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**SECURING ANIMAL
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GLOBAL CHALLENGES****OXIDATIVE STATUS OF BROILER CHICKENS FED UZIZA LEAF (*PIPER GUINEENSE*) AND UDA SEED (*XYLOPIA AETHIOPICA*) SUPPLEMENTED DIETS AS AN ALTERNATIVE TO ANTIBIOTIC-SUPPLEMENTS**

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ABSTRACT

*Herbal plants like Uziza (*Piper guineense*) and Uda (*Xylopiya aethiopica*) can be used for its biological, therapeutic and pharmacological properties without any toxicity. The use of antibiotics as growth promoter in animal production poses a threat to human health due to its residual effects in animal products. The study determines effect of Uziza leaf and Uda seed supplemented diets on oxidative status of broiler chickens. One hundred and twenty broiler chicks (Ross 308) were randomly allocated to five dietary treatments (24 birds/replicate). The Treatment 1 was fed with basal Treatment (positive control), Treatment 2, Treatment 3, Treatment 4 and Treatment 5 were received the basal diet plus Oxytetracycline 1g/kg (negative control), Uziza leaf powder 5g/kg, Uda seed powder 5g/kg and Uziza leaf and Uda seed 3g/kg each, respectively. The experimental trial lasted for six weeks. Blood samples of broiler chickens were collected and evaluated for oxidative status. The data were subjected to statistical analysis of one-way analysis of variance using completely randomized design. The total antioxidant activity, catalase, glutathione peroxidase, superoxidase dismutase, glutathione and malonyldialdehyde were influenced ($p < 0.05$) by Uziza leaf and Uda seed supplemented diets in broiler chickens. This study has shown that supplementation of Uda seed and Uziza leaf improved oxidative status of broiler chickens when added at the rate of 5g/kg in poultry ration.*

Keywords: Broiler chickens, Feed additives, Supplementation, Uziza leaf, Uda seed.

INTRODUCTION

In the recent times, animal nutrition has focused more on the utilization of plant nutrients for feed cost reduction, easy digestibility and absorption of nutrients. Natural feed additives like medicinal plants could be used to replace antibiotic growth-promoter in broiler chicken production due to the health-enhancing and nutritional properties owing to their pharmaceutical and medicinal properties with strong efficacy and low cost. The use of antibiotics as growth promoting in animal production poses a threat to human health due to its residues in animal products and is a major threat in the commercial poultry sector. The ban in the use of antibiotic growth-promoters has led to the manipulation of poultry feed ingredients by the feed mill industry to improve and balance up their profit margin. African medicinal plant herbs and spices like Uziza (*Piper guineense*) and Uda (*Xylopiya aethiopica*) within piperaceae and annonaceae are known for their biological, therapeutic and pharmacological properties with little or no toxicity (Mgbeahuruike et al., 2019). Their leaves, seeds, fruits, barks, and roots are known and used for various nutraceuticals and functional effects according to African folk medicine. For instance, Uziza (*Piper guineense*) and Uda (*Xylopiya aethiopica*) have been known to contain



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phytochemical substances such as Alkaloids, Tannins, Saponins and Flavonoids (Fategbe et al., 2021). The leaves, seeds, barks, and roots of *Piper guineense* and *Xylopi aethiopica* has been reported to have antibacterial, antifungal, anti-plasmodial, anti-hypertensive and diuretic effects (Butt et al., 2013). Previous reports have shown that the seed extracts of *Piper guineense* and *Xylopi aethiopica* contain plant lipids that enhance healing from diverse ailments due to their antioxidant and anti-inflammatory properties (Obiri and Osafo, 2013). Interestingly, *Piper guineense* and *Xylopi aethiopica* seeds are beneficial to man's health as immunostimulants and immune boosters (Nwozo et al., 2011). The study aimed to investigate the effects of oxidative status of broiler chickens fed Uziza leaf (*Piper guineense*) and Uda seed (*Xylopi aethiopica*) supplemented diets.

MATERIALS AND METHODS

Experimental location

The institutional research committees of Alvan Ikoku Federal College of Education, Owerri approved the animal study and its experimental protocols which was performed in accordance with standard guideline of college animal scientific procedure. The feed trial was conducted at the poultry unit of the teaching and research farm of AIFCE, Imo State, Nigeria.

Experimental birds

A total of 120 commercial day-old broiler chicks (Agrided breed: Ross 308) with an initial body weight of $366\text{g} \pm 2\text{g}$ (after 14 days brooding) was randomly assigned to 5 dietary treatments such that each treatment have 24 broiler chicks/pen/treatment (50:50 males and females) housed (7 x 7 x 4 feet) in pen. At the initiation of the study, broiler chicks in each treatment was blocked by body weight and gender. The broiler chicks were vaccinated of against infectious bursal disease and Newcastle disease (Hester Bioscience limited, Gujarat, India) at day 7 and 15 in their drinking water. Coccidiostat was not included in the basal feed. The birds were kept in a deep litter system (t). Feed delivery were limited to 83g/bird at the initiation of the study and gradually increased until *ad libitum* consumption was achieved. The experimental feeds and water (free from antibiotics or any drugs) were both available.

Experimental diets

The starter and finisher diets were prepared in a mash form and made throughout the feeding trial. The graded level of Uda seed (*Xylopi aethiopica*) and Uziza leaf (*Piper guineese*) (test ingredients) were added and mixed thoroughly after feed formulation according to each treatment inclusion rate. The basal diet was composed of feed ingredients that meet the nutrient requirements of broiler chicks based on the (National Research Council, 2012) recommendation. The experimental diets include: - Treatment 1: Basal diet with no treatment (positive control), Treatment 2: Basal diet with Oxytetracycline 1g/kg (negative control), Treatment 3: Basal diet with Uziza leaf 5g/kg, Treatment 4: Basal diet with Uda seed 5g/kg, Treatment 5: Basal diet with Uziza leaf 3g/kg and Uda seed 3g/kg.

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Blood collection and oxidative status analysis

At the end of the experimental trial, blood samples were collected from the birds' wing vein using a 5mL syringe and placed in sterilized bottles without ethylenediaminetetraacetic acid (EDTA: anticoagulant) for determining the oxidative status.

Statistical analysis

The data values were presented as mean $\pm$ SEM (standard of error of mean) from replicated experiments. All blood analysis were replicated six times (n=6/treatment). The result data were subjected to statistical analysis in a one-way analysis of variance (ANOVA) using completely randomized design. Treatment means were compared by Least Significance Difference test through IBM SPSS statistics, version 21. Differences at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The oxidative status of broiler chickens fed Uziza leaf (*Piper guineense*) and Uda seed (*Xylopia aethiopica*) supplemented diets is presented in Table 1. All oxidative status (total antioxidant activity, catalase, glutathione peroxidase, superoxidase dismutase, glutathione and malonyldialdehyde) of broiler chickens were influenced ($p < 0.05$) by Uziza leaf (*Piper guineense*) and Uda seed (*Xylopia aethiopica*) supplemented diets.

Antioxidants are compounds and reactions that remove scavenge or suppress the formation of reactive oxygen species (ROS) or oppose their actions. The role of antioxidants has been demonstrated in ameliorating membrane lipid peroxidation caused by ROS (Dutta *et al.*, 2020). Natural herbal plants and their seed, leaf or root extracts like Uziza leaf and Uda seed are good sources of phytochemical nutrients include steroid, alkaloid, flavonoid, saponin, tannin, phenolic acid and phytate. They have proved to function as singlet and triplet oxygen quenchers, free radical scavengers and peroxide decomposers (Anghel *et al.*, 2010). The total antioxidant activity, catalase, glutathione peroxidase, superoxidase dismutase, glutathione and malonyldialdehyde of broiler chickens were influenced by the dietary Uziza leaf and Uda seed supplemented diets. The total antioxidant activity of broiler chickens fed Uda seed diet was significantly higher than other diets. This result could be due to the antioxidants present in Uda seeds that reduce free radical and reactive oxygen species accumulation, thereby inhibiting lipid peroxidation. These mitigating effects of Uziza leaf and Uda seed could be attributed to its rich phytonutrients, antioxidant content, and vitamins which provide protective roles against oxidative stress (Olagunju and Sandewa, 2018). The antioxidant system is based on the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malonyldialdehyde (MAD) which together with metal-binding proteins are responsible for prevention of free radical formation and keep this process under control and there are other antioxidant defence which is based on chain-breaking antioxidants vitamins E, C, carotenoids. The CAT and SOD of broiler chickens fed Uda diet was significantly higher compared to other diets in the study. SOD is a major antioxidant enzyme that protects cells and organisms from the damaging effects of superoxide anion.



Table 1: The oxidative status of broiler chickens fed Uziza leaf (*Piper guineense*) and Uda seed (*Xylopia aethiopica*) supplemented diets

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	SEM	P-value
Total antioxidant activity (mM)	3.67 ^a	3.67 ^a	3.20 ^{ab}	3.71 ^a	2.25 ^b	0.29	0.05
Catalase (mmol/ml)	4.47 ^b	10.78 ^a	8.77 ^{ab}	8.31 ^{ab}	5.29 ^b	1.06	0.04
Glutathione peroxidase (u/l)	32.63 ^b	33.76 ^b	40.70 ^{ab}	44.26 ^a	41.07 ^{ab}	9.92	0.05
Superoxidase dismutase (u/ml)	1.12 ^{ab}	0.86 ^b	1.18 ^{ab}	1.51 ^a	0.95 ^b	0.09	0.05
Glutathione (mM)	0.22 ^b	0.35 ^a	0.28 ^{ab}	0.36 ^a	0.19 ^b	0.03	0.04
Malonyldialdehyde (μM)	12.36 ^{ab}	15.31 ^a	12.35 ^{ab}	13.74 ^a	10.84 ^b	0.65	0.02

a, ab, b: Mean in the same row with different superscripts are significantly ($p < 0.05$) different.

Diets (n = 6). SEM: Standard error of mean.

Treatment 1: Basal diet (Positive control)

Treatment 2: Basal diet + Oxytetracycline 1g/kg (Negative control)

Treatment 3: Basal diet + Uziza leaf 5g/kg

Treatment 4: Basal diet + Uda seed 5g/kg

Treatment 5: Basal diet + Uziza leaf 3g/kg and Uda seed 3g/kg

SOD works in conjunction with CAT and GSH-Px to diminish the harmful effects of ROS. SOD and CAT are of cellular defence against oxidative damage; they play a role in the enzymatic mechanisms. SOD converts highly toxic superoxide radicals into hydrogen peroxide while CAT is responsible for the breakdown of hydrogen peroxide. GSH-Px works in tandem with CAT to scavenge excess hydrogen peroxide and lipid peroxides in response to oxidative stress. Our findings agree with turmeric supplementation improved the antioxidant capacity of broilers by increasing SOD and GSH-Px activities and decreasing serum MDA concentrations in broilers (Wang *et al.*, 2015). Phenolic compounds support antioxidant system through direct scavenging of ROS produced after oxidative stress and/or prevent the formation of ROS by inhibiting enzymes. Similarly, supplementation of ginger at 0.5% or 1% level or in combination decreased the lipid peroxidation levels of broiler birds. Sadeghi *et al.* (2013) reported that supplementation of ginger powder significantly reduced MDA content in the serum of broilers at the ages of 21 and 42 day of age which corroborates with our findings. GSH-Px activity depends on the balance between the levels of glutathione and glutathione disulfide. The GSH-Px of broiler chickens fed Uda diet was significantly higher compared to other diets. Our study revealed increased glutathione peroxidase contents in Uda diet implying the accumulation of free radicals in the

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blood serum. Plasma GSH-Px catalyses the reduction of H_2O_2 and lipid hydroperoxides using GSH as a reducing agent (Ottaviano *et al.*, 2008). Our study found that there was a significantly increase of GSH contents in Uda diets compared to other diets in the study. Hence, the increased GSH-Px activities may be due to the accumulation of free radicals and the high availability of GSH in Uda seeds.

CONCLUSION AND RECOMMENDATION

Based on the obtained results, it is concluded that supplementation of Uziza leaf and Uda seed diets improved the oxidative stress indicators of broiler chickens when added at the rate of 5g/kg of broiler ration. Uziza leaf and Uda seed had positive effect and can be a viable alternative feed additive to antibiotics in the nutrition of broiler chickens.

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