FUDMA Journal of Agriculture and Agricultural Technology
ISSN: 2504-9496
Vol. 11 No. 2, June 2025 Special Issue: Pp.66-72

https://doi.org/10.33003/jaat.2025.1102.009

EFFECT OF FEEDING PAWPAW (*Carica papaya*) AQUEOUS EXTRACT AS REPLACEMENT TO ANTIBIOTICS USE ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS IN BROILER CHICKEN (Starter Phase)

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ABSTRACT

Poultry farming faces challenges from rising feed costs and concerns about antibiotic use. This study investigated pawpaw (*Carica papaya*) leaf extract as a natural alternative to antibiotics in broiler production. Three hundred, day-old broiler chicks were randomly assigned to four treatments: control (T1) and pawpaw leaf extract at 20ml (T2), 40ml (T3), and 60ml (T4) per liter of drinking water. The experiment followed a completely randomized design with four replications per treatment. At day 21, blood samples were collected from selected birds for hematological and serum biochemical analysis. Broilers receiving pawpaw extract showed significantly higher ($p<0.05$) total protein (7.16-7.93 g/dL vs. 4.20 g/dL) and albumin (4.43-5.03 g/dL vs. 2.56 g/dL) levels compared to the control group. Cholesterol levels were lowest in T4 (127.03 mg/dL) compared to control (163.83 mg/dL). Creatinine increased with extract dose, with T4 showing the highest value (1.88 mg/dL). Heamatological parameters remained within normal ranges across treatments, though Mean Corpuscular Hemoglobin Concentration was significantly higher in the control group. The extract's antioxidant properties likely protected liver cells against oxidative damage, enabling more efficient protein synthesis while supporting immune function through improved globulin production. Pawpaw leaf extract significantly improved broiler serum protein profiles, suggesting enhanced metabolic efficiency and immune function. The 40ml/L dose (T3) is recommended for optimal health benefits without the potential renal stress observed at higher concentrations, providing a promising natural alternative to antibiotic growth promoters in broiler production.

Keywords: Broiler, Serum biochemistry, Heamatology, Pawpaw leaf extract

INTRODUCTION

Poultry production is a fundamental component of world agriculture and a key source of cheap animal protein (Adene and Oguntade, 2006). Broiler chickens, which are specifically bred for meat, are appreciated for their high growth rate and feed conversion efficiency. Nevertheless, the poultry sector is confronted with rising challenges, most notably concerning feed production, which constitutes about 60–70% of overall cost of production. The rise in feed costs is attributed to competition for major ingredients such as maize and soybean, which are also major food sources for human beings (Ologbon & Ambali, 2012). As a result of these issues, farmers and researchers have been prompted to look for alternative feed ingredients that are cheap, readily available, and nutritionally sufficient.

Pawpaw (*Carica papaya*) leaves have been of interest as a possible feed ingredient. Rich in protein and bioactive compounds, pawpaw leaves have been utilized in traditional herbal medicine due to their health-promoting activities Okpe and Ibrahim (2024). In livestock, the bioactive compounds may enhance immune status, boost growth rates, and reduce the need for synthetic additives (Oloruntola, 2019). In spite of their potential attributes, the use of pawpaw leaves in broiler diets has not been extensively investigated, particularly with regard to their impact on growth performance, carcass quality, hematological parameters, and serum biochemistry. This research seeks to bridge this gap by presenting empirical evidence on the use of pawpaw leaf meal in the dietary regimen of broiler chickens.

Feed cost represents most of the financial outlay in broiler production, boosted by high demand and prices of traditional feed ingredients Osman *et al.,* (2010). Maize and soybean, which are components of poultry feeds, are also essential for human consumption and industrial use. This competition has caused successive price increases, to the detriment of the profitability of poultry production. Besides, the use of synthetic growth promoters and additives has also raised safety concerns about poultry products and their residues in the human food chain Lee *et al*., (2012).

The investigation into natural feed additives like pawpaw leaf meal offers a solution that can address these issues. Pawpaw leaves are readily available, widespread in tropical and subtropical regions, and frequently regarded as farm waste. Their high nutritional content and medicinal attributes such as antioxidant and antimicrobial properties qualify them to be part of broiler diets (Oloruntola, 2019). But the dearth of a broad base of studies on their impacts on broiler performance, carcass characteristics, and health indices is a limitation to their acceptance. The objective of this study is to establish a scientific foundation for the utilization of pawpaw leaf meal as an inexpensive and sustainable feed ingredient.

Antibiotics are included in animal feeds at sub-therapeutic levels to avert zoonotic infections and improve product quality, although there has been a growing discontent with their inclusion as animal growth promoters (Letlhogonolo *et al.,* 2020).

As the world human population continues to increase, the principal aim of broiler producers is to raise chickens that are ready for consumption in the shortest time possible. Phytogenic nutritional additives represent a promising alternative to be included in livestock feed to enhance poultry performance and strengthen the poultry immune system. The pawpaw tree, or *Carica papaya*, is the most prolific natural source of papain. Apart from that, the utilization of pawpaw leaves (Carica papaya) in broiler feeding has been utilized effectively (Onyimonyi & Ernest 2009). Antibiotic growth promoters enhance feed consumption and inhibit the growth of pathogenic microorganisms. Therefore, through research, it has been found that Carica papaya leaves contain all the essential constituents that guarantee improved feed consumption, effective utilization of feed, and inhibition of growth of pathogenic organisms.

Nutritional supplementation using phytobiotics, phytogenic feed additives, phytochemical feed additives, and herbal supplements or ingredients is gaining acceptance globally as a viable substitute for synthetic growth promoters (Fallah *et al*. 2013). The antimicrobial, anti-stress, antioxidant, and immunomodulatory characteristics of phytochemicals qualify them as ideal growth promoters in animal production (ValenzuelaGrijalva *et al*. 2017). Parts of various plants that are of medicinal importance had been used as supplements and or ingredient in the production of livestock to achieve various purposes of performance and health importance (Oloruntola, 2018). Seeds and leaves of pawpaw were reported to neutraceutical and antioxidant properties (Kadiri *et al.,*2016). Pawpaw contains a high content of vitamin A, B, and C; papain; and chymopapain. Seeds and leaves of papaya contain 2,2-diphenyl-1-piccrlhydrazyhydrate (7.8 mg/ml and 1.0 mg/ml), phenol (424.89 mg GAE/100 g dry weight and 30.32 mg GAE/100 g dry weight), and flavonoid (333.14 mg GAE/100 g dry weight and 59.54 mg GAE/100 g dry weight), respectively (Maisarah *et al.,* 2014). Medicinal plants were reported to be used as anti-fertility agents (Nurcahyani *et al*. 2018). Despite the evidence of the positive impacts associated with the consumption of pawpaw plant parts as additives or ingredients in animal feed (Hassan *et al.,* 2014; Oloruntola *et al*. 2018a), there is limited pathological study exploring the impact of the use of pawpaw leaves as broiler chicken diet supplements or ingredients. Hence, the aim of this research was to evaluate the impact of pawpaw leaf extract on growth performance, hematological and serum biochemical profiles, and carcass and organoleptic traits in broiler chickens.

Experimental site

The research will be conducted at the Prof. Lawal Abdu Saulawa Livestock Teaching and Research Farm of the Animals Science Department, Federal University Dutsin-ma. Dutsin-ma lies on latitude $12^0$26'N and longitude $07^0$29'E. Rainfall is between May and September with a peak in August. The average annual rainfall is about 700mm. The mean annual temperature ranges from 29^0C – 31^0C. The highest air temperature normally occurs in April/May and the lowest in December through February (Abaje *et al.,* 2014)

Source of Experimental Materials

The broiler chickens, feeders, drinkers, pawpaw leaf extract will be sourced from Dutsin-Ma and Katsina metropolis.

Processing of Pawpaw leaves extract

Fresh Pawpaw leaves will be collected from Kastina and environ. They will be washed, cut, air-dried for 5 days till crispy dried. The dried leaves were blended into powder. The powdered product was then reconstituted by mixing 60 grams of powder dried pawpaw leaves with 1000 ml of water. The liquid product was left to sit for 15 minutes after which it was strained and served to the birds. The phytochemicals in pawpaw leaves extract will be determined according to the methods described by Okwu & Ezenagu (2008).

Experimental design and management

A total of 300 'day-old' broiler chicks were used for the study. Upon arrival, the chicks were weighed and randomly distributed into 24 pens. The pens will be randomly allotted to the following treatments in a completely randomized design;

Treatment one – Negative Control
Treatment two – 20 ml Pawpaw leaf extract
Treatment three – 40 ml Pawpaw leaf extract
Treatment four – 60 ml Pawpaw leaf extract

Each treatment had four replications of 15 chicks each. A starter (1–28 days) diet was formulated according to the National Research Council (NRC, 1994) requirements. The extract was included in their drinking water at a rate of 20, 40 and 60 ml per 1000ml of water for treatment 2,3 and 4 respectively. The birds were vaccinated against infectious bursa disease on 7 and 21 days, and Newcastle disease on 14 and 28 days. The birds were kept at 34^0C for the first 7 days. Afterwards, the temperature were reduced by 3^0C per week until it reached 24^0C. During the first week, 22h of light will be provided.

Table 1: Composition of the Experimental Diet (Starter and finisher diet)

INGREDIENTS g/kg	STARTER DIET
Maize	560.00
Soyabean Meal	300.00
Fish Meal	75.00
Soya Oil	33.80
Dicalcium phosphate	19.20
Premixes	2.50
Limestone	4.00
Methionine	1.00
Lysine	2.00
Salt	2.50
Pawpaw leaf extract	0.00
TOTAL	1000.00
CALCULATED ANALYSIS	
Crude protein g/kg	23.40
Energy ME, kcal/kg	3040.42
fat g/kg	36.28
crude fibre g/kg	33.85
calcium g/kg	10.52
Total phosphorus g/kg	8.91
Non-phytate P, g/kg	4.49
Ca:NPP	2.34

Composition of premix per kg of diet: Vitamin A, 12500 I.U; vitamin D3, 255000 I.U; vitamin K3, 2mg; vitamin B1, 3mg; vitamin B2, 5.5mg; calcium pantothenate, 11.5mg; vitamin B12, 0.025mg; choline, chloride, 500mg; folic acid, 1mg; biotin, 0.08mg; manganese, 120mg; iron, 100mg, zinc, 80mg; copper, 8.5mg; iodine, 1.15mg; cobalt, 0.3mg; selenium, 0.12mg; anti-oxidant, 120mg.

Haematological and serum biochemical parameters

At the 3rd and 6th weeks of the experiment, between the hours of 7:00am – 9:00am 2 birds were randomly selected from each replicate (10 poults per treatment) for blood collection. About 5mls of blood were collected from the sub-clavicular vein of each bird using a scalp vein needle set after swabbing with mentholated spirit. The blood was placed into sterilized glass tubes/bottles containing Ethylene Diaminetetra-acetic Acid (EDTA) for Haematological studies. Blood samples for serum biochemical studies were collected into plain containers (i.e without anticoagulant) for serum separation. Serum enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) was determined by standard methods using Randox test kits (Randox Laboratories, Antrim, UK).

Statistical analysis

Data will be subjected to analysis of variance using the general linear model procedure of SPSS software 20 software package. Means will be separated using the Duncan Multiple Range Test.

Impact of reconstituted pawpaw leaf extract on the serum biochemical parameters in 21-day-old starter broiler chickens.

RESULTS

The effect of serum components of broiler chicken fed water with reconstituted pawpaw leaf extract at 3 weeks is shown in Table 2. The total protein, Albumin, and Creatinine were significantly ($p<0.05$) affected by pawpaw leaf extract, while globulin, cholesterol, triglycerides, very low-density lipoprotein, high-density lipoprotein, low-density lipoprotein, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and uric acid, were not significantly ($p>0.05$) affected by the diets. The total protein and albumin levels were found to be statistically significantly increased ($p<0.05$) in the starter broilers treated with

pawpaw leaf extract in drinking water when compared with the control group not treated with any pawpaw leaf extract in drinking water. Control group broilers (T1) at three weeks of age had the lowest serum total protein (TP) level at 4.20 g/dL; however, those in T2 (7.16 g/dL), T3 (7.26 g/dL), and T4 (7.93 g/dL) had greater levels. The control group (T1) also recorded the highest cholesterol level, which was 163.83 mg/dL, while group T4 had the lowest level at 127.03 mg/dL. Furthermore, the highest triglyceride level was in T4, which was 131.53 mg/dL.

Creatinine levels exhibited a significant increase, especially within the T4 group, where the measurements approached twice that of the control group (1.88 mg/dL versus 0.90 mg/dL).

Table 2: Effect of reconstituted pawpaw leaves extract on the serum biochemical profile of starter broiler chickens at 21 days

Parameters	T1	T2	T3	T4	SEM
Total protein(g/dL)	4.20[b]	7.16[a]	7.26[a]	7.93[a]	0.31
Albumin (g/dL)	2.56[b]	4.43[a]	4.70[a]	5.03[a]	0.41
Globulin (g/dL)	1.63	2.76	2.56	2.86	0.56
Cholesterol(mg/dL)	163.83	154.66	136.06	127.03	12.10
Triglyceride(mg/dL)	111.53	116.26	116.26	131.53	10.33
VLDL (mg/dL)	22.30	23.23	23.23	26.33	2.07
HDL (mg/dL)	86.23	71.06	70.36	67.30	9.82
LDL (mg/dL)	55.36	60.33	42.46	33.40	9.59
ALP (U/L)	41.00	39.00	40.00	38.33	2.34
AST (U/L)	90.33	89.00	119.33	88.00	10.55
ALT (U/L)	28.66	22.00	32.66	25.00	3.69
Uric acid (mg/dL)	8.23	8.26	8.63	9.83	1.01
Creatinine (mg/dL)	0.90[b]	1.06[b]	1.34[b]	1.88[a]	0.132

[ab] Means on the same row having different superscripts are significantly ($p<0.05$)
SEM: Standard Error of means
VLDL= Very Low-Density Lipoprotein, HDL= High-Density Lipoprotein, LDL= Low-Density Lipoprotein, ALP= Alkaline Phosphatase, AST= Aspartate Aminotransferase, ALT= Alanine Aminotransferase

Table 3: Effect of reconstituted pawpaw leaves extract on the Hematological Indices of broiler starter chickens (21 days)

Parameters	T1	T2	T3	T4	SEM
PCV (%)	29.00	25.67	28.00	29.00	3.32
HB (g/dL)	9.73	8.77	9.43	9.57	1.05
RBC (x103/L)	2.63	2.43	2.60	2.40	0.30
WBC (x103/L)	14.27	12.53	11.97	12.63	1.29
Heterophil (%)	34.67	33.67	29.67	30.00	2.87
Lymphocyte(%)	63.00	63.33	66.67	67.00	3.07
Eosinophil (%)	1.00	1.67	1.33	1.33	0.5
Monocytes (%)	1.33	1.33	2.33	1.67	1.04
MCV (fl)	106.30	151.09	130.56	158.64	26.74
MCH (pg)	36.93	36.19	36.39	40.05	3.03
Basophil (%)	0.00	0.00	0.00	0.00	0.00
MCHC (g/dL)	35.13^{a}	24.40^{b}	28.11^{ab}	25.92^{b}	4.18

^{abc} Means on the same row having different superscripts are significantly ($p<0.05$)
SEM: Standard Error of means. PCV: Packed Cell Volume, HB: Hemoglobin, RBC: Red Blood Cell, WBC : White Blood Cell, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration

The results of hematological indices in broiler chicken administered water with reconstituted pawpaw leaf extract at 3 weeks are presented in Table 3, PCV Values ranging from 25.67% (T2) to 29.00% (T1 and T4) were recorded, with no significant difference ($p<0.05$), indicating a stable red blood cell mass across treatments. The mild reduction of PCV in T2 could be a mild dilutional or nutritional effect but not clinically relevant.

. The Red Blood Cell (RBC) counts were between 2.40 and 2.63 x10³/L. All the ranges were identical for all groups, thereby suggesting the absence of adverse hematopoietic effects of the extract. Hemoglobin levels also showed a similar trend, with T2 recording the lowest (8.77 g/dL) and T1 the highest (9.73 g/dL). In spite of the difference, all the values were within normal physiological ranges for broilers and point to the fact that pawpaw extract did not have any adverse effect on hemoglobin production.

MCV and MCH were higher in the extract-supplemented groups, particularly T4 (MCV: 158.64 fl; MCH: 40.05 pg), indicating that the size of red blood cells and hemoglobin went up. Interestingly, the Mean Corpuscular Hemoglobin Concentration (MCHC) of T1 (35.13 g/dL) was markedly higher than the rest of the groups (e.g., T2: 24.40 g/dL, T4: 25.92 g/dL), indicating a potential dilution effect or the phenomenon of red blood cell regeneration in the higher dose-treated group. The statistical significance ($p<0.05$) suggests that pawpaw extract may have an effect on hemoglobin encapsulation by erythrocytes, but further research is needed to ascertain its physiological relevance.

Leukocyte counts were not negatively affected significantly by the extract. T1 had the highest reading (14.27 x10³/L) and T3 the lowest (11.97 x10³/L), both of which were within normal. This is a non-pathological reaction, even suggesting mild immunomodulation.

Heterophils decreased to a certain degree in extract-treated groups (from 34.67% in T1 to 29.67–30.00% in T3 and T4), while lymphocytes increased (from 63.00% in T1 to 67.00% in T4), which may reflect a tendency to a more adaptive immune profile. Eosinophils, monocytes, and basophils did not vary in values during the treatments, which meant that the extract did not provoke allergic or inflammatory responses. The minimal rise in lymphocyte value with the fall in heterophils can denote positive regulation of the immune system, with probable improvement in resistance to disease without provoking hyperactive immune reactions. Red blood cell (RBC) levels, hemoglobin (Hb) level, and packed cell volume (PCV) are crucial indicators of oxygen transport as well as erythropoiesis in bird species.

DISCUSSION

The hematological changes observed are in line with the findings reported by Okpe and Ibrahim (2024), who reported significant changes in PCV and RBC counts in broiler chickens fed diets supplemented with pawpaw leaf meal. However, variation existed in the MCHC values, which were significantly higher in the control group of the current study, differing from the findings reported by Oloruntola, (2019), where MCHC values did not vary significantly across the treatment groups.

Biochemically, the increase in the content of serum total protein and albumin after supplementation with pawpaw leaf extract is consistent with that of

Ezenwosu *et al.,* (2022) in which significant effects on serum biochemical indices were obtained with the use of pawpaw leaf extract. However, the increase in cholesterol level reported in this study contrasts with what Oloruntola.,(2019) observed, where no treatment differences existed for cholesterol content.

The antioxidative property present in the pawpaw extract also seemed to have played a principal role in safeguarding hepatocytes (liver cells) against oxidative injury, thereby enabling greater protein synthesis. This finding is consistent with research conducted by those who established that medicinal plants with high phenolic content have the potential to greatly enhance liver function and protein biosynthesis. Aside from this, the immunomodulatory action attributed to pawpaw can also account for the high globulin fraction observed in the total protein, an indicator of a boosted immune response.

These biochemical enzyme results obtained via this study point out that the pawpaw leaf extract had no negative impact on liver health to hamper its function. Rather, it seems to have been easily tolerated by the broilers, facilitating the positive impacts of protein synthesis and lipid modulation observed. The results are consistent with accumulating evidence for the safe application of plant-based additives in poultry production as alternatives for synthetic growth promoters and therapeutics. Therefore, pawpaw leaf extract is a potential phytogenic feed additive that can promote metabolic health and improve the productivity of broilers via several different physiological mechanisms. This calls for additional studies to optimize dosage regimens and widen its advantages

Conclusion

Reconstituted pawpaw leaf extract significantly improved total protein, albumin, and creatinine levels in broilers at 3 weeks, with treated groups showing superior values compared to the control group.

RECOMMENDATION

Treatment 3 (40 ml extract per 1000 ml water) is recommended for optimal health benefits without compromising kidney function. While higher doses (Treatment 4) showed additional benefits for protein levels and reduced LDL cholesterol, the elevated creatinine suggests potential renal stress.

ACKNOWLEDGEMENT

The Authors gratefully acknowledge Tertiary Education Trust Fund (Tetfund) and Federal University Dutsin-Ma, Kastina State, Nigeria for sponsoring this research work.

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