration of 241.18 mg/dL observed in the control group. No significant variations ($p > 0.05$) were observed among treatment means for triglyceride, creatinine, BUN, ALT, and total protein.

Triglyceride values ranged from 122.35±11.38 to 126.98±9.39 mg/dL, falling within the normal range of 45.7 to 172 mg/dL (Meluzzi et al., 1991, as cited in Odunitan-Wayas et al., 2018). Similarly, alanine aminotransferase (ALT) levels ranged from 10.26±2.04 to 11.29±4.39 mg/dL, which is within the normal range of 9.50-37.2 mg/dL as reported by Roa et al., (2020).

Serum concentrations of cholesterol and triglycerides serve as indicators of lipid metabolism (He et al., 2015). The significant reduction in cholesterol concentration observed in the present study suggests that *Ficus nota* fruit juice may possess hypocholesterolemic properties attributed to its phytobiotic contents. The results are consistent with Zhou et al. (2015) and Yu et al., (2019), who reported that dietary supplementation of broilers with fermented Ginkgo biloba rations and fermented Ginkgo biloba leaves can significantly reduce the serum levels of cholesterol. Comparable results were noted by Gilani et al., (2018), who demonstrated that the use of phytobiotics, organic acids, and their combinations led to a significant reduction in serum levels of cholesterol and triglycerides in broiler chickens.

Phytobiotics have been shown to decrease circulating cholesterol levels and inhibit lipid oxidation (Crouse et al., 1999; Fki et al., 2005; Starčević et al., 2020; Zeni et al., 2020). Additionally, studies by Chen et al., (2013) and Laka et al., (2022) elucidate their role in modulating cholesterol metabolism and improving lipid profiles. Additionally, phytobiotics are known for their ability to reduce the absorption of dietary cholesterol (Ahmed et al., 2017; Sarika et al., 2009), enhance the excretion of cholesterol (Koochaksaraie et al., 2011), and inhibit enzymes involved in cholesterol synthesis (Lee et al., 2007), thereby effectively lowering cholesterol levels in the animal's body.

Furthermore, the absence of significant changes and variations among treatment groups in BUN, creatinine, ALT, and total protein in the present study implies normal kidney and liver functions. These results indicate that the bioactive compounds from *Ficus nota* fruit juice were well-tolerated by the birds, without inducing any adverse effects on kidney and liver functions.

Growth Performance

The impact of fermented *Ficus nota* fruit juice on the growth of broilers, as presented in Table 4, was assessed using key parameters such as final weight, weight gain, and feed conversion ratio (FCR). Additionally, feed and water consumption were meticulously recorded and evaluated to provide a comprehensive analysis of the treatment's effects on broiler performance.

Statistical analysis revealed no significant differences ($p>0.05$) among treatment means for initial weights, indicating a high level of uniformity among the experimental birds. This uniformity is crucial for completely randomized experiments, as it minimizes the influence of weight variations on the results. The incorporation of fermented *Ficus nota* fruit juice in the drinking water of broilers resulted in significantly higher ($p<0.05$) final weight, weight gain, and feed conversion ratio (FCR) compared to the control group, with concentrations of 60-80 ml demonstrating the highest impact. However, no significant variation ($p>0.05$) was observed in the feed and water intake of the birds, indicating similar patterns of consumption across all groups.

Table 4. Growth performance indices (mean±SD) of broiler chickens supplemented with fermented *Ficus nota* fruit juice.

Parameters	T1 (Control)	T2 (40 ml)	T3 (60 ml)	T4 (80 ml)	CV (%)
Initial weight (g)	324.67±8.96	325.00±2.64	324.33±6.02	324.33±4.04	3.45
Final weight (g)	1335.33±5.68 ^c	1696.00±17.78 ^b	1992.00±10.78 ^a	2030.00±73.07 ^a	3.46
Gain in weight (g)	1010.67±14.57 ^c	1371.33±18.90 ^b	1668.33±16.19 ^a	1706.33±76.79 ^a	3.46
Feed intake (g)	2813.33±67.14	2871.00±49.72	2843.67±10.96	2835.67±46.05	3.46
FCR	2.78±0.10 ^c	2.09±0.06 ^b	1.71±0.01 ^a	1.70±0.05 ^a	3.61
Water intake (ml)	13851±63.31	13896±32.14	13910±21.79	13939±53.67	3.46

*Values with different superscripts differ significantly at the 5% level

The inclusion of fermented *Ficus nota* fruit juice as a source of phytobiotics has demonstrated a positive effect on body weight and feed efficiency in experimental broilers. This aligns with existing literature emphasizing the benefits of phytobiotics in broiler feeds. Numerous studies, including those by Abdel-Wareth et al., (2019), Al-Sagan et al., (2020), Devi et al., (2018), Gheisar et al., (2015a, 2015b), Starčević et al., (2020), Toghyani et al., (2011), and Yan et al., (2011), consistently demonstrate the significant and observable economic benefits of phytobiotic supplementation on body weight (BW) and feed conversion ratio (FCR) in broiler development.

The similar patterns of feed and water intake among the treatment groups are crucial, as they imply that the observed differences in weight gain and feed efficiency are more likely attributed to the introduction of the fermented fruit juice treatment rather than differences in consumption patterns among the groups. This consistency strengthens the validity of the findings, indicating that the enhanced growth performance can be directly linked to the effects of fruit juice supplementation.

CONCLUSION

Supplementing broiler drinking water with 60-80 ml of fermented *Ficus nota* fruit juice enhances hematological indices and promotes broiler growth without causing harmful changes in blood biochemical parameters. These findings highlight the plant's potential as a safe and effective alternative for promoting health and growth in broilers. Furthermore, the results emphasize the importance of conducting further studies across different animal species to fully explore the benefits and applications of *Ficus nota* fruit juice in animal nutrition and health.

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