The effects of replacing synthetic antibiotics with bay leaf, tamarind and West African pepper aqueous solutions on haematology, serum metabolites and organoleptic properties of broiler chickens

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Abstract

Food quality and safety have been a significant and critical issue due to the residual effects of Antibiotic Growth Promoters (AGPs) used in animal diets to improve growth rates. Natural alternatives are needed to improve growth without compromising consumers' health. This study was aimed at evaluating bay leaf, tamarind pulp and West African pepper as replacement for synthetic antibiotics in the broiler chicken. A total of 150 1- day old Arbor acre broiler chickens were divided into five treatments (Control, Bay leaf, Tamarind pulp, West African pepper seed and Mixture of bay leaf, tamarind pulp and West African pepper seeds), three replicates per treatment with 10 chicks per replicate. The bay leaf and West African pepper seed were ground into powder and added into water at 10g/L. Tamarind pulp was used at 10g/L. The experiment lasted for 56days. Haematological parameters measured were significantly different ($p<0.05$) across the treatments except the monocytes. The control group (34.00%; 3.37x 10^6uL) and the mixture of bay leaf, tamarind pulp and West African pepper seed group (33.00%; 3.35x 10^6uL) had the highest packed cell volume and red blood cells compared to other treatment groups. The mixture of bay leaf, tamarind pulp and West African pepper seed group had the highest lymphocyte value (68.00%) and lowest heterophils levels (23.00%) compared to the control group (62.00%; 30.00%). All biochemical parameters measured were significantly($p<0.05$) different across the treatment groups. The treatments had no significant effects ($p<0.05$) on the organoleptic properties of the chicken meat. This study concludes that the use of 10g/L solution of bay leaf, tamarind pulp and West African pepper seed aqueous solutions, individually and in combination had positive effects on health indices without any adverse effects.

Keywords: Broiler chickens, natural antibiotics, serum biochemistry, bay leaf, tamarind pulp
Running title: Natural alternatives to antibiotics in broiler chickens

Effets du remplacement des antibiotiques synthétiques par des solutions aqueuses de laurier, de tamarin et de poivre d'Afrique de l'Ouest sur l'hématologie, les métabolites sériques et les propriétés organoleptiques des poulets de chair

Résumé

La qualité et la sécurité des aliments sont devenues un enjeu important et critique en raison des effets résiduels des promoteurs de croissance antibiotiques (PCA) utilisés dans l'alimentation animale pour améliorer les taux de croissance. Des alternatives naturelles sont nécessaires pour améliorer la croissance sans compromettre la santé des consommateurs. Cette étude visait à évaluer le laurier, la pulpe de tamarin et le poivre d'Afrique de l'Ouest comme substituts aux antibiotiques synthétiques chez le poulet de chair. Un total de 150 poussins de chair Arbor acre âgés de 1 jour ont été répartis en cinq traitements (Témoin, Laurier, Pulpe de tamarin, Graines de poivre d'Afrique de l'Ouest et Mélange de laurier, pulpe de tamarin et graines de poivre d'Afrique de l'Ouest), avec trois répétitions par traitement et 10 poussins par répétition. Les feuilles de laurier et les graines de poivre d'Afrique de l'Ouest ont été broyées en poudre et ajoutées à l'eau à raison de 10g/L. La pulpe de tamarin a été utilisée à 10g/L. L'expérience a duré 56 jours. Les paramètres hématologiques mesurés étaient significativement différents ($p<0,05$) entre les traitements, à l'exception des monocytes. Le groupe

témoin (34,00 % ; 3,37x 10^6/µL) et le groupe mélange de laurier, pulpe de tamarin et graines de poivre d'Afrique de l'Ouest (33,00 % ; 3,35x 10^6/µL) ont présenté l'hématocrite et le nombre de globules rouges les plus élevés par rapport aux autres groupes de traitement. Le groupe mélange de laurier, pulpe de tamarin et graines de poivre d'Afrique de l'Ouest a présenté la valeur de lymphocytes la plus élevée (68,00 %) et les niveaux d'hétérophiles les plus bas (23,00 %) par rapport au groupe témoin (62,00 % ; 30,00 %). Tous les paramètres biochimiques mesurés étaient significativement différents (p<0,05) entre les groupes de traitement. Les traitements n'ont eu aucun effet significatif (p>0,05) sur les propriétés organoleptiques de la viande de poulet. Cette étude conclut que l'utilisation d'une solution à 10g/L de solutions aqueuses de laurier, de pulpe de tamarin et de graines de poivre d'Afrique de l'Ouest, individuellement et en combinaison, a eu des effets positifs sur les indices de santé sans aucun effet indésirable.

Mots-clés : Poulets de chair, antibiotiques naturels, biochimie sérique, feuille de laurier, pulpe de tamarin

Titre courant : Alternatives naturelles aux antibiotiques chez les poulets de chair

Introduction

Food quality and safety have been a significant and critical issue in the past years. In 2022, World Health Organization defined food security as access to enough safe and nutritious food to sustain life and promote good health. Animal feed and feeding is the central crux of livestock industry, Synthetic Antibiotic Growth Promoters (AGPs) have been widely employed at sub-therapeutic concentrations to improve the growth performance and overall health of the animal (Rickie *et al*., 2020; Azizi *et al*., 2024). AGPs are medicines that destroy or inhibit bacterial growth and are administered at a low sub therapeutic dosage (Hughes *et al.*, 2004).

In past decades AGPs have been used to improve production parameters and strong evidence have been linked to the presence of residues of synthetic growth promoters (i.e. antibiotics, anabolic compounds, hormones, and beta-agonists) in feed components and animal diets to negative effects in both human and animal health (Robles *et al.*, 2019). Due to the residual effect of these AGPs, the concern about food safety is increasing as awareness of the consumer increases around the world (Tiwari *et al*., 2014a; Samad, 2022; Alward *et al*., 2024).

Therefore, to protect the planet effectively, reduce antibiotic resistance and allergies, the reduction of AGPs is essential. Commercial additives of plant origin such as phytogenic feed additives have been proposed and developed as alternatives to synthetic antibiotics to help animals maintain good health and reach their growth potential (Al-Sagheer *et al*., 2019; Abou-Elkhair *et al*., 2020).

Phytogenic Feed Additives (PFAs) also known as botanicals, are plant derived compounds which contain herbs, leaves and flowers (non woody); spice or non-leafy parts such as fruits, seed, root and bark; essential oil and oleoresins (Windisch *et al*., 2008; Biswas and Kim, 2025). PFAs have the potential to enhance palatability, stimulate gastrointestinal activity, improve intestinal morphology and meat quality (Biswas, 2024). These effects could be attributed to their antimicrobial (Yadav *et al*., 2016); antioxidant properties (Oni *et al*., 2024) and immune-modulatory properties (Dong *et al*., 2024). Bay leaf, tamarind pulp and West African pepper seeds contain bioactive components and are one of these PFAs.

This study was designed to determine the effect of replacing synthetic antibiotics with bay leaf, tamarind pulp and West African pepper seed aqueous solutions on haematological parameters, serum metabolites and organoleptic properties of broiler chickens.

Materials and Methods

Experimental location

The experiment was carried out at the Poultry Unit, Teaching and Research Farm, College of Agriculture, Osun State University, Ejigbo Campus, Osun State. The study area is located

at latitude 7°54N and longitude 4°18E with an altitude of 426 m above sea level. Ejigbo is located 35 km north-east of Iwo, 30 km north of Ogbomoso and about 24km east of Ede (Google Earth, 2025).

Sample collection and preparation

Dried bay leaf, West African pepper seeds (WAPS) and fresh tamarind pulp were purchased from the herbs market in Ibadan, Oyo State. The tamarind pulp were air dried for 3 weeks. The bay leaf and WAPS were ground into powder separately, the tamarind pulp were used without grinding. 10g/L of each additives were immersed in hot water separately at 60^{o}C for two hours, the whole solution was used. Triplicate samples were taken to the laboratory for proximate and phytochemical analysis.

Experimental animals and management

One hundred and fifty 1-day-old Arbor acres broiler chicks were purchased from a reputable hatchery. The pen was cleaned and fumigated two weeks before the arrival of the birds. Charcoal was provided as a source of heat and a thermometer to monitor the temperature. The pen was partitioned for the five treatments and three replicates per treatment. The birds were grouped into five treatments and three replicates per treatment in a completely randomized design. The experimental layout are; T1- Synthetic antibiotic (oxytetracycline - control), T2- 10g/L solution of bay leaf powder, T3- 10g/L solution of tamarind pulp, T4 - 10g/L solution of West African pepper seeds powder, T5- 10g/L of mixture of bay leaf powder, tamarind pulp, and West African pepper seeds. The birds were brooded for 7 days and on the 8th day to 56th day experimental additives were added in their water. The birds were fed daily with measured feed and water throughout the study.

Experimental diets

The birds were offered commercial pre starter (day old - 14), starter (day 15- 24), and finisher (day 25- 56) diets in accordance with nutritional requirements of the birds at each phase of growth as presented in table 1. Feed and water were supplied *ad libitum* throughout the experimental period. The treatments involved adding bay leaf, tamarind pulp and West African pepper seed powder at 10g/L, singly and in combination at 3.33g per additive, while the control group received oxytetracycline at 1g/L following the manufacturer's recommended dose. The additives and antibiotics were added to water from day 8 to day 56.

Table 1: Composition of the commercial feeds fed to the broiler chickens

Composition	Pre starter	Starter	Finisher
Crude protein (%)	22.50	20.5	17.00
Crude fat (%)	4.00	5.00	4.00
Crude fibre (%)	5.00	5.00	5.00
Calcium (%)	0.95	0.90	0.85
Available phosphorous (%)	0.45	0.40	0.38
Metabolizable energy (kcal)	3000	3100	3000
Lysine (%)	1.30	1.15	0.90
Methionine (%)	0.58	0.52	0.39

Data collection

Haematology and serum biochemical indices

One bird was randomly selected from each replicate, 10 milliliters of blood samples were collected from the birds after feed deprivation, via the jugular vein on day 56 of experimental period for haematological and blood biochemical assays. 5 milliliters of blood was introduced into the test tube without anticoagulant for serum total protein, albumin, globulin, glucose, cholesterol, and creatinine. 5 milliliters of blood samples was introduced into the second test tube, which contain Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant and was used to analyse Haemoglobin (Hb) concentration, Packed Cell Volume (PCV), White Blood Cell (WBC) count, Red Blood Cell (RBC), Platelets, lymphocyte, neutrophil and monocytes. The blood samples were taken to the laboratory after collection in a sample holder placed in an ice container. The Red blood cells and White blood cells were measured using the improved Neubauer hemocytometer (Kelly, 1979). Wintrobe's microhaematocrit was used to determine the packed cell volume (Kelly, 1979). Haemoglobin was measured using cyanometaemoglobin techniques (Mitruka and Rawnsley, 1977). For serum biochemical profile, the blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum. Serum enzyme activities such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured spectrophotometrically following the method described by Reitman and Frankel (1957).

Sensory appraisal

At the end of the 8th week, 3 birds per treatment (with weights closer to the average weights of the respective treatments), were selected, weighed and slaughtered. Prior to slaughtering, the sampled birds were denied feed and water for 12 hours. The slaughtered birds were scalded using hot water. Breast muscle of each treatment was cut into chunks and boiled for 30 minutes, allowed to cool and then served to ten panelists for sensory assessment. A well-structured questionnaire was designed using the nine hedonic scale to assess attributes such as appearance, taste, aroma, texture and overall acceptability.

Statistical analysis

The data collected were analyzed using descriptive statistics and one-way analysis of variance (ANOVA) with the procedure of SAS (2020). Statistically, means were compared using Duncan multiple range test of the same package at 5% level of probability.

Results and Discussion

Phytochemical composition of bay leaf, tamarind and West African pepper

Table 2 presents the phytochemical composition of bay leaf, tamarind pulp and West African pepper seeds. The result showed the amount of each bioactive compounds present in the bay leaf, tamarind pulp and West African pepper seeds. Alkaloid, saponin and glycoside value observed in West African pepper seeds was the highest compared to bay leaf and tamarind pulp. Alkaloids are essential for their antimicrobial, analgesic, and properties that stimulate growth in animals (Heikens *et al*., 1995; Ezenobi *et al*., 2016). Glycosides are responsible for various metabolic and pharmacological functions (Dada *et al*., 2013; Balogun *et al*., 2016). Their presence were confirmed by Rana and Sharma (2018) for tamarind pulp and Ogbuagu (2021) for West African pepper seeds. Saponins have been shown to improve nutrient utilization and possess antimicrobial activity (Trease and Evans 2002; Isikhuemen *et al*., 2020). The presence of saponins in tamarind pulp was supported by Rana and Sharma (2018) and it was noted in West African pepper seeds by Ogunmefun *et al*. (2017). The highest value of tannin was shown in bay leaf (95.38 mg/100 g), followed by West African pepper seed (82.87 mg/100 g) and tamarind pulp (48.57 mg/100 g). Tannins have antimicrobial and antioxidant properties, and in low-moderate levels can support intestinal health. Dziri *et al*. (2020) reported tannin levels of 98.4 mg/g in bay leaf aqueous extracts. Dobroslavić *et al*. (2021) reported high phenolic and tannin

concentrations in bay leaf using green extraction techniques.

Flavonoids are known for their antioxidant, anti-inflammatory, and immunomodulatory roles (Okoye and Ebeledike, 2013). The highest value was recorded in West African pepper seeds compared to bay leaf and tamarind pulp. The presence of flavonoids in bay leaf and West African pepper seeds was also reported by Dobroslavić *et al*. (2021) and Ogunmefun *et al*. (2017) respectively. Bay leaf showed the highest phenols (134.56 mg/100 g), followed closely by West African pepper seeds (127.29 mg/100 g), and tamarind pulp (84.68 mg/100 g). Phenols are strong antioxidants that reduce oxidative stress and support immune responses. Dobroslavić *et al*. (2021) reported a phenolic content of up to 17.32 mg GAE/g in bay leaf, which is 1,732 mg/100 g higher than the one obtained in this study. The highest content of terpenoids was shown West African pepper seeds (48.51 mg/100 g), followed by bay leaf (44.25 mg/100 g), and tamarind pulp (38.28 mg/100 g). Terpenoids contains antimicrobial, antifungal, and anti-inflammatory properties. Their presence in bay leaf was also confirmed by Khodja *et al*. (2023).

Table 2: Phytochemical composition of bay leaf, tamarind pulp and West African pepper

Phytochemical(mg/100g)	Bay leaf	Tamarind	West African pepper	SEM
Alkaloid	7.28^{b}	4.36^{c}	9.92^{a}	0.80
Saponin	42.37^{b}	28.55^{c}	58.36^{a}	4.31
Tannin	95.38^{a}	48.57^{c}	82.87^{b}	6.99
Flavonoid	77.32^{b}	40.22^{c}	98.33^{a}	8.49
Glucoside	52.08^{b}	28.68^{c}	66.37^{a}	5.52
Phenol	134.56^{a}	84.68^{c}	127.29^{b}	7.79
Terpenoid	44.25^{b}	38.28^{c}	48.51^{a}	1.49

Haematological properties of broiler chickens administered aqueous solutions of bay leaf, tamarind and West African pepper as replacement for synthetic antibiotics

Table 3 presents the haematological properties of blood samples of birds administered aqueous solutions of bay leaf, tamarind pulp and West African pepper seeds. Haematological indices were influenced by the treatments in most parameters. In this study PCV value ranged from 30-34%, haemoglobin ranged from 9.60-11.30g/dl and RBC ranged from 2.78 to 3.37. All values are within reference values, PCV (22-35%), haemoglobin (7-13g/dl) and RBC ($2.5\text{-}3.5\text{x}10^{6}$) reported for healthy birds (Bonous and Stedman, 2000). PCV and Haemoglobin are critical indicators in diagnosis of anaemia (Peter *et al*., 2011) as well as bone marrow capacity to produce RBC (Chineke *et al*., 2006; Aikipitanyi and Egweh 2020). The higher value in the mixture of bay leaf, tamarind pulp and West African pepper seed group suggests the oxygen carrying capacity of the blood was enhanced by the synergistic effect of the combined additives compared to individual treatment groups. Ijoma *et al*. (2024) also observed an increase in haemoglobin and RBC when pepper and turmeric were added individually and in combination to broilers diet 5g/kg. This study aligns with Joseph (2021) who observed a significant decrease in PCV values compared to control group when tamarind pulp was administered to noiler cocks at 0,5,10 and 15g/liter. Onyemaechi *et al.* (2025) reported PCV range of 32-36%, also observed increased in the PCV values when 1g and 2g of black pepper was supplemented in broiler's diet which contradict this study.

WBC were significantly affected by the treatment. The values in this study ranged from 14.40 (Tamarind pulp group) - 16.70x10^3uL (Bay leaf group). Bay leaf group is similar to the control group, West African pepper seed group and Mixture of bay leaf, tamarind pulp and West African pepper seed group but significantly different from tamarind pulp group. Increased white blood cell counts are often associated with infection in healthy animals, it also reflects immunostimulatory activity (Alagawany *et al.,* 2021). The elevated WBC count in bay leaf group suggests an enhanced immune response. The values are within the reference (12-30x10^3ul) value in healthy birds (Bounous and Stedman, 2000). This study aligns with Alaa *et al.* (2020) who observed elevated WBC in broilers compared to control group when bay leaf was added at 1,2,3g/kg up to 5 weeks of age. This study is in agreement with Ijoma *et al.* (2024) who reported no significant difference in WBC of the treatment groups compared to control when broilers were fed 5g/kg of West African pepper seed and turmeric in their diet singly and in combination. Contrary to this study Aikipitanyi and Egweh (2020) observed significant difference in WBC of broiler chicken fed ginger and black pepper at 0.5% singly and in combination but reported a higher value 76.20 - 83.20x10^3ul than this study. The lower value in tamarind pulp group compared to the control group in this study supports Jena *et al* (2005) who observed significant decrease in WBC in birds fed diet supplemented with tamarind seed. In this study Lymphocytes values ranged from 60-80% and heterophils ranged from 23-35%. All values are within normal range, lymphocytes (45-70%), heterophils (15-50%) as reported by Jain (1993). Lymphocytes were significantly higher in the mixture of bay leaf, tamarind pulp and West African pepper group compared to other treatment groups but had the lowest heterophils value. Lymphocytes are adaptive immunity, and their increase means improved immune readiness (Gross and Siegel, 1993; Nwaigwe *et al*., 2020). The higher value suggests the combined additives provided a balanced immunomodulatory effect. Stress leads to a decrease in the number of lymphocytes and increase in the number of heterophils (Aengwanich *et al.,* 2003; Nurmeiliasari *et al.,* 2021). This finding supports Alaa *et al.* (2020) which observed a lower heterophils and lymphocytes ratio compared to the control group when bay leaf was added at 1-3g/kg in broiler diets. West African pepper seed group had the highest heterophils value. Heterophil is a stress indicator. However, the values are within normal range. This suggests no stress or inflammation. Monocytes, eosinophil and basophil are also within reference value, monocytes (2-5%), basophil (0-2%) reported by Nanbol *et al.* (2016).

Table 3: Haematological properties of broiler chickens administered aqueous solutions of bay leaf, tamarind and West African pepper as replacement for synthetic antibiotics

Parameter	T1	T2	T3	T4	T5	SEM
PCV (%)	34.00^a	32.00^{ab}	30.00^b	30.00^b	33.00^a	0.49
Haemoglobin (g/dL)	11.30^a	10.30^c	9.80^d	9.60^d	10.60^b	0.16
RBC (x10^6uL)	3.37^a	3.31^b	2.78^d	2.81^c	3.35^a	0.07
WBC (x10^3uL)	15.85^{ab}	16.70^a	14.40^b	15.10^{ab}	16.10^{ab}	0.31
Platelets (x10^4uL)	11.20^c	13.20^a	12.40^b	12.60^b	10.60^d	0.26
Lymphocytes (%)	62.00^c	65.00^b	61.00^c	60.00^c	68.00^a	0.83

Heterophils (%)	30.00[b]	28.00[b]	30.00[b]	35.00[a]	23.00[c]	1.07
Monocytes (%)	4.00	3.00	4.00	4.00	3.00	0.25
Eosinophil (%)	4.00[ab]	3.00[b]	4.00[ab]	0.00[c]	5.00[a]	0.49
Basophil (%)	0.00[b]	0.00[b]	1.00[a]	1.00[a]	1.00[a]	0.13

[abc] means with different superscripts along the rows were significantly different ($p< 0.05$)
PCV- Pack cell Volume, RBC- Red blood cell count, WBC- White blood cell count
T1- Antibiotics, T2- Bay leaf, T3- Tamarind pulp, T4- West African pepper, T5- Bay leaf, Tamarind pulp and West African pepper

Serum biochemistry of broiler chickens administered aqueous solutions of bay leaf, tamarind and West African pepper as replacement for synthetic antibiotics

Table 4 shows the serum biochemistry of blood samples of birds administered aqueous solutions of bay leaf, tamarind pulp and West African pepper. Biochemical indices indicate internal physiological processes such as protein metabolism, liver and kidney function, immune status, lipid regulation and balance. The serum biochemistry parameters were significantly ($P<0.05$) different across the treatments. In this present study, total protein of the mixture of bay leaf, tamarind pulp and West African pepper seed group was significantly higher than other treatment groups. The increased value (4.0g/dL) in mixture of bay leaf, tamarind pulp and West African pepper seed group as compared to other treatments suggest enhanced protein metabolism and nutrient absorption, this could be due to the combined phytochemical actions of each additive. This study observed a lower value compared to Obadire *et al. (*2023) who reported a higher value of total protein (6.82) in broilers feed diet supplemented with West African pepper seed and in combination with tumeric (6.64) compared to control. Makeri *et al.* (2024) also noted that at day 42 broilers fed tamarind pulp combined with avocado in their diet had higher value of total protein compared with the control group. Total protein values in bay leaf group, tamarind pulp group and West African pepper seed group were similar to control group. This supports the study of Ali (2020) who reported that adding bay leaf to broilers diets at 1 to 3g/kg for 35days, total protein were not significantly affected compared to control. This result contrasts with the study of El Gogary *et al*. (2024) who found that adding black pepper to broiler diets significantly improve total protein as compared to control group. Across all the treatment groups the total protein falls within the normal (2.58-5.22g/dL) range (Meluzzi *et al.*, 1992).

Albumin results showed significant difference across all treatments. The value ranged from (0.5-0.8) which is within the range (0.5 to 1.4g/dl) reported by Senanayake *et al.* (2015). This suggests that liver and other hepatics organs involved in protein synthesis are in normal condition. The higher value in mixture of bay leaf, tamarind pulp and West African pepper seed group compared to control group suggests a synergistic effect in the combined additives, thereby enhancing synthesis of albumin in the liver. Obadire *et al.* (2023) supplemented broilers diet wsith turmeric and West African pepper seed at 2 and 4% levels and observed no significant difference in the albumin. This could mean combining West African pepper seed with other additives can influence liver function in different ways based on the phytochemical interactions. Bay leaf group and West African pepper seed group were similar to control group. Ali (2020) reported that broiler fed diets with bay inclusion at 1-3g/kg for 35 days, their albumin level did not show significant difference compared to control. Aikpitanyi and Egweh (2020) also observed that ginger and black pepper at 0.5% level individually and in combination had no significant difference in their albumin

compared to control. Globulin is for immunity in livestock (Dairo *et al.* 2016). Values of globulin in this study showed significant difference among the treatment groups. Albumin and globulin depend on dietary protein availability (Akazu *et al.,* 2024). Globulin values of this present study across the treatment falls within the refences range 1.05-6.40g/dl. (Mitruka and Rawnsley, 1977). The increased value of globulin (3.20g/dL) in the mixture of bay leaf, tamarind pulp and West African pepper seed group compared to bay leaf group and tamarind pulp group could be due to the phytochemical present in the additive combination stimulating the bird's immune system. The reduced value (3.00g/dL) in the bay leaf group and tamarind pulp group suggest moderate bioactivity from each additive compared to the combination group. This supports Akazue *et al.* (2024) who supplemented turmeric and negro pepper in the diet of broiler and reported significant difference among the treatment groups. The globulin values showed that birds' immunity were not affected across the treatment group as they were all within the reference value. Akinfola *et al.* (2007) reported that higher globulin levels in birds supplemented with black pepper in their diets means an improved resistance to infection.

The values of AST and ALT were significantly different, AST and ALT are indicators of how liver functions and damage (Ambrosy *et al.* 2015). Liver is the center of the digestive, metabolic and productive activities (Aikpitanyi and Egweh, 2020). Ekine *et al.* (2021) reported that increased level of AST and ALT above normal level indicate liver damage. AST and ALT values in this study are within the normal range 70-220u/l (Meluzzi *et al.,* 1992) and 7 - 55u/l (Clinical diagnostic division, 1990) respectively. The higher AST and ALT value in mixture of bay leaf, tamarind pulp and West African pepper group compared to other treatments suggests strong synergistic activation of hepatic metabolism. The lower value in control group suggests minimal hepatocellular cells. Akazue *et al.* (2024) reported a significant difference but lower value than this study in AST (46.59-51.37) in broilers fed diets supplemented with turmeric and negro pepper at 1.5-4% levels at finisher stage. Makeri *et al.* (2024) study also showed increased in metabolic activities in broilers under stress when tamarind pulp pulp was added to their water. Damanhouri and Ahmad (2014) observed that the inclusion of black pepper in broiler diets enhanced activity of the liver. ALP values in this study were significantly different among treatment groups. ALP values ranged from 229 (control group) - 327u/l (Mixture of bay leaf, tamarind pulp and West African pepper group). ALP is an enzyme found in the liver, bones kidney and intestine; but intestinal ALP does not contribute much to the ALP levels in the serum (Hoffman *et al*., 2008). A reduction in ALP activity could be as a result of slow bone growth (Szabo *et al.,* 2005). ALP values in control group, bay leaf group, tamarind pulp group and West African pepper seed group are within the reference values of 167-305u/l for healthy birds (Oleforuh Okokeh *et al.,* 2015) except the mixture of bay leaf, tamarind pulp and West African pepper group (327). The higher value in the mixture of bay leaf, tamarind pulp and West African pepper group compared to other treatments suggests that the combined additives could have induced hepatic stress or stimulated bone increased since ALT and AST are within normal range, this suggests no actual liver damage. This study contradicts Obadire *et al.* (2023) study who reported no significant difference in ALP, AST and ALT when broilers diet was supplemented with turmeric and West African pepper seed at 0-4% individually and in combination. Creatinine is a by product of creatinine phosphate metabolism that is exerted by the kidney, it is produced as result of energy production by the skeletal muscles (Esubonteng, 2011). Creatine is a biochemical marker for the diagnosis of renal (Akande *et al*., 2013) creatine value in mixture of bay leaf, tamarind pulp and West African pepper group differed significantly from other treatments. Glucose values were significantly different

across the treatment which means each additives influence the birds in different ways, the value ranged from 205-306mg/dl which falls within the normal (200-500mg/dl) range for healthy broilers (Thrall, 2007) but higher than the reference value of 180-250mg/dl reported by (Sturkie, 1986; Hazelwood, 2000; Hernawan *et al.,* 2012). The increased value in the mixture of bay leaf, tamarind pulp and West African pepper group could be due to mild stress from the additives combined. The lower value in bay leaf group compared to other treatments could be due to an increase in proteins that are not associated with glutathione. Bay leaf has been reported to improve insulin function (Khan *et al.,* 2017). Thakare (2004) noted that decrease in glucose concentration with the use of bay leaf might be as a result of increase in protein that are not associated with glutathione but contribute to metabolism of other nutrients in the blood of the birds. This study is in support with Ali (2020) who reported lower values of glucose compared to control when bay leaf was supplemented up to 3g/kg in broiler diet. West African pepper seed group were similar to control group. This study aligns with Aikpitanyi and Egweh (2020) who reported a significant difference in glucose ranged from 201-220mg/dl in broilers fed supplemented diets of black pepper and ginger, singly and in combination at 0.5% level. Cholesterol is an indicator for lipid metabolism. In this study cholesterol values range from 105-153mg/dl and all are within the normal reference range (87-192mg/dl) of healthy broilers (Meluzzi *et al.,* 1992). The higher value in mixture of bay leaf, tamarind pulp and West African pepper group compared to control group could be attributed to synergistic effect from the combined additives. Piperine in West African pepper seed is known as a bio enhancer when added to other nutrients (Singh *et al.,* 2010). The higher value suggests increased lipid metabolism rather than lipid disorder, as it is still within the normal range for healthy broilers. Birds in bay leaf group, tamarind pulp group and West African pepper seed group were significantly different. Cholesterol value in bay leaf group was higher compared to control group. Bay leaf is known to reduce liver enzymes that form fatty acids (Musa *et al.,* 2011, Titilayo *et al.,* 2018). Ali (2020) reported a higher value of cholesterol (154.06-170.3) in broilers fed diet supplemented with 1-3g/kg of bay leaf up to 35 days of age and also observed a reduced cholesterol values compared to control group which contradicts this study. The higher value of cholesterol (119mg/dL) in bay leaf group compared to control (105mg/dL) could be due to the mode of application(water). Tamarind pulp group and West African pepper seed group also showed a higher value in cholesterol compared to the control group. This result contradicts with Aikpitanyi and Egweh (2020) who reported that ginger and black pepper combination at 0.25+0.25% level suppressed cholesterol. Since all values are within normal range, across the treatment groups, this indicates the bird's metabolism was stable.

Table 4 Serum biochemistry of broiler chickens administered aqueous solutions of bay leaf, tamarind and West African pepper as replacement for synthetic antibiotics

Parameter	T1	T2	T3	T4	T5	SEM
TP (g/dL)	3.60^{b}	3.60^{b}	3.70^{b}	3.70^{b}	4.0^{a}	0.04
ALB (g/dL)	0.50^{c}	0.60^{bc}	0.70^{ab}	0.60^{bc}	0.80^{a}	0.03
GLO (g/dL)	3.10^{ab}	3.00^{b}	3.00^{b}	3.10^{ab}	3.20^{a}	0.03
AST (u/L)	177.00^{c}	179.00^{c}	181.00^{b}	180.00^{bc}	194.00^{a}	1.63
ALT (u/L)	19.00^{d}	20.00^{cd}	21.00^{bc}	22.00^{ab}	23.00^{a}	0.44

ALP (u/L)	229.00^{e}	233.00^{d}	236.00^{c}	249.00^{b}	327.00^{a}	9.82
Creatinine (mg/dL)	0.40^{c}	0.50^{b}	0.50^{b}	0.50^{b}	0.60^{a}	0.02
Glucose (mg/dL)	207.00^{c}	205.00^{d}	210.00^{b}	208.00^{c}	306.00^{a}	10.54
Cholesterol (mg/dL)	105.00^{e}	119.00^{b}	108.00^{d}	113.00^{c}	153.00^{a}	4.65

^{abc} means with different superscripts along the rows were significantly different ($p< 0.05$)

TP- Total Protein, ALB- Albumin, GLO- Globulin, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, ALP- Alkaline Phosphate

T1- Antibiotics, T2- Bay leaf, T3- Tamarind pulp, T4- West African pepper, T5- Bay leaf, Tamarind pulp and West African pepper

Organoleptic properties of broiler chickens administered aqueous solutions of bay leaf, tamarind and West African pepper as replacement for synthetic antibiotics

Table 5 shows the organoleptic properties of birds administered aqueous solutions of bay leaf, tamarind and West African pepper. Sensory attributes of broiler chicken give insight into consumer preference and meat quality. Results of organoleptic properties of broiler meat showed that all the treatment groups including the control group had similar effects on the appearance, tastes, aroma, texture and overall acceptability. Prasetyo *et al.* (2024) reported no significant difference in organoleptic properties when broiler thigh meat was braised with 20%, 25% and 30% of indigenous herbs and spices (ginger and garlic). Alagbe (2023) reported that the colour, flavour, tenderness and overall acceptability of broiler meats were influenced when phytogenic additive (Prosopis Africana oil) were included at 200, 600, 800mg/kg. Contrary to this study, in quail, Al rubaee (2018) study reported significant differences and noted that sensory traits of breast meat showed that 3% bay leaf powder recorded the best, taste, tenderness and acceptability than other treatments.

Table 5 Organoleptic properties of broiler chickens administered aqueous solutions of bay leaf, tamarind and West African pepper as replacement for synthetic antibiotics

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Appearance	6.40	6.70	7.10	6.40	6.10	0.23
Taste	6.40	6.50	7.10	6.90	5.70	0.21
Aroma	6.90	6.80	6.80	6.50	6.40	0.20
Texture	7.10	7.20	7.50	7.10	6.40	0.19
Acceptability	6.90	6.70	7.00	6.70	6.20	0.20

T1- Antibiotics, T2- Bay leaf, T3- Tamarind pulp, T4- West African pepper, T5- Bay leaf, Tamarind pulp and West African pepper

Conclusion and Recommendation

The biochemical parameters measured though significantly different across the treatments are within normal range for healthy broiler chicken. Mixture of bay leaf, tamarind pulp and west african pepper seed improved serum protein, albumin and globulin. Bay leaf reduce glucose level compared to other treatments. Tamarind pulp reduced WBC, Heterophils level was lowered in birds given mixture of bay leaf, tamarind pulp and west african pepper seed compared to other treatments. Further studies

are recommended to be carried out to evaluate the long term effect of the mixture of bay leaf, tamarind pulp and west african pepper seed at a lower dose.

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Date received: 23rd June, 2025

Date accepted: 18th October, 2025