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EFFECT OF *Tetrapleura tetraptera* ON BLOOD PROFILES AND NUTRIENT DIGESTIBILITY COEFFICIENT OF BROILER CHICKENS

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ABSTRACT

This study was conducted out of the quest to promote fast growth and better performance in livestock and aimed at determining the effect of Tetrapleura tetraptera on blood profiles and nutrient digestibility coefficient of broiler chickens. One hundred and sixty day-old (Abor acre) strains of broiler chickens were used for the study. Two diets were formulated, one at the starter phase and the other at the finisher phase. The diets were divided into four portions and labelled T1, T2, T3, and T4. T1 (Control) had no Tetrapleura tetraptera fruit powder (TTFP), while T2, T3, and T4 contained 300, 400, and 500 g of TTP per 100 kg feed, respectively. The birds were allocated into four groups of 40, each containing four replicates of 10 birds. Treatment diets were randomly assigned in a completely randomized design, and the birds had access to feed and water ad libitum. The research lasted 56 days. All the data collected were statistically analyzed. The result of the haematological assay showed that the diets did not have any significant (P>0.05) influence on the birds. No significant (P>0.05) differences were observed in the values of serum biochemical parameters analyzed except cholesterol and triglyceride. Birds in T1 recorded the highest significant values for the two parameters compared to other treatments. The nutrient digestibility in all the parameters measured (crude protein, crude fibre, ether extract, and ash) indicated significant differences (P≤0.05) across treatments. However, birds in the in T4 group recorded higher digestibility values in all parameters accessed compared to other treatments. It was therefore concluded that Tetraptera tetraptera fruit powder could be incorporated into a broiler diet of up to 500 g as a feed additive to enhance its blood profiles and nutrient digestibility.

Keywords: Broiler chicken, Cholesterol, Serum protein, *Tetrapleura tetraptera*, and Triglyceride.

INTRODUCTION

Over the years, antibiotics have been widely employed as feed additives for enhanced livestock and poultry performance (Okon *et al.,* 2023a). Regrettably, their utilization has been associated with various adverse effects, encompassing microbial drug resistance, liver injury, teeth discolouration, and gastrointestinal disorders in both humans and animals (Jing*et al*., 2009; Isikwenu and Udomah, 2015; Essien *et al.,* 2023). The global ban on this product (Cardozo *et al*., 2004; Okon *et al*., 2023c) has prompted feed producers worldwide to adopt healthier, plant-based alternatives called phytobiotics, minimizing side effects. Phytobiotics include spices and herbs from plant origin. They can be incorporated into animal diets to boost their performance and improve the quality of their products, such as meat and eggs (Windisch *et al*., 2008; Okon*et al.,* 2022). These natural herbs and spices are readily available, cheaper, and have no side effects. They could come from leaves, bulbs, rhizomes, seeds, and fruit, and they have been used in animal diets with promising results. Examples of such known spices include *Xylopia aethiopica* (Solomon *et al*., 2022), *Monodora myristica* (Okon *et al*., 2023b), turmeric and clove (Ayodele *et al.*, 2021), *Ocimum gratissimum* (Essien and Udoh, 2021). However, these herbs and spices have been reported to contain an array of bioactive compounds such as *saponin, alkaloid, tannin, glycoside, phenols, and terpenoids which* could be responsible for their nutritional and pharmacological activities (Okwu, 2003; Essien *et al*., 2022).

Tetrapleura tetraptera, commonly known as Aridan, Uho in Igbo, and Uyayak in Efik and Ibibio, is a lowland forest plant in tropical Africa. The plant is highly sought because of its nutritional and medicinal properties. The tree grows to approximately 25 meters in height. The fruit is brownish, and the pod contains tiny hard seeds that measure up to 8mm long. It is used in many Nigerian cuisines because of its strong but pleasant aromatic fragrances (Orwa *et al*., 2009). Aladesanmi (2007) reported that the fruit could control and prevent many ailments, such as diabetes, arthritis, inflammation, hypertension, convulsion, Jaundice, rheumatism, and malaria. The proximate composition of *T. tetraptera* reveals its possession of crude protein (7.51 %), crude fibre (10.33 %), ether extract (4.11 %), ash (6.14 %), nitrogen-free extract (61.11 %), and a cocktail of minerals which include iron, calcium, magnesium potassium and zinc (Okwu, 2003; Essien, 2021). However, information on the value of *T. tetraptera* as a feed

additive is limited; hence, this research was conducted to assess the effect of *T. tetraptera* on nutrient digestibility and blood profiles of broiler chickens.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, Akwa Ibom State University, Obio Akpa Campus, Nigeria. Obio Akpa lies between latitudes 5017 and 50171North of the equator and longitudes 70271 and 71581east with a temperature of 25oC annual rainfall ranging from 3500 to 5000mm and a relative humidity of between 60 and 90 % (SLUS-AK, 1994).

Sourcing and Processing of Tested Material

Tetrapleura tretraptera fruits used in this research were purchased from Abak market, Abak Local Government area of Ibom State. The fruit was sliced into small pieces to aid in the drying process, and it was left to sun-dry for seven days. Subsequently, the dried fruit was manually blended into a powder using a hand-operated blender (Corona 1016, Landersy Y. CIA, South Africa).

Proximate and Phytochemical Composition of *Tetrapleura tetraptera*

A sample of the ground *T. **tetraptera*** was subjected to proximate analysis for the determination of crude protein ether extract, crude fibre ash, and moisture using the guide provided by AOAC (1995), while the phytochemical assay was adapted from the studies of (Dosunmu, 1997; Ojewole and Adewunmi, 2004).

Experimental Diets

Two experimental diets were specifically formulated for the starter and finisher phases of the study. Each diet was divided into four portions and labelled T1, T2, T3, and T4. T1(control) had no *T. tetraptera* powder, while T2, T3, and T4 contained 300, 400, and 500 g of *T. **tetraptera*** powder per 100 kg feed, respectively. Table 1 represents the ingredients and nutrient composition of the experimental diets.

Management of Experimental Birds and Design

A total of one hundred and sixty (Abor acre) day-old chicks were used for the research. The birds were purchased from a reputable poultry distributor at Abak, Abak Local Government

Area of Akwa Ibom State. On arrival, the birds were weighed equally and divided into four groups of forty birds. Each group was randomly assigned to one of the treatment diets using a completely randomized design (CRD). Each group was further divided into four replicates of ten birds per replicate housed in a pen measuring 2 m × 2 m. The experiment followed standard brooding procedures, providing feed and water *ad libitum*. In the fifth week, the diet was switched to broiler finisher mash. Routine medication and vaccines were administered as scheduled. Biosecurity measures and other management practices were adhered to throughout the 56-day duration of the experiment.

Table 1: Gross Composition of Experimental Diet

Ingredient	Starter Phase	Finisher Phase
Yellow maize	50.00	55.00
Soya bean meal	25.00	20.00
Blood meal	3.00	3.00
Fish meal	3.00	3.00
Palm kernel cake	5.00	5.00
Wheat offal	9.00	9.00
Bone meal	4.00	4.00
Common salt	0.25	0.25
Premix	0.25	0.25
Lysine	0.25	0.25
Methionine	0.25	0.25
Total	100.00	100.00
Calculated chemical composition		
Crude protein	22.92	21.37
Ether extract	4.09	4.32
Crude fibre	3.15	3.96
Ash	3.57	5.25
NFE	66.27	65.10
ME (Kcal/kg	2879.85	2911.02

Blood Collection and Analysis

On the 56th day, 10 ml of blood samples were drawn from two birds per replicate through the wing vein into different bottles for haematological and serum biochemical indices. From the 10 ml collected, 5 ml was transferred into labelled and sterilized bottles containing ethylene diamine tetraacetic acid (EDTA) with anticoagulant for haematological assay. Haemoglobin concentration (Hb), red blood cell (RBC), and white blood cell (WBC) counts were determined by the Cyanmethemoglobin method as described by Coles (1986). Mean corpuscular volume (MCV), mean corpuscular Haemoglobin (MCH), and mean corpuscular Haemoglobin

concentration (MCHC) were calculated according to the formula described by Sharma (1990) as shown below:

MCV (%) = PCV × 10/RBC

MCH (%) = HB × 10/RBC

MCHC (%) = HB × 100/PCV

The remaining 5ml of the blood sample was transferred into anticoagulant-free bottles and was used to determine blood biochemical components, which include total protein, albumin globulin, cholesterol, and triglyceride; serum enzymes aspartate aminotransferase (AST), Alanine amino transaminase (ALT) and alkaline phosphatase (ALP) using Cobas Mira Automatic Analyzer (Roche Diagnostic system, Basel Switzerland) with the aid of commercial kits (Lab Test Diagnostica Lagoa Santa, MG Brazil) samples reading were performed using spectrophotometer (Lasany single Beam visible spectrophotometer (L1 – 720), Lasany International Panchkula) with light wavelength adequate for each parameter.

Nutrient Digestibility Coefficient

A nutrient digestibility trial was conducted on the 49th day of the experiment. Two birds per replicate were randomly selected and housed separately in individual metabolic cages for seven days. A known quantity of feed was given to the birds. Polyethylene was placed under the cage for easy collection of faeces. The birds were allowed to acclimatize for three days before their excreta were collected. Faeces collected per bird per day were oven-dried at 85 0C until a constant weight was obtained. The faeces were bulked according to treatment and taken to the laboratory for proximate composition analysis.

$$\text{Nutrient digestibility coefficient} = \frac{\text{Nutrient in feed consumed} - \text{Nutrient in faeces voided} \times 100}{\text{Nutrients in feed consumed}}$$

Data Analysis

Data collected from various parameters measured were subjected to a one-way analysis of variance according to Steel and Torrie (1980). The analysis of variance indicated significant treatment effects, and the means were compared using Duncan's New Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The proximate and phytochemical composition of *Tetrapleura tetraptera*is is presented in Table 2. The protein, fat, fibre, and ash values were comparable to values (7.80 %, 4.45 %, 18.56 %, and 9.42 %), respectively, as reported by Olubunmi (2013) except for Nitrogen-free extract and dry matter, which was comparable to N'zibo *et al*. (2019). The plant also contains

phytochemicals, including tannin, saponin, flavonoid, alkaloid, and phenol. Phenols were the highest constituents, followed by flavonoids, tannins, saponins, and alkaloids. Studies have detected these phytochemicals as medicinal, antioxidant, anti-inflammatory, and antimicrobial properties (Yadav and Agarwala, 2011). The values reported in this study were comparable to those reported by Adusei *et al*. (2019) but lower than those reported by Igwe and Akabuike (2016). These values may be influenced by the soil characteristics and climatic conditions where the plant was cultivated, genetics, and analytical procedure variation (Okon *et al*., 2022). The results of the haematological parameters determined in this research are presented in Table 3. The results revealed that the diet did not significantly($P>0.05$) affect all the haematological parameters analyzed. Numerous studies have outlined typical haematological parameters in avian species. Mitruka and Rawnsley (1977) and Campbell (2013) documented the following normal ranges for haematological measures in broiler chickens: Packed cell volume (35.9-41.0 %), haemoglobin (11.60-13.68 g/dl), red blood cell (2.21-4.84 × 106 /ml), white blood cell (4.07-4.32 × 103 /ml), mean corpuscular volume (81.60-89.10 FI), mean corpuscular haemoglobin (27.20-28.90pg) and mean corpuscular haemoglobin concentration (32.41-33.37 %). The values observed in this study aligned to a great extent with the normal ranges previously reported by the same author.

The diet did not negatively influence the birds' red blood cell (RBC). The red blood cell values obtained in this research did not vary between the experimental diet and the control, indicating that including *T. tetraptera* powder did not alter its normal functions. No significant increase ($P>0.05$) was observed in the value of packed cell volume (PCV) and Haemoglobin count (Hb) of the birds across treatment. Blood haematological parameters help evaluate the animal's physiological state (Etim *et al*., 2013). Ekpo and Okon (2023) reported that RBC, Hb, and PCV are sensitive to dietary type and quality.

Also, Adejumo (2004) indicated that there was a correlation between PCV, Hb, and the quality of the diet. Packed cell volume (PCV) is known as an index for blood toxicity. Its abnormal level could suggest the presence of toxic factors in the diet (Oyawoye and Ogunkunle, 1998). Low PCV usually portrays iron deficiency. The result showed the absence of toxic substances, an adequate level of iron in the feed, and the ability of the birds to perform normal erythropoietin activity.

The white blood cells (WBC) of the birds were not significantly ($P>0.05$) influenced by the diet. White blood cells play a major role in fighting against disease-causing micro-organisms

in the body, such as bacteria, fungi, and viruses (Britannia, 2020). Therefore, the non-significance in values of the birds could suggest an enhanced immune system. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin count (MCHC) of the birds were not affected by the diet. These parameters are used to measure or evaluate anaemic conditions in humans and animals. However, all the haematological parameters analyzed fell within the normal range reported by Abdulazeez*et al*. (2016). The results of the serum biochemical composition of the birds are presented in Table 4. The diets across treatments did not significantly ($P>0.05$) affect serum total protein, albumin, and globulin. Total protein measures the amount of protein in the blood and its balance in amino acid levels or profile. Albumin is produced in the liver, and it helps to prevent the blood from leaking out of blood vessels, while globulin binds with haemoglobin to transport iron in the blood. High levels of globulin indicate liver damage, chronic infection, and kidney dysfunction, while a low level of albumin depicts poor health conditions in animals. This result revealed the absence of disease conditions in the experimental birds and adequate protein levels in the feed. Total protein, albumin, and globulin values reported in this study fell within the normal ranges (2.50-5.50 g/dl; 2.10-3.45 g/dl; 2.13-3.02 g/dl) reported by Howlett (2000), Mitruka and Rawnsley (1977), and Adeyemo and Sani (2013), respectively.

The liver enzymes aspartate aminotransferase (AST), Alanine transaminase (ALT), and alkaline phosphatase (ALP), though present in negligible concentrations, are important as they help in the determination of the proper functioning of the liver of animals. These enzymes were not affected by the diet. The values reported in this study were within the normal range reported by Meluzzo *et al*. (1992), suggesting that the liver of the birds functioned optimally. The liver is the centre of all bodily metabolic activities and is subject to chemical and biological damage (Aikpitanyi and Egweh, 2020). These damages are made obvious by the levels of these enzymes. Increased levels of these enzymes are associated with liver damage, which could result from toxic substances in feed. Therefore, the similarities in values between the control and the rest of the group indicate normal or proper functioning of the enzymes and could suggest an absence of toxic substances in the feed. The result of this study agrees with the findings of Emadi and Kermanshali (2007), who reported non-significant AST, ALP, and ALT values in broilers fed turmeric powder.

Significant ($P\leq0.05$) variations were observed in the value of cholesterol and triglyceride. Triglycerides are synthesized in the liver from fatty acids, protein, and glucose when they are

above the body's current needs and then stored in adipose tissues (Esubonteng, 2011). Values reported in this study fell within the normal range (87 – 192 mg/dl) reported by Meluzzi *et al.* (1992). Birds in T4 had the lowest cholesterol and triglyceride values. Their values decreased significantly with an increase in the test ingredient or feed additive level. The lower values obtained for the two parameters could suggest the antioxidant effect of *T. tetraptera*. The spice has been reported to possess antioxidant properties which inhibit lipid peroxidation. The results align with other researchers' reports on other spices and herbs (Ekpo and Okon, 2022; Okon *et al*., 2023c). Studies by Ekine *et al*. (2020) and Ayodele *et al*. (2016) recorded significant decreases in triglyceride and cholesterol values of broilers fed ginger, black pepper, turmeric, and clove. These findings confirmed the antilipidemic and hypolipidemic properties of *the Tetrapleura tetraptera* fruit.

Table 2: Proximate and Phytochemical Composition of *Tetrapleura tetraptera*

Parameter	TTFP
Crude protein	7.51
Crude fibre	10.13
Ether extract	4.11
Ash	6.14
NFE	61.11
Dry matter	89.0
Anti-nutrient (mg/100g)	
Tannin	1.82
Saponin	1.63
Flavonoid	2.57
Alkaloid	1.30
Phenol	3.21

TTFP = *Tetrapleura tetraptera fruit powder*

Table 3: Haematological Indices of Broiler Chickens fed *Tetrapleura tetraptera* Fruit Powder

Parameter	T_1 (0 gTTFP)	T_2 (300 gTTFP)	T_3 (400 gTTFP)	T_4 (500 gTTFP)	SEM
Red blood cell ($x10^6$/NL)	2.55	2.51	2.62	2.58	0.04
Haemoglobin (g/dl)	13.01	12.54	12.51	13.32	0.03
PCV (%)	35.78	35.81	36.23	36.34	0.03
White blood cell ($X10^3$/u)	9.31	9.05	9.34	9.23	0.01
MCV (fl)	91.31	90.55	91.14	91.20	0.01
MCH (pg)	37.01	36.52	37.13	36.55	0.02
MCHC (g/dl)	34.00	33.56	33.57	34.11	0.01

SEM = Standard Error of the mean, MCV = mean corpuscular volume, MCH = mean corpuscular Haemoglobin, MCHC = mean corpuscular haemoglobin concentration, PCV = Packed cell volume, TTFP = *Tetrapleura tetraptera* fruit powder

Table 4: Serum Biochemical Indices of Broiler Chickens fed *Tetrapleura tetraptera* Fruit Powder

Parameter	T_1 (0 gTTFP)	T_2 (300 gTTFP)	T_3 (400 gTTFP)	T_4 (500 gTTFP)	SEM
Total protein (g/dl)	5.15	5.07	5.13	5.13	0.03
Albumin (g/dl)	2.84	2.67	2.41	2.52	0.01
Globulin (g/dl)	2.31	2.40	2.82	2.51	0.01
AST (u/l)	80.12	80.31	80.43	80.11	0.01
ALT (u/l)	24.51	24.62	25.01	25.10	0.03
ALP (u/l)	35.10	34.71	35.23	34.81	0.03
Cholesterol (mg/dl)	140.31^a	121.44^b	117.03^b	91.02^c	1.34
Triglyceride (mg/dl	150.51^a	138.56^b	111.53^c	90.20^d	1.21

abcd: means along rows with different superscripts are significantly (P<0.05) different.

SEM = Standard Error of Mean, ALT = Alamine transaminase, ALP = Alkaline phosphatase, AST = Aspartate amino transferase, TTFP = *Tetrapleura tetraptera* fruit powder

Table 5: Apparent Nutrient Digestibility of Broiler Chickens Fed *Tetrapleura Tetraptera* Fruit Powder

Parameter	T_1 (0 gTTFP)	T_2 (300 gTTFP)	T_3 (400 gTTFP)	T_4 (500 gTTFP)	SEM
Dry matter	80.56^d	82.61^c	84.31^b	85.67^a	0.03
Crude protein	70.13^d	75.65^c	78.02^b	80.21^a	0.10
Crude fibre	60.78^d	62.41^c	63.10^b	65.37^a	0.02
Ether extract	75.61^d	76.37^c	77.59^b	80.11^a	0.01
Ash	82.11^d	83.92^c	85.37^b	88.53^a	0.01
Nitrogen free extract	71.53^d	73.41^c	74.51^b	76.29^a	0.03

abcd = means along rows with different superscripts are significantly (P<0.05) different.

SEM = Standard Error of Mean

TTFP = *Tetrapleura tetraptera* fruit powder

The result of the nutrient digestibility coefficient of the birds, as shown in Table 5, indicated significant (P≤0.05) differences in all the parameters across treatments. Digestibility was higher in T4 than in the rest of the group. Digestibility increased with the test material levels (*T. tetraptera*). Spices and herbal products are useful to humans and animals because of their nutritional and medicinal properties (Czarra, 2009). They act directly against microorganisms in the gastrointestinal tract, creating an enabling environment for protein and energy digestion, absorption, and utilization (Frankic *et al*., 2009). The birds effectively utilized the crude protein, fibre, ether extract, and ash contents of the broiler feed. The birds of T4 recorded the highest significant values, followed by T3, T2, and T1. Spices also increase the secretion of endogenous enzymes that help indigestion.

CONCLUSION AND RECOMMENDATIONS

Based on the results obtained in this research, Tetrapleura *tetraptera* could be incorporated into broilers' diets at 500 g/100kg feed for normal haematological and serum biochemical indices and to promote nutrient digestibility in broiler chickens.

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