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Original Research

Effects of bitter leaf (Vernonia amygdalina) powder on liver enzymes, lipid profiles and carcass traits of finisher broiler chickens

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ABSTRACT
Thirty-two (32) three-week old Anak Acre unsexed broiler chicks were used to evaluate the effects of bitter leaf powder (BLP) on liver enzymes, lipid profile and carcass characteristics. The birds were acclimatized for one week and thereafter allocated to 4 treatments: T1 (Control), T2 (2.5g BLP/bird), T3 (5g BLP/bird), and T4 (7.5g BLP/bird) in a completely randomized design and each treatment was replicated twice, with each replicate containing 4 birds. The birds received BLP through drinking water (2ml drench) for 4 weeks. At the end of the treatment, data obtained from serum parameters and carcass traits were subjected to analysis of variance and significance was determined at $P \leq 0.05$. Results showed that BLP supplements had no significant effect ($P>0.05$) on liver enzyme markers and lipid profiles. The group treated with BLP at 2.5 g/bird had lowest ALP and ALT liver enzymes and lowest lipid and highest good cholesterol (HDL). The carcass characteristics showed that T3 differed ($P<0.05$) from other groups in live, slaughter, plucked, eviscerated and dressed weights and body parts including the thigh, breast, neck and the shank weights. The drumstick, back and the gizzard weights did not differ significantly ($P>0.05$) across the groups. The control group outperformed ($P <0.05$) other groups in intestinal and liver weights whereas the abdominal fat was numerically ($P>0.05$) highest in the group treated with 2.5 g/bird. It can therefore be concluded that liver enzyme markers and lipid profile of broiler chickens were at their best in broilers treated with BLP at 2.5 g/bird while the carcass characteristics showed superiority in broilers treated with BLP at 5 g/bird.

Keywords: Bitter leaf powder, broilers, carcass-traits, lipid profile, liver enzyme

INTRODUCTION
Globally, there has been a steady rise in the production of food of animal origin, particularly from poultry sector owing to high consumer demand. In this regard, FAO (2011) reported that the contribution of poultry meat is around 33% of the total global meat production. However, this phenomenon is not true for developing countries in Africa; rather it is dwindling (Okey & Egede, 2021). The poultry industry is faced with a number of challenges including but not limited to diseases/parasites, antibiotic resistance, inclement weather, lack of adequate feed ingredients to formulate feed, expensive drugs/vaccines and many others.

Feed additives are ingredients added to animal diets to enhance production efficiency, improve health and reduce morbidity (FAO, 2008). Khan *et al*. (2012) also reported increased net returns and improved carcass quality with the use of various feed additives in poultry. Antibiotics have been the major feed additives used for decades in poultry production. However, several bans on antibiotics usage owing to the emergence of resistant bacteria strains as well as public concerns about drug residues in poultry products as feed additives have accelerated and led to investigations on suitable natural alternatives (Polat *et al*., 2011). Biogenic and plant-derived products have proven to be less toxic, residue free and ideal for animal feed production (Okey & Egede, 2021). One of the herbal leaves which have been used in poultry nutrition as extract or leaf meals is bitter leaf (Owen & Amakiri, 2012; Oleforuh-Okoleh *et al*., 2015). *Vernonia amygdalina* leaf extract contains anthocyanin, proanthocyanin, tannin, alkaloid, phenolic acid and flavonoid antioxidants (Omede *et al*., 2018; Oyesola *et al*., 2022; Nowak *et al*., 2022) which increase digestibility and absorption of nutrients (Abbasi *et al*., 2020; Shilov *et al*., 2020) and combat oxidative stress (Oyesola *et al*., 2022). Phenolic acid is an immunomodulator, anti-mutagenic, and anti-inflammatory agent (Abdel-Moneim *et al*., 2020). The

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inclusion of flavonoids to broiler diets increased villus height and ratios, duodenum and jejunum crypt depth, growth, and carcass composition (Prihambodo *et al*., 2021; Ugokwe and Ugokwe, 2022). Thus nutrient absorption and growth indices in broilers were better.

Previous studies revealed *Vernonia amygdalina* increased broiler carcass performance (Japhet & Godgift, 2021; Mandey *et al*., 2021; Tokofai *et al.* 2021; Kismiati *et al*., 2023) whereas some reported no effect on carcass weight (Mandey *et al*., 2021; Rusli *et al*., 2022). Alanine transaminase (ALT) is used to identify acute liver failures as the enzyme is released into the serum immediately after a hepatocellular damage (Orlewick & Vovchuk, 2012). Increased Aspartate transaminase (AST) and ALT levels may cause chronic liver damage in fast growing broilers (Dudley *et al.*, 1982) and this might result to an occurrence of sudden death syndrome as reported by Qujeq & Aliakbarpour (2005). Furthermore, Radwan *et al*. (2007) reported that with high cholesterol levels, broilers tend to develop fatty deposits in their blood vessels. This eventually leads to difficulty in blood flow and consequently lameness and sudden death (Okpe *et al*., 2022). High levels of cholesterol, particularly LDL (Low-density lipoprotein) cholesterol, are mainly responsible for hypercholesterolemia (Krieger, 1998). It is known that hypercholesterolemia is a risk factor for cardiovascular diseases such as atherosclerosis, which is a common cause of mortality and morbidity (Wald & Law, 1995; Krieger, 1998). Hypercholesterolemia has been associated with enhanced oxidative stress related to increased lipid peroxidation (Adaramoye *et al*., 2005). Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels (Pritchard *et al*., 1995). Hence, the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach and efforts have been made to explore the antioxidant functions of various medicinal plants (Tomotake *et al*., 2006; Visavadiya & Narasimhacharya, 2007). High density lipoprotein cholesterol (HDL-C) level is a desirable constituent which impart positive health outcomes known to have resulted from the use of some plant materials (Ojiako & Nwanjo, 2009). Suhaemi & Hidayati (2020) showed that some African leaves reduced cholesterol levels in broiler meat. Blood consists of important components that maintain and regulate the body's physiological state (Molnar & Gair, 2015). Therefore, this study was designed to investigate the effects of bitter leaf powder on liver enzyme markers, lipid profile and carcass traits of broiler chickens.

MATERIALS AND METHODS

EXPERIMENTAL SITE

The experiment was carried out at the Teaching and Research Farms of the Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. Umudike lies on latitude 05^{o} 21´ N and longitude 07^{o} 33´ E in the rainforest zone of Southeastern Nigeria with Relative Humidity of about 50-90%, ambient temperature range of 17 to 36^{o} C and annual average rainfall of 2177 mm (NRCRI, 2018).

COLLECTION AND PREPARATION OF BITTER LEAF POWDER (BLP)

Vernonia amygdalina plant was identified by a Botanist in the Department of Botany, MOUAU, and the leaves were collected around the surroundings of the study area. The leaves were washed and rinsed with clean water, and air dried under shade until they were crispy to touch, while still retaining their green colour. The dried leaves were ground with electric grinding machine (Sonik®, Model SB-464) to produce the bitter leaf powder (BLP). The BLP was stored in an air tight container till used.

EXPERIMENTAL ANIMAL MANAGEMENT, DESIGN AND DURATION

Thirty-two, three-week old Anak Acre broiler chickens were used for the study. Ethical approval was obtained from ethical committee of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State with code MOUAU/CVM/REC/2024010 assigned to the study. The birds were purchased from a reputable commercial "brood and sell" dealer within Umudike metropolis. Prior to the birds' arrival, the poultry house was washed properly with detergent solution, disinfected using saponated cresol, after which was left for a week so as to reduce the residues of the disinfectant prior to the arrival of the birds. The floor of the deep litter pen was laid with 5 mm thick wood shavings. On arrival of the birds, they were given a mixture of clean water, glucose and multivitamin (Vitalyte®) for stability against stress. Commercial finisher ration Top Feed® (commercial Top feed® has Metabolisable energy of 3,200 /Kcal/Kg and Crude protein of 22%) and water were given *ad-libitum* during the one-week acclimatization period. Routine vaccinations (Marek's, i/o, Lasota and Gumboro) were up to date. After one week acclimatization, the birds were fed with commercial feed (Top feed) and allocated to 4 groups which were treated through drinking water for 4 weeks as follows: T1 (Control), T2 (2.5 g BLP/bird, drenched with 2 mL water), T3 (5 g BLP/bird, drenched with 2 mL water), and T4 (7.5 g BLP/bird, drenched with 2 mL water) using completely randomized design and each treatment was replicated twice, with each replicate containing 4 birds. The experimental study lasted for four weeks (ended at 8 weeks of age).

SERUM BIOCHEMISTRY

At the end of the study, one bird was randomly selected from each replicate for blood collection. Through the wing vein, 5 mL of blood was collected and deposited in a well labeled vacutainer plain sample bottle for coagulation of blood and eventual decanting to get the serum for biochemical analyses for ALP, ALT, AST, Cholesterol, Triglycerides, HDL (High-density lipoprotein), LDL (Low-density lipoprotein) and VLDL (Very low-density lipoprotein).

CARCASS AND ORGAN MEASUREMENTS:
At the end of the experiment, two birds were selected, fasted overnight and weighed prior to slaughtering. The live weight, slaughter weight, plucked weight, eviscerated weight, dressed weight and weight of the organs and cut-up parts were obtained.

STATISTICAL ANALYSIS

All data obtained were subjected to analysis of variance (ANOVA) using a Statistical Package for Social Sciences *(SPSS) version 20.0*. Significant differences among means were separated at 5% probability level using Duncan's New Multiple Range Test as described by Obi (2002).

RESULTS

EFFECT OF BITTER LEAF (*VERNONIA AMYGDALINA)* POWDER ON LIVER ENZYMES AND LIPID PROFILE OF FINISHER BROILER CHICKENS

Table 1 shows the effect of bitter leaf powder (BLP) on the serum biochemistry of broiler chickens. There was no significant difference (P>0.05) amongst the serum biochemical parameters measured. The liver enzyme markers (ALP, ALT and AST) showed that T3 group had numerically the highest of the values. This group could be the concentration of BLP in which maximum absorption of bitter leaf constituents occurred. Likewise, the lipid profile showed the same trend in which the cholesterol and triglyceride values were numerically highest in birds in T3 and lowest in birds in T2 groups except the high density lipoprotein (HDL) which numerically reduced progressively as the BLP concentration gradually increased, with T2 having the highest quantity even greater than T1. This possibly shows that bird on 2.5 g BLP/bird was the best since HDL is a lipid that is good for the health of the heart and it was highest in this group. The low density lipoprotein and very low density lipoprotein (VLDL) were numerically highest in T3 and lowest in T2 which further pointed to the superiority of birds on 2.5 g BLP/bird.

EFFECT OF BITTER LEAF *(VERNONIA AMYGDALINA)* POWDER ON CARCASS CHARACTERISTICS OF FINISHER BROILER CHICKENS

Table II shows the effect of bitter leaf powder (BLP) on the carcass characteristics of broiler chickens. The results showed that T3 group was significantly greater (P≤0.05) than other groups with respect to live, slaughter, plucked, eviscerated and dressed weights as well as the body parts' weights including the thigh, breast, neck and the shank. The T3 alongside T2 were also superior (P≤0.05) to other groups with respect to the weight of the head. The drumstick, back and the gizzard weights though were not statistically different (P>0.05) across the groups, nonetheless, the T3 had superior numerical weight acquisition. Meanwhile, T1 outperformed significantly (P≤0.05) other groups with respect to intestinal weight whereas with respect to liver weight, T1 and T3 significantly (P≤0.05) did better than other groups in the present study. Though with no statistical difference (P>0.05), the abdominal fat was numerically highest in T2. The above trend showed that T3 actually performed best.

DISCUSSION

EFFECT OF BITTER LEAF (*VERNONIA AMYGDALINA)* POWDER ON LIVER ENZYMES AND LIPID PROFILE OF FINISHER BROILER CHICKENS

Liver enzymes (ALT, ALP, and AST) are important in the determination of the proper functioning of the liver **(**Ambrosy *et al*., 2015**).** An increase in the concentration of these enzymes particularly ALT occurs due to damaged or diseased hepatoctye cells. ALT is found in highest amount in the liver and is used to identify acute liver failures (Orlewick & Vovchuk, 2012) as the enzyme leaks into the serum immediately after a hepatocellular damage. The present result is similar to the result of Okukpe *et al*. (2020) and Okpe *et al*. (2022) who recorded no significant difference in ALT and AST across bitter leaf extract treatments. *Vernonia amygdalina* is widely used in the tropics for its hepatoprotective property (Tokofai *et al.*, 2021). There is a possibility that a lower quantity of BLP could impart positively on liver enzymes since its inclusion at 2.5 g/bird/day had the most lowering effect on the liver enzymes. Radwan *et al.* (2007) reported that with high lipid levels, broilers tend to deposit fat in their blood vessels. This eventually leads to difficulty in blood flow and eventually lameness and sudden death of broiler chickens. Similar to the present result of T2, Owen *et al*. (2011) reported numerical reduction in cholesterol level occasioned by bitter leaf supplementation in the diet. The present finding partly aligns with the reports of reduction in lipid profile of animals given bitter leaf supplements (Yokozawa *et al.*, 2006; Ekpo *et al.*, 2007; Adaramoye *et al.*, 2008; Ojiako & Nwanjo, 2009). The higher the quantity of serum HDL, the better the function of the heart of the birds (Ojiako & Nwanjo, 2009). HDL which was found highest in T2 in the present study exerts part of its anti-atherogenic effect by counteracting LDL oxidation. HDL promotes the reverse cholesterol transport pathway, by inducing an efflux of excessively accumulated cellular

Table 1: Serum biochemistry of broiler chickens fed graded levels of bitter leaf meal

	Parameters	T1(Control)	T2 (2.5g BLP)	T3 (5g BLP)	T4 (7.5g BLP)
Liver Enzymes	ALP (µ/L)	32.35±6.90	30.35±0.50	39.35±3.60	30.35±8.05
	ALT (µ/L)	11.35±2.15	10.35±0.85	18.35±2.80	16.35±8.50
	AST (µ/L)	127.35±2.85	127.35±2.25	138.35±3.85	127.35±8.95
Lipid Profile	Cholesterol (mg/dL)	97.35±12.45	95.35±1.80	122.35±6.20	105.35±27.65
	Triglyceride (mg/dL)	29.35±0.70	28.35±1.80	38.35±1.50	33.35±11.20
	HDL (mg/d L)	59.35±5.60	62.35±1.80	50.35±20.95	41.35±6.35
	LDL (mg/dL)	31.58±6.68	27.67±0.37	63.77±26.83	57.85±31.75
	VLDL (mg/dL)	5.9±0.14	5.72±0.36	7.66±0.30	6.72±2.24

Legend: ALP (alkaline phosphatase), ALT (Alanine transaminase), AST (Aspartate transaminase), HDL (High-density lipoprotein), LDL (Low-density lipoprotein), VLDL (Very low-density lipoprotein).

cholesterol and prevents the generation of an oxidatively modified LDL (Yokozawa *et al.,* 2006). Excess LDL-cholesterol as evidenced in T2 of the present study can be deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions (Adaramoye *et al.,* 2008). Low triacylglycerol and increased HDL-cholesterol (HDL-C) levels as seen in T2 are desirable health outcomes known to result from the use of some plant materials (Ojiako & Nwanjo, 2009). Many authors have reported that *V. amygdalina* contained high flavonoid levels, which reduced bird meat fat (Praptiwi *et al*., 2020; Nath & Aravindkumar, 2021; Nufer & Shatskikh, 2021; Nowak *et al*., 2022; Tan *et al*., 2022).

EFFECT OF BITTER LEAF *(VERNONIA AMYGDALINA)* POWDER ON CARCASS CHARACTERISTICS OF FINISHER BROILER CHICKENS

Oyesola *et al*. (2022) stated that *V. amygdalina* contained tannin, alkaloid, phenol, and flavonoid antioxidants and were effective against oxidative stress. Antioxidant feed supplementation increases feed nutrient digestibility and absorption (Abbasi *et al*., 2020; Shilov *et al*., 2020). The present results are in tandem with other authors that got increases in live weight of experimental animals (Daramola *et al*., 2018; Mandey *et al*., 2021; Okey & Egede 2021] Tokofai *et al*., 2021). Similar to the present results, Japhet & Godgift (2021) and Kismiati *et al*. (2023) reported that *V. amygdalina* leaf extract supplementation increased broiler carcass performance significantly unlike Mandey *et al*. (2021) who reported only numerical increase. Unlike the current study, Rusli *et al*. (2022) reported that *V. amygdalina* leaf powder (2–6%) did not significantly affect carcass weight. Okukpe *et al*. (2020) reported improvement in carcass weight, fore-leg, fore-muscle, shoulder bone, rib, chest muscle, loin bone and loin muscle in animals that consumed bitter leaf treated diets with a significantly (p <

Table II: Carcass characteristics of broiler chickens fed bitter leaf meal

Parameters	T1(Control)	T2 (2.5g BLP)	T3 (5g BLP)	T4 (7.5g BLP)
Live weight (g)	$3102.00±57.00^{b}$	$3056.00±36.00^{b}$	$3679.50±101.50^{a}$	$3159.00±47.00^{b}$
Slaughter weight (g)	$3042.00±32.00^{b}$	$2895.50±116.50^{b}$	$3425.00±32.00^{a}$	$2991.50±19.50^{b}$
Plucked weight (g)	$2944.00±14.00^{b}$	$2774.00±120.00^{b}$	$3317.00±42.00^{a}$	$2857.50±0.50^{b}$
Eviscerated weight (g)	$2465.00±70.00^{b}$	$2336.00±63.00^{b}$	$2801.50±59.50^{a}$	$2454.50±30.50^{b}$
Dressed weight (g)	$2326.50±76.50^{b}$	$2215.00±90.00^{b}$	$2643.50±50.50^{a}$	$2344.00±57.00^{b}$
Wing (g)	113.50±1.50	163.50±51.50	121.50±5.50	108.00±1.00
Drumstick (g)	148.50±0.50	141.00±1.00	157.50±10.50	135.50±5.50
Thigh (g)	$155.50±5.50^{b}$	$160.50±8.50^{b}$	$193.50±5.50^{a}$	$159.50±0.50^{b}$
Breast (g)	$813.00±4.00^{c}$	$814.50±14.50^{c}$	$923.50±8.50^{a}$	$870.50±2.50^{b}$
Back (g)	486.50±3.50	461.00±36.00	535.50±12.50	464.50±4.50
Gizzard (g)	76.50±12.50	56.50±3.50	77.00±15.00	70.00±2.00
Neck (g)	$75.50±0.50^{b}$	$91.00±7.00^{b}$	$119.00±9.00^{a}$	$78.00±3.00^{b}$
Shank (g)	$43.50±1.50^{b}$	$44.50±2.50^{b}$	$51.50±1.50^{a}$	$38.50±0.50^{b}$
Intestine (g)	$258.50±1.50^{a}$	$203.50±1.50^{d}$	$238.00±5.00^{b}$	$221.50±5.50^{c}$
Liver (g)	$87.50±6.50^{a}$	$55.50±3.50^{c}$	$83.00±1.00^{ab}$	$66.50±4.50^{bc}$
Abdominal fat (g)	33.00±0.00	55.00±26.00	42.00±5.00	51.00±1.00
Head (g)	$49.00±1.00^{b}$	$53.50±1.50^{a}$	$54.00±1.00^{a}$	$48.50±0.50^{b}$

^{abcd} Means along the same row with different superscripts are significantly different (p≤0.05)

0.05) better performance in treatment with 3g of bitter leaf per kg of feed unlike 4 and 5g of bitter leaf per kg of feed treatments.

CONCLUSION AND RECOMMENDATION

It can be concluded that supplementation at 2.5 g BLP/bird, drenched with 2 mL of water performed the best with respect to liver enzyme markers and lipid profiles whereas the 5 g BLP/bird, drenched with 2 mL of water performed best in acquisition of more carcass weights due to positive effect of bitter leaf powder.

CONFLICT OF INTEREST

There was no conflict of interest.

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