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Research Article

The Phytogenic Effects of *Xylopia aethiopica* and *Tetrapleura tetraptera* Fruit Meals on Serum Biochemical, Antioxidant and Oxidative Profiles of Layer Chickens Following Outbreak of Gumboro Disease

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Abstract

The effect of Xylopia aethiopica and Tetrapleura tetraptera fruit meals on blood serum biochemistry and oxidative and antioxidant parameters of layer chickens following an outbreak of infectious bursal disease in layer chickens were investigated. A total of four hundred and fifty (450) day old Isa Brown pullet chicks procured from 'Agrited' were used for the study. The birds were vaccinated against Newcastle disease on arrival and infectious bursal disease vaccine (IBDV) on the 10th day. On day 18 before the second dose of IBDV could be given, high morbidity and a few mortalities were observed in the flock. Clinical signs and post mortem examination revealed IBD outbreak. Supportive therapy was instituted and by day 24 (6 days of clinical disease), the birds stopped dying. By then, a total of 138 birds (30.67 %) had died leaving a total of 300 birds which were then used for the study. The birds were randomly allotted into 10 treatments groups of 3 replicates each containing 10 chicks. Treatment 1 was the control, while T2, T3 and T4 were fed diets supplemented with 0.5, 1.5 and 2.5g of X. aethiopica fruit meal per kg of feed. Treatment 5, T6 and T7 were fed diets supplemented with 0.5, 1.5 and 2.5g of T. tetraptera fruit meal per kg of feed, while T8, T9 and T10 were fed diets supplemented with a combination of 0.5, 1.5 and 2.5g of X.

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aethiopica and T. tetraptera fruit meal per kg of feed. At the end of 50 and 73 weeks of age (woa), three birds were selected for serum biochemical and antioxidant studies. Data collected were subjected to analysis of variance (ANOVA) and separation of means was made using Duncan's Multiple Range Test. The results showed significantly higher serum total protein in the treated groups; while serum cholesterol and triglyceride levels were lower in the treated groups than the control.

Keywords: Serum biochemistry, antioxidant, phytochemical, *X. aethiopica, T. tetraptera,* layers

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Introduction

Interest in the role of alternative medicines for the treatment of acute and chronic diseases has been on the increase (Chengbo *et al.*, 2015). Of particular interest to researchers are the anti-inflammatory and antioxidant properties of botanicals and various classes of phytochemicals. Acceptance of plants and vegetables used in traditional medicine as sources of prophylactic and chemotherapeutic drugs is on the increase (Hannah *et al.*, 2016).

Phytogenics are defined as compounds of plant origin incorporated into animal feeds to improve livestock productivity through the enhancement of digestion, nutrient absorption and suppression of pathogens present in the gastrointestinal tract (Hafeez *et al*., 2016). They are extracts of plants derived from herbs and spices, which have profound effects on animal health and production. The use of phytogenics results in improved feed intake, nutrient digestion, body weight gain, and feed conversion ratio (Reisinger *et al.*, 2011; Hafeez *et al.*, 2016), reduce incidence of diarrhea, and improved reproductive performance, feed efficiency and profitability (Kim and Kang, 2022). They act as metabolic enhancers, stimulating the production of endogenous digestive enzymes (Applegate *et al.*, 2010; Shires *et al.*, 2012). Consequently, phytogenics have gained traction in the feed industry and to an increasing extent, producers are incorporating them into their feeding programs (Steiner, 2010). The bio-active compounds contained in phytogenics include alkaloids, flavonoids, bitters, saponins, glycosides, mucilage, tannins, phenolics, polyphenols, terpenoids, polypeptides, thymol, cineole, linalool, piperine, allicin, capsaicin, allylisothiocyanate, and anethole (Ilusanya *et al.*, 2012;

Upadhaya and Kim, 2017). Due to variability in performance results from animal studies evaluating phytogenic feed additives (PFA), and limited understanding of their modes of action, there is limited application of phytogenics as feed additives (Yang *et al.*, 2015). The future of phytogenic feed additives will hence depend on the characterization of the materials to gain insight into their major and minor constituents, mode of action and value in production and welfare of animals (Upadhaya and Kim, 2017).

Many of the naturally occurring antioxidants have been characterized, and are available for use as prophylactic and therapeutic agents to inhibit the adverse effects of ROS (Weiss and Landauer, 2003). Compounds rich in antioxidant activities has the ability to alleviate oxidative stress when consumed (Ganesan *et al.*, 2011; Pisoschi *et al.*, 2016). Natural products containing at least 0.1% of antioxidants can be accepted as dietary supplements with antioxidant properties (Flieger *et al.*, 2021). Antioxidants are widely used in foods, especially meat and meat products because they protect lipids and proteins from oxidative degradation (Lara *et al.*, 2011; Hoffman *et al.*, 2014; Longato *et al.*, 2017).

Early attempts to enhance the feeding value of feed stuffs by chickens include the addition of antibiotic growth promoters (AGP) to enhance intestinal health and function and hence the performance of chickens. However, issues regarding antimicrobial resistance, as well as, antimicrobial residues in meat and eggs have led to the ban on the use of AGP in chicken production, necessitating the evaluation of other feed additives such as prebiotics, probiotics, and synbiotics believed to exert beneficial effects on health and performance of chickens. Most diseases of chickens adversely affect health, egg production and quality. Viruses have the most dominant immunosuppressive effect on chickens. These viruses include *chicken infectious anaemia virus* (CIAV), *reovirus, adenovirus, avian leukosis* virus (ALV), *marek's disease virus* (MDV), *reticuloendotheliosis virus* (REV), and *infectious bursal disease* virus (IBDV) (Schat *et al.*, 2014). The commercial feed additives are however, not readily accessible and affordable to the small-scale and resource poor farmers who constitute a greater percentage of chicken farmers in Nigeria. Consequently, there is need to evaluate locally available phytogenic materials believed to exert beneficial effects on intestinal health and function, growth and reproduction.

Xylopia aethiopica and *Tetrapleura tetraptera* are locally available plant spices which have been used in folklore medicine in the management of convulsion, leprosy, stomach ache, rheumatoid pains, cough, lumbargo, neuralgia and colic pain (Woode *et al.*, 2013). There is however, dearth of information on the possible value of *X. aethiopica* and *T. tetraptera* on health and performance of layer chickens.

Consequently, the objectives of this research were to evaluate the effects of dietary levels of *X. aethiopica* and *T. tetraptera* fruit meals and their combinations on blood serum

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biochemistry and antioxidant parameters of layer chickens affected by an outbreak of infectious bursal disease.

Materials and Methods

Location of study

The study was carried out at the Teaching and Research Farm of the College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike. Umudike is located between latitude 05^{o} 29^{I} north and longitudes 07^{o} 23^{I} east and at an altitude of 122.0m above sea level (Akpan and Osodeke, 2015).

Preparation of test materials

Riped fruits of *X. aethiopica* and *T. tetraptera* were purchased from Orie-Ugba a local market in Umuahia North Local government area of Abia State, Nigeria. The fruits were properly identified by Dr. Nwajiobi Benson of Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike. Thereafter, the materials were washed, rinsed and dried to constant weight under shed, ground to powder with an electric grinder, and stored in air tight containers at 4^{o}C until used. A sample of each test material was taken to the laboratory for proximate and phytochemical analysis.

Experimental chicks

The study was carried out using 300 twenty-four days old layer *Isa Brown* pullet chicks that survived an IBD outbreak. The chicks were originally 450 in number and were purchased at day old from Agrited Nigerian Limited, a reputable hatchery in Ibadan, Oyo State, Nigeria. The chicks were housed on a deep litter brooding pen with kerosene stove and electric bulbs as sources of heat. They were fed chick mash and water ad libitum and were vaccinated against Newcastle disease (NCD) at day old using NCD vaccine intra-ocular and against IBD on day 10 of age using IBD vaccine administered through drinking water. Eight days post IBDV administration (18 days of age), high morbidity and a few mortalities were observed in the flock. Clinical signs and post mortem examination revealed infectious bursal disease outbreak. Mortality ceased after six days of onset of clinical disease by which time a total of 138 birds (30.67 %) had died from the outbreak.

Experimental design

The 300 IBD surviving pullet chicks were randomly allocated to 10 experimental groups of 30 chicks per group with each group shared into three replicates containing 10 chicks each. The study was a 2 x 3 factorial in completely randomized design. That is, two phytogenic additives each at three levels of dietary inclusion namely, 0.5, 1.5 and 2.5 g/kg

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feed. The experimental groups were as follows:
Treatment 1 or Control: Basal diet (no dietary supplementation)
Treatment 2: 0.5 g *X. aethiopica* per kg basal diet
Treatment 3: 1.5 g *X. aethiopica* per kg basal diet
Treatment 4: 2.5 g *X. aethiopica* per kg basal diet
Treatment 5: 0.5 g *T. tetraptera* per kg basal diet
Treatment 6: 1.5 g *T. tetraptera* per kg basal diet
Treatment 7: 2.5 g *T. tetraptera* per kg basal diet
Treatment 8: 0.5 g *X. aethiopica* + 0.5 g *T. tetraptera* per kg basal diet
Treatment 9: 1.5 g *X. aethiopica* + 1.5 g *T. tetraptera* per kg basal diet
Treatment 10: 2.5 g *X. aethiopica* + 2.5 g *T. tetraptera* per kg basal diet

Experimental diet
The chick mash contained 20.05 % crude protein and 2653 kcal ME/kg; grower mash contained 16.25 % CP and 2650 kcal ME/kg, while layer mash contained 18.26% CP and 2656 kcal ME/kg. The ingredient and proximate composition of the experimental diets are presented in Table 1. *Xylopia aethiopica* and *T. tetraptera* fruit meals were added to the diets after compounding.

Table 1: Percentage and calculated nutrient composition of basal diet

	Basal diet		
Ingredient	**Chick mash**	**Grower mash**	**Layer mash**
Yellow Maize	51.00	57.00	55.50
Soya bean meal	26.40	15.30	20.00
Wheat offal	18.10	24.00	11.50
Fish meal	-	-	2.70
Bone meal	2.50	1.70	2.50
Oyster shell	-	-	7.00
Limestone	1.30	1.30	-
Common salt	0.35	0.35	0.35
Lysine	0.10	0.10	0.10
Methionine	0.10	0.15	0.20
Premix	0.15	0.10	0.15
TOTAL	**100**	**100**	**100**
Calculated nutrient composition			
Crude protein (%)	20.05	16.25	18.26
Metabolizable energy (Kcal/kg)	2653	2650	2656
Calcium	1.30	1.05	3.39
Phosphorus	0.58	0.70	0.88

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Management of experimental chicks
The chicks were brooded until 42 days of age when they were allocated to the dietary treatments. They were reared in an open-sided house providing 0.15 m^2/bird. Chick mash was fed to the birds *ad libitum* until ten weeks of age. Grower mash was fed at 110g/bird/day from 11 weeks of age to onset of egg production, while layer mash was fed at 120g/bird/day fed from onset of lay to the end of the study. Water was given *ad libitum* throughout the experimental period. From onset of egg production, the birds were housed in single bird laying cages. Other vaccinations recommended for layer chickens in the study environment were administered as and at when due and other management practices were adopted to ensure optimal health and performance of the birds.

Proximate analysis of test materials
The proximate analysis of the test materials was determined using the techniques of the Association of Official Analytical Chemists (AOAC, 2012). These included determination of the moisture, ash, crude protein, crude fibre and carbohydrate contents of the *X. aethiopica* and *T. tetraptera*.

Phytochemical analysis of test materials
The determination of alkaloids was as described by Harborne (1973). The determination of flavonoids was according to Lucas and Markakes (1975). Determination of phenolic contents was conducted according to the Folin–Ciocalteu method (Meda *et al.*, 2005) was used to determine total phenolic content. Saponin determination followed the method of Harborne (1973). Determination of terpenoids was done according to the method of Harborne (1973). Determination of phytate was determined according to Lucas and Markakes (1975). Oxalate content of the samples was determined by titration method as described by Lucas and Markakes (1975). Tannins was determined and calculated using the method of Harborne (1973). Determination of glycosides was done according to the method of Solich *et al.* (1992).

Determination of serum biochemical indices
Blood in sample bottles without anticoagulant was allowed to clot and then centrifuged at 10000g for 5 min for harvest of serum. Biochemical indices determined were total serum protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, creatinine, uric acid, triacylglycerol and cholesterol levels, as well as serum malondialdehyde, super oxide dismutase, catalase and glutathione peroxidase levels.

Aspartate aminotransferase (AST)
Determination of AST was done using a commercial test kit (Randox, UK). It is based on the principle that L-aspartate combines with α-oxoglutarate to form oxaloacetate. Aspartate aminotransferase is measured by the concentration of oxaloacetate hydrazine

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formed with 2,4-dinitrophenylhydrazine. Two Reagents, denoted R1 and R2 were used. R1 is a combination of 100 mmol/L of phosphate buffer at pH 7.4, 100 mMol/L of L-aspartate and 2 mMol/L of α-oxoglutarate. R2 is 2, 4-dinitrophenylhydrazine and 5% sodium hydroxide (NaOH) solution.

A 0.5 ml of R1 was put into each of two clean test tubes labelled sample and blank. 0.1 ml of the sample was added to the test tube labelled sample and an equal volume of distilled water was added to the test tube labelled blank. The contents of each test tube were mixed properly by shaking the test tubes vigorously and incubated for 30 minutes at 37^{o}C with a Laboratory biochemical incubator (Model AUCMA spx. Z11). Thereafter, 0.5 ml of R2 was added to each of the test tubes and the set up allowed to stand for 20 minutes at room temperature. Next, 5 ml of 5% NaOH solution was added to the contents of each test tube and again left to stand for 5 minutes at room temperature. The absorbance of sample and blank were read on an Ultraviolet and visible light (UV-VIS) spectrophotometer (Model 166uv) at 546 nm after zeroing with the blank at the same wavelength. To get the AST activity of the sample, the absorbance for sample was interpolated with the ones on the calibrated table. The table shows standard AST concentrations expressed in international units per litre (IU/L) (Ugbogu *et al.,* 2021).

Alanine aminotransferase (ALT)
Alanine aminotransferase (ALT) was determined using Randox commercial test kit (Randox, UK) based on the reaction between L-alanine and α-oxoglutarate to form L-glutamate and pyruvate hydrazone. ALT is measured by the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine. Two Reagents, denoted R1 and R2 were used. R1 is a combination of 100 mmol/L of phosphate buffer at pH 7.4, 200 mMol/L of L-alanine and 2 mMol/L of α-oxoglutarate. R2 is 2, 4-dinitrophenylhydrazine and 5% sodium hydroxide (NaOH) solution.

0.5 ml of R1 was put into each of two clean test tubes labelled sample and blank. 0.1 ml of the sample was added to the test tube labelled sample and an equal volume of distilled water was added to the test tube labelled blank. The contents of each test tube were mixed properly and incubated for 30 minutes at 37^{o}C. Thereafter, 0.5 ml of R2 was added to each of the test tubes which were then allowed to stand for 20 minutes at room temperature. Next, 5 ml of 5% NaOH solution was added to the contents of each test tube and the tubes left to stand for 5 minutes at room temperature. The absorbance of both sample and blank were read on a spectrophotometer at 546 nm. To get the ALT activity of the sample, the absorbance for sample was interpolated with values on the calibrated table. The table shows standard ALT concentrations expressed in international units per litre (IU/L) (Ugbogu *et al.,* 2021).

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Creatinine

This was determined using a commercial test kit (Randox, UK) and it is based on the reaction between creatinine in alkaline solution with picric acid to form a coloured complex. Creatinine concentration is proportional to the amount of the coloured complex. Two Reagents, denoted R1 and R2 were used. R1 is 35 mMol/L of picric acid, while R2 is 0.32 Mol/L of sodium hydroxide (NaOH) solution. Equal volumes of R1 and R2 were mixed to form the working reagent. Two clean test tubes labelled test and standard were set up. 2 ml of the working reagent was added to each of the test tubes. 0.2 ml of sample was added into the test tube labeled test. To the test tube labelled standard was added 0.2 ml of serum of known creatinine concentration. The contents of each test tube were mixed thoroughly. Absorbance A1 was obtained for test and standard, respectively at 546 nm within 30 seconds interval using a spectrophotometer, while absorbance A2 was read after 2 minutes (Ugbogu *et al.,* 2021). Creatinine concentration was calculated as follows:

$$\text{Absorbance of test} = \text{A2} - \text{A1 for test}$$

Absorbance of creatinine standard = A2 – A1 for creatinine standard

$$\text{Creatinine conc. (mg/dl)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} x \frac{\text{Conc. of standard}}{1}$$

Where, concentration of standard is 2.05 mg/dl

Uric acid

The method used was the modified Trinder's enzymatic colorimetric method according to Prencipe *et al.* (1978) for in vitro determination of uric acid in serum using a Quimica Clinica Aplicada (QCA) uric acid test kit (QCA, Spain). Three clean test tubes were labelled sample and serially arranged. Two standards (SD1 and SD2) and one blank test tubes were also labelled and arranged. 1.5 ml of uric acid working reagent was put into each of the six test tubes. 0.03 ml of uric acid standard was added into the test tubes labelled sample, as well as into SD1 and SD2 tubes. The contents of each test tube were thoroughly mixed and the tubes incubated at 37^{o}C for 10 minutes. Thereafter, the absorbance of the sample and standard against the blank were read at 505 nm using a digital spectrophotometer. The uric acid concentration (mg/dl) was obtained using the formula:

$$\text{Uric acid (mg/dl)} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} x \frac{5}{1}$$

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Total serum protein

Concentration of total protein was determined using a commercial test kit (Randox, UK). It is based on the reaction between cupric ions in an alkaline medium with protein peptide bonds resulting in the formation of a purple coloured complex. The intensity of the coloured complex is directly proportional to the concentration of protein in the sample ((Ugbogu *et al.*, 2021). A reagent denoted R1 was used. R1 contains a mixture of 100 mMol/L of NaOH, 16 mMoL/L of Na-K-tartrate, 15 mMol/L of potassium iodide and 6 mMol/L of $CuSO_4$. 1.0 ml of R1 was reconstituted in a test tube by adding 4 ml of distilled water to get a volume of 5 ml. Three test tubes labelled test, blank and standard were set up. 1.0 ml of reconstituted R1 reagent was pipetted into each of the test tubes; then 20µl of the serum sample was added into the test tube labelled test. 20µl of the protein standard reagent was added to the test tube labelled standard and 20µl of distilled water was added to the test tube labelled blank. The contents of each tube were thoroughly mixed and then allowed to stand at room temperature for 30 minutes. Thereafter, the absorbance of each test tube was read on a spectrophotometer at 560nm after zeroing with the blank (Ugbogu *et al.*, 2021). Total protein was calculated using the formula:

$$\text{Total protein (g/dl)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard}\ x\ \frac{\text{Conc. of standard}}{1}$$

Where, concentration of standard is equal to 5.95 g/dL

Total cholesterol

Total cholesterol was determined using enzymatic colorimetric chod-pad test method described by Allain *et al.*, (1974) with Randox laboratory test kits. The principle is as follows: Free and esterified cholesterol in the sample originates by a means of a coupled reaction where serum cholesterol reacts with enzymes to produce a coloured complex whose intensity is proportional to the concentration of serum cholesterol and is measured spectrophotometrically.

d. Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ cholesterol + fatty acids
e. Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ cholestene-3-one + H_2O_2
f. $2H_2O_2$ + phenol + 4-Aminoantipyrine $\xrightarrow{\text{peroxidase}}$ quinoneimine (colored derivative) + $4H_20$

The procedure is as follows: The working cholesterol reagent (1 mL) was added into tubes labeled blank, standard and test groups. The test sample (10 µL) was added to the appropriate tube, mixed properly and allowed to stand for 10 minutes at room temperature. Readings were then taken with the aid of a spectrophotometer at 505 nm. A

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standard was provided in the kit. The total cholesterol (TC) in the sample was calculated using the formula below:

$$TC = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \text{x } 200 \ (\text{mg/dl}).$$

Triglycerides

The principle for the determination of triglycerides is based on the reaction of peroxidase with hydrogen peroxide, 4-aminophenazone and 4-chlorophenol to form the indicator quinoneimine. The amount of indicator formed correlates with the concentration of triglycerides (Ugbogu *et al.,* 2021). A commercial test kit (Randox, UK) was used. Two reagents denoted R1a and R1b were used. R1a is a combination of 40mMol/L of pipes buffer at pH 7.6, 5.5mMol/L of 4-chlorophenol and 17.5mMol/L of magnesium ions. R1b comprises 0.5mMol/L of 4-aminophenazone, 1.0mMol/L of ATP, 150 U/ml of lipases, 0.4 U/ml of glycerol-kinase, 1.5 U/ml of glycerol-3-phosphate oxidase and 0.5 U/ml of peroxidase. One vial of R1b was reconstituted with 15 ml of R1a and mixed properly. Three test tubes labelled test, blank and standard were set up for each serum sample. From the reconstituted reagent, 1.0 ml was pipetted into each of the test tubes. 0.01ml of test serum was added into the test tube labelled test. The same volume serum of known triglyceride concentration (standard) was added to the test tube labelled standard and also the same volume of distilled water was added to the test tube labelled blank. The contents of each test tube were mixed thoroughly and the tubes incubated at 37^{o}C for 5 minutes. Thereafter, the absorbances of standard and test were read at 546nm on a spectrophotometer after zeroing with the blank (Ugbogu *et al.,* 2021). The triglyceride concentration of the sample was calculated using the formula:

$$\text{Triglyceride (mg/dl)} = \frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ standard} x \frac{\text{Conc. of standard}}{1}$$

Where, the triglyceride concentration of standard is 192mg/dl.

Total bilirubin

Bilirubin combines with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex in the presence of caffeine. The test was conducted using a commercial test kit (Randox, UK). Four reagents denoted R1, R2, R3 and R4 were used. R1 is a combination of 29 mMol/L sulphanilic acid and 0.17N Hydrochloric acid. R2 is 38.5 mMol/L of sodium Nitrite. R3 is a combination of 0.26 mol/L of caffeine and 0.52 mol/L of sodium benzoate. R4 is a combination of 0.39 Mol/L of Tartrate and 1.9N NaOH. Two test tubes labelled blank and sample were set up. 200µl of R1, 1000µl of R3 and 200µl of the serum sample were pipetted into each test tube. 50µl of R2 was added only to the test tube labelled sample. The test tubes were allowed to stand for 10 minutes at room

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temperature. Thereafter, 1000µL of R4 was added into each of the test tubes which were again allowed to stand for 30 minutes at room temperature. The absorbance of sample against blank was read at 578nm wavelength (Ugbogu *et al.,* 2021). Total bilirubin was calculated as:

Total bilirubin (mg/dl) = 185 x absorbance of serum sample

Oxidative and antioxidant profile
Malondialdehyde (MDA)
Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA) as described by Draper and Hadley (1990).

Malondialdehyde reacts with thiobarbituric acid (TBA) to form a red or pink colored complex which absorbs maximally in acid solution at 532 nm. The Procedure is as follows: The serum (50 µL) was deproteinized by adding 1 mL of 14% trichloroacetic acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 minutes to complete the reaction and then cooled on ice pack for 5 minutes. After centrifugation at 2000 g for 10 minutes, the absorbance of the colored product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56 × 105 mol/L/cm) using the formula, $A = \Sigma CL$, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in nmol/mg of protein.

Superoxide dismutase (SOD)
Adrenaline (10 mg) was dissolved in 17 mL of distilled water to make adrenaline solution. Serum sample (0.1 mL) was added to 2.5 mL of phosphate buffer (pH 7.8). Adrenaline solution (0.3 mL) was added, mixed well and absorbance was read at 450 nm at 30 seconds interval for 5 times (Xin *et al*., 1991).

Glutathione peroxidase (GSHPx)
The determination of glutathione peroxidase levels involves the measuring of its activity in a serum sample by monitoring the decrease in reduced gluthathione (GSH) or the oxidation of hydrogen peroxide (H_2O_2). 0.05ml of serum sample was mixed with a phosphate buffer containing GSH and H_2O_2. The mixture was incubated for 2 h to allow the reaction to occur. After incubation, 2ml of Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid), DTNB) was added to the mixture.

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This reacted with GSH to form a coloured complex that was measured. The absorbance was measured at 412 nm using a spectrophotometer. The intensity of the colour is proportional to the amount of GSH remaining, which inversely correlates with GSHPx activity. GSHPx activity was calculated based on the change in absorbance over time, using a standard curve generated from known concentrations of GSH.

Catalase (CAT)

Catalase activity was assayed by the method of Sinha (1972). Dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of chromic acid as unstable intermediate. The chromic acetate formed is measured at 570nm. To 0.9ml of distilled water and 0.1ml of plasma in a test tube was added 2ml of H_2O_2 and 2ml of phosphate buffer. The reaction was initiated by adding 2ml of dichromate acetic acid reagent to this mixture. Absorbance of the set up was taken at 30 seconds interval over 2 minutes. The activity of catalase was expressed as U/ml of plasma.

Data Collection

Serum biochemical and oxidative and antioxidant indices

At 50 and 73 woa, blood was collected from three randomly selected birds per group for determination of serum biochemical, oxidative and antioxidant parameters.

Statistical analysis

Data collected were analyzed by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS). Significant means were separated using Duncan New Multiple Range Test (DNMRT) according to Steel and Torrie (1980). The statistical model is shown below:

$$Y_{ijk} = \mu + T_i + e_{ijk}$$

Where, Yij is the k^{th} response variable of the jth bird in the ith treatment, μ is the overall mean, T_i is the effect of the ith treatment while e_{ijk} is the k^{th} random error associated with the jth bird in the ith treatment.

Results and Discussion

Phytogenic effects of the test materials on serum biochemistry of layer chickens

The total protein, total cholesterol, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, bilirubin, and creatinine at 50 and 73 woa are presented in Table 2.

The Table shows that the evaluated serum biochemical indices differed significantly

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between treatments across the age periods. At 50 woa, T6 had the highest total protein (4.51±0.13 g/dl) which differed significantly ($p < 0.01$) from the values observed in T2, T4, T5 and T3, but was statistically similar to values for other treatments. The least value for total protein was recorded in T3 (3.34±0.13 g/dl). Total cholesterol was highest in T5, T6, T1, T2, and T9 and these were statistically similar to values observed in the other treatments except T10 which had the lowest value for this variable (74.37±6.31 mg/dl) ($p < 0.05$). Hens in T1 had the highest value for triglycerides which was similar to the values observed in T5, T2, T6 and T9, but significantly higher than the values recorded by the other treatments. The least value for triglycerides was recorded in T10 (66.93±3.67 mg/dl). The highest value for AST was observed in T10 (82.73±1.21 U/L) followed by T1, T8, T2, T3, T7 and T5 while the least value was recorded in T9 (74.21±1.21 U/L). Hens in T1, T7, T6 and T5 recorded the highest ALT values ($p < 0.01$). These were followed by T2 and T10 which were similar to T3 and T8 but higher compared to T4 and T9 (40.27±0.65 and 38.82±0.65 U/L, respectively). Uric acid was highest in T6 and T1 and these were statistically similar to T4, T2, T9, T5, and T7 but higher ($p < 0.01$) compared to T8, T10 and T3 which had the least value (5.24±0.31 mg/dl). Bilirubin was significantly ($p < 0.01$) higher in T1 compared to the other treatments except T8, T2 and T6 which in turn were statistically similar to T9, T7, T3 and T10 but significantly higher compared to T5 and T4 with the least value. Hens in T1 and T8 recorded the highest creatinine level which were statistically higher ($p<0.01$) compared to the values for other treatments except T9 and T7. The lowest creatinine levels were observed in T3, T5, and T6. At 73 woa, hens in T6 and T3 recorded the highest total protein values which differed significantly ($p<0.01$) from the values in the other treatments except T1 and T7. The least total protein level was recorded by hens in T2 (3.57±0.19 g/dl). The highest total cholesterol was recorded in T3 with statistically similar values observed in T1 and T7 but significantly lower values observed in the other treatments. The least level of total cholesterol was recorded in T10 (74.13±3.76 mg/dl). Triglycerides was highest in T3 and T1 with statistically similar values observed in T4, T7, T2, and T5 but significantly ($p<0.00$) lower values recorded in the other treatments. Hens in T10 recorded the lowest triglyceride level (62.73±2.23 mg/dl). Hens in T1 recorded the highest AST and ALT activities (93.70±1.15 and 49.98±0.84 u/l, respectively) while the least value for AST was observed in T5. Hens in T10, T9 and T5 recorded the least values for ALT. Uric acid and total bilirubin were highest in hens belonging to T10. The least value for uric acid was observed in T8 (3.83±0.31 mg/dl) while T6, T7 and T3 recorded the least levels of bilirubin.

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Table 2: Serum biochemical indices of layer chickens fed varying dietary levels of *X. aethiopica and T. tetraptera* fruit meal

	Treatment											
Age/parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**SEM**	**p-value**
50 woa												
Total Protein (g/dl)	4.23^{abc}	4.01^{bc}	3.34^{d}	3.88^{bc}	4.00^{bc}	4.51^{a}	3.82^{c}	4.22^{abc}	4.29^{ab}	4.23^{abc}	0.13	<0.01
Cholesterol (mg/dl)	103.55^{a}	100.67^{a}	91.64^{ab}	88.12^{ab}	104.41^{a}	103.99^{a}	92.37^{ab}	85.00^{ab}	97.28^{a}	74.37^{b}	6.31	0.05
Triglyceride (mg/dl)	88.60^{a}	79.43^{abc}	75.72^{bcd}	69.61^{cd}	82.59^{ab}	81.60^{abc}	75.06^{bcd}	72.43^{bcd}	76.84^{abcd}	66.93^{d}	3.67	0.02
AST (u/l)	82.32^{ab}	81.27^{abc}	80.85^{abc}	78.55^{bc}	78.84^{abc}	78.05^{c}	78.99^{abc}	81.46^{abc}	74.21^{d}	82.73^{a}	1.21	<0.01
ALT (u/l)	46.25^{a}	42.52^{b}	41.28^{bc}	40.27^{cd}	44.60^{a}	45.26^{a}	45.81^{a}	41.48^{bc}	38.82^{d}	42.39^{b}	0.65	<0.01
Uric acid (mg/dl)	7.10^{a}	6.84^{ab}	5.24^{d}	7.05^{ab}	6.39^{abc}	7.14^{a}	6.36^{abc}	6.07^{bcd}	6.81^{ab}	5.45^{cd}	0.31	<0.01
Bilirubin (mg/dl)	0.53^{a}	0.49^{ab}	0.44^{bcd}	0.39^{d}	0.41^{cd}	0.48^{ab}	0.45^{bcd}	0.50^{ab}	0.46^{bc}	0.44^{bcd}	0.02	<0.01
Creatinine (mg/dl)	0.98^{a}	0.80^{bc}	0.76^{c}	0.87^{bc}	0.76^{c}	0.75^{c}	0.88^{abc}	0.97^{a}	0.92^{ab}	0.79^{bc}	0.04	0.01
73 woa												
Total Protein (g/dl)	4.49^{ab}	3.57^{c}	4.92^{a}	4.17^{bc}	4.02^{bc}	4.94^{a}	4.39^{ab}	3.99^{bc}	3.57^{c}	3.99^{bc}	0.19	<0.01
Cholesterol (mg/dl)	106.67^{ab}	96.02^{bcd}	111.06^{a}	93.37^{cd}	87.49^{de}	78.85^{ef}	102.62^{abc}	88.42^{de}	76.73^{ef}	74.13^{f}	3.76	<0.01
Triglyceride (mg/dl)	78.14^{a}	74.89^{abc}	79.11^{a}	76.09^{ab}	73.79^{abc}	69.80^{bc}	74.91^{abc}	70.59^{bc}	68.34^{cd}	62.73^{d}	2.23	<0.01
AST (u/l)	93.70^{a}	83.56^{ef}	90.65^{abc}	82.09^{efg}	79.33^{g}	87.28^{cd}	91.58^{ab}	89.47^{bc}	80.47^{fg}	84.95^{de}	1.15	<0.01
ALT (u/l)	49.98^{a}	42.09^{de}	46.73^{bc}	42.75^{de}	40.77^{e}	44.41^{cd}	46.60^{bc}	47.36^{b}	40.89^{e}	42.52^{e}	0.84	<0.01
Uric acid (mg/dl)	5.89^{ab}	5.88^{ab}	4.85^{cd}	5.47^{abcd}	4.59^{de}	4.89^{bcd}	5.81^{abc}	3.83^{e}	4.99^{bcd}	6.20^{a}	0.31	<0.01
Bilirubin (mg/dl)	0.50^{ab}	0.49^{ab}	0.38^{c}	0.49^{ab}	0.44^{bc}	0.40^{c}	0.38^{c}	0.48^{ab}	0.47^{ab}	0.51^{a}	0.02	<0.01
Creatinine (mg/dl)	1.02^{ab}	0.90^{cd}	1.09^{a}	0.99^{abc}	1.08^{a}	0.94^{bcd}	0.83^{d}	0.91^{bcd}	0.95^{bc}	0.90^{cd}	0.04	<0.01

a, b, c, d, e, f, g: means on the same row bearing different superscripts differ significantly (P≤0.05); SEM: Standard error of mean; woa: weeks of age; AST - Aspartate aminotransferase; ALT - Alanine aminotransferase; Treatment 1 or Control (No dietary supplementation); T 2: 0.5 g *X. aethiopica* per kg basal diet; T 3: 1.5 g *X. aethiopica*; T 4: 2.5 g *X. aethiopica*; T 5: 0.5 g *T. tetraptera*; T 6: 1.5 g *T. tetraptera*; T 7: 2.5 g *T. tetraptera*/ T 8: 0.5 g *X. aethiopica* + 0.5 g *T. tetraptera;* T 9: 1.5 g *X. aethiopica* + 1.5 g *T. tetraptera;* T 10: 2.5 g *X. aethiopica* + 2.5 g *T. tetraptera*.

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Hens in T3 and T5 recorded the highest creatinine level which were statistically similar to the values observed in T1 and T4 but higher ($p<0.01$) compared to the values for the other treatments. The least value for serum level of creatinine was recorded in T7 (0.83±0.04 mg/dl).

The higher serum total protein observed in T6, T7, T8, T9, and T10 compared to other treatments suggests that *T. tetraptera* fruit meal at 1.5g/kg and above as well as the combination of *X. aethiopica* and *T. tetraptera* fruit meals could enhance cellular protein synthesis. This is probably as a result of the moderate to high contents of saponin, phenol and flavonoid in these phytogenics. These phytochemicals have been shown to have immunomodulatory effects and as such could enhance serum protein through the production of immunoglobulins (Duarte *et al.*, 2010). The results agree with Williams *et al.* (2023) who reported higher total serum protein and albumin in broilers fed a composite of *X. aethiopica* and *Clove* compared to control group. Solomon *et al.* (2024) however, reported non-significant differences in serum total protein in broilers fed varying dietary levels of *X. aethiopical* fruit meal. The lower serum cholesterol and triglyceride levels in the treated groups compared to the control group across the age periods are in agreement with Christofoli *et al.* (2023) who reported lower levels of cholesterol and triglycerides in broilers fed diets containing essential oils of *Citrus sinensis* and *Xylopia aromatica* fruits compared to the control. Solomon *et al.* (2024) reported a dose dependent reduction in serum cholesterol, triglycerides, low density lipoprotein, and very low-density lipoprotein in broilers fed increasing levels of *X. aethiopical* fruit powder compared to the control while Sung *et al.* (2011) had reported that supplementation of peppermint and *Geranium thunbergii* lowered cholesterol levels in laying hens. These studies attributed the lowering effects on serum cholesterol and triglycerides to the powerful antioxidant factors such as glutathione, SOD, Vit. C, Vit. E, and flavonoids in the phytogenics. The reduced serum cholesterol and triglycerides in the treated groups is favourable for health and performance. In addition to the antioxidants, *X. aethiopica* and *T. tetraptera* contain tannins, saponins, and phenols reported to reduce body cholesterol and low-density lipoprotein cholesterol in animals (Jing *et al.*, 2022). According to Kim, *et al.* (2014) and Zeng *et al*. (2019), tannins bind to fats and cholesterol, enhance cholesterol catabolism and their excretion as faecal bile. Saponins increase fatty acid oxidation, inhibit lipid synthesis, and modulate gut–liver lipid metabolism (Cao, *et al.*, 2024). As reported by Yao *et al*. (2024), polyphenols reduce intracellular triglycerides, total cholesterol, LDL, AST, and ALT. Wang, *et al.* (2014) indicated that polyphenols limit preadipocyte differentiation, adipocyte proliferation and lipogenesis and promote lipolysis. Serum AST and ALT levels is used to evaluate liver health status and elevated levels above normal range indicate hepathic damage (Odesanmi *et al.,* 2009). The lower

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AST and ALT levels in most of the supplemented groups indicate that *X. aethiopica* and *T. tetraptera* fruit meal and their composite enhanced liver health and protected hepatocytes against damage. It also indicates absence of antinutritional factors in the test materials as alluded to by Solomon *et al.* (2024). The results are in conformity with Adegoke *et al.* (2018) who reported reduced ALT values following dietary inclusion of turmeric and cayenne pepper in diet of broilers. Serum level of metabolites such as uric acid, bilirubin, and creatinine enables evaluation of internal organ function and body metabolic activities. Elevated bilirubin level indicates pathologic proteolysis, impaired liver uptake and/or conjugation of bilirubin, and/or bile clearance defects. Elevated blood uric acid level may signal renal dysfunction while elevated creatinine level could indicate muscle protein catabolism. The observed lower serum levels of these metabolites in the treated groups indicates optimized body metabolic activities, internal organ function and health in the treated groups compared to the control. Williams *et al.* (2023) reported lower uric acid at 28 days of age in broilers fed fruit powder of *X. aethiopica* compare to control group while no difference was observed in serum creatinine level.

Phytogenic effects of the test materials on serum oxidative and antioxidant profiles of layer chickens

The serum profile of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSHpx), and catalase (CAT) of the experimental groups at 50 and 73 woa are presented in Table 3.

At 50 woa, only SOD level differed significantly between treatments with hens in T1 having the highest level (1.79±0.03 U/ml). This was statistically similar to T2, T4, T6, T8, and T10, but significantly ($p = 0.05$) higher compared to other treatments. The least SOD level was observed in T5 (1.63±0.03 U/ml). Hens in T8 recorded the highest MDA level (1.88±0.03 nMol/ml) at 73 woa. This was statistically similar to T1, T4, and T6, but significantly ($p < 0.01$) higher compared to the other treatments. The lowest MDA level was observed in T5 (1.66±0.03 nMol/ml). Hens in T8 also recorded the highest serum SOD level (1.78±0.03 U/ml) which did not differ significantly from T1, T4, T6, T10 and T2, but was higher ($p < 0.01$) compared to T9, T3, T7, and T5. The least SOD value was observed in T5 (1.59±0.03 U/ml). Serum GSHpx did not differ significantly ($p > 0.05$) between treatments, while hens in T10 recorded the highest level of CAT (49.74±1.20 U/ml). This did not differ significantly ($p > 0.05$) from T6, T8, T7, and T2 but was significantly higher compared to other treatments. Hens in T3, T5, and T9 recorded the least CAT activity.

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Table 3: Serum profile of oxidative and antioxidant parameters of layer chickens fed varying dietary levels of *X. aethiopica and T. tetraptera* fruit meal

	Treatment											
Age/parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**SEM**	**p-value**
50 woa												
MDA (nMol/ml)	1.88	1.78	1.82	1.82	1.75	1.82	1.75	1.78	1.78	1.83	0.03	0.20
SOD (U/ml)	1.79^{a}	1.74^{ab}	1.69^{bc}	1.74^{ab}	1.63^{c}	1.72^{ab}	1.66^{bc}	1.69^{ab}	1.66^{bc}	1.72^{ab}	0.03	0.05
GSHpx (U/ml)	42.98	41.21	42.12	41.78	43.12	42.38	42.83	42.76	41.50	41.01	0.75	0.45
CAT (U/ml)	45.45	43.96	44.17	45.35	45.70	44.10	45.25	44.48	42.82	43.05	0.82	0.21
73 woa												
MDA (nMol/ml)	1.85^{ab}	1.79^{bc}	1.76^{bc}	1.83^{ab}	1.66^{d}	1.83^{ab}	1.72^{cd}	1.88^{a}	1.78^{bc}	1.77^{bc}	0.03	<0.01
SOD (U/ml)	1.74^{ab}	1.70^{abc}	1.65^{bcd}	1.73^{ab}	1.59^{d}	1.73^{ab}	1.62^{cd}	1.78^{a}	1.69^{bc}	1.72^{ab}	0.03	<0.01
GSHpx (U/ml)	45.17	43.47	42.30	42.39	40.87	45.23	42.71	42.62	40.15	44.65	1.29	0.14
CAT (U/ml)	49.36^{ab}	45.83^{ab}	45.20^{c}	45.51^{bc}	45.19^{c}	49.22^{ab}	47.59^{ab}	48.30^{ab}	44.34^{c}	49.74^{a}	1.20	0.02

a, b, c, d: means on the same row bearing different superscripts differ significantly (P<0.05); SEM: Standard error of mean; woa: weeks of age; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSHpx: Glutathione peroxidase; CAT: Catalase; Treatment 1 or Control (No dietary supplementation); T 2: 0.5 g *X. aethiopica* per kg basal diet; T 3: 1.5 g *X. aethiopica*; T 4: 2.5 g *X. aethiopica*; T 5: 0.5 g *T. tetraptera*; T 6: 1.5 g *T. tetraptera*; T 7: 2.5 g *T. tetraptera*/ T 8: 0.5 g *X. aethiopica* + 0.5 g *T. tetraptera;* T 9: 1.5 g *X. aethiopica* + 1.5 g *T. tetraptera;* T 10: 2.5 g *X. aethiopica* + 2.5 g *T. tetraptera.*

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Serum MDA is a biomarker for lipid peroxidation, free radical production, and oxidative stress (Yousef *et al.,* 2009). The non-significant differences in serum MDA level between the control and most of the treated groups across the age periods suggest that *X. aethiopica* and *T. tetraptera* fruit meals did not induce oxidative stress in the treated chickens while the significantly lower MDA levels in T5, T7, T9, and T10 compared to the control group at 73 woa indicate antioxidant effects by the phytogenics. Adusei *et al.* (2019) and Nwafor *et al*. (2024) demonstrated the in vitro antioxidant activities of *T. tetraptera*. According to Adusei *et al.* (2019), antioxidant capacity of *T. tetraptera* was in the order: pulp > whole fruit > seed. Oyedeji *et al.* (2018) reported the free radical scavenging activities of *X. aethiopica* in vitro. According to Ndoye *et al*. (2024), essential oils of *X. aethiopica* showed low antioxidant capacity when compared to ascorbic acid. The low antioxidant capacity of *X. aethiopica* may be responsible for the non-statistically lower serum MDA observed in *X. aethiopica* treated groups compared to the control group at 50 woa. The non-significant differences in serum antioxidants levels between the control group and most of the treated groups across the age periods, as well as, the significantly lower levels in some treated groups compared to the control could be as a result of the antioxidant effects of bioactive chemicals such as saponins, flavonoids, phenols, and polyphenols in *X. aethiopica* and *T. tetraptera*. Polyphenols are the most common antioxidants found in fruits and vegetables. Phenolic and polyphenolic compounds, along with plant carotenoids, vitamin E, and vitamin C serve as antioxidants that can protect body tissues from oxidative stress (Rahman, *et al*., 2022). The results of the present study align with the findings of *An et al.* (2019) who reported non-significant differences in glutathione peroxidase activity of broiler chickens whose diet was supplemented with ginger extract but disagrees with Wen *et al.* (2019) who reported increased superoxide dismutase activity in hens supplemented with ginger. Balami *et al.* (2021) also reported increased catalase and superoxide dismutase activity in broilers whose feed was supplemented with *Moringa oleifera* leaf meal. A similar finding was earlier recorded by Zhao *et al.* (2011) who reported increased catalase and superoxide dismutase activity in hens whose feed was supplemented with ginger root (*Zingiber officinale*). The discrepancies in results may be related to different plant materials and poultry species (broiler versus layer chickens).

Conclusion

Results from this study showed that the supplementation of layer rations with fruit meal of *X. aethiopica and T. tetraptera* did not adversely affect serum biochemistry and antioxidant profile. Supplementation with *X. aethiopica* and *T. tetraptera* is beneficial to the health of layer chickens because this study revealed reduced oxidative stress marker, better antioxidant profile, and reduced serum cholesterol levels in the supplemented groups than the control. Based on these findings, it is recommended that *X. aethiopica and*

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T. tetraptera can be incorporated in the ration of layer chickens for improved performance especially during recovery from immunosuppressive diseases like Gumboro disease.

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