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Antioxidant Enzyme Status in Broiler Chicken: Role of Dietary Supplementation of Pawpaw Leaf and Seed Meal Composite Mix

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Abstract

This study was conducted to examine the role of dietary supplementation of pawpaw leaf and seed meal composite mix on antioxidant enzyme status in broiler chicken. Pawpaw leaves were harvested, chopped into smaller pieces, air-dried and milled to pawpaw leaf meal (PLM). Seeds of ripe pawpaw fruits were also air-dried and ground to pawpaw seed meal (PSM). The PLM and PSM were mixed together at ration 3:1 to form a pawpaw leaf and seed meal composite mix (PLSMM) and analysed for chemical analysis. Two basal diets were formulated for the starter (0 to 21 days of age) and grower/finisher phase (22 to 42 days of age). Thereafter, basal diets were divided into six (6) portions. Portion one (Diet 1), contained 0g/kg PLSMM supplementation, portion two (Diet 2), was supplemented with 2 g/kg PLSMM, while portions three (Diet 3), four (Diet4), five (Diet 5) and six (Diet 6) were supplemented with 4, 6, 8 and 10 g/kg respectively. On day 42 of the experiment, blood was collected from 3 birds per replicate into aplain bottle for antioxidant enzymes determination. The superoxide dismutase (SOD) activity significantly ($p<0.05$) increased in the broilers fed diets supplemented with 6, 8 and 10g/kg PLSMM. Significantly ($p<0.05$) increased glutathione peroxidase (GPx) activity was recorded at 4, 6, 8 and 10g/kg PLSMM dietary supplementation when compared to 0g/kg and 2g/kg PLSMM dietary supplementation. Catalase (CAT) activity significantly ($p<0.05$) increased in birds fed 8g/kg PLSMM supplemented diets when compared to the other diets.

Keywords: Antioxidant, enzyme, broiler chicken, pawpaw leaf/seed

Introduction

Heat stress was reported to exert negative effects on production performance and health status of commercial broilers raised in the tropics (Tirawattanawanich *et al*., 2011). According to Akbarian *et al.* (2016), the effect of heat exposure on poultry is now a global challengeand heat stress is now a major factor significantly affecting the well-being and production of these species of animal. In addition, the presence of reactive species (RS) in animals in excess of their available antioxidant was recently linked with heat stress (Halliwell and Whiteman, 2004). These reactive species i.e. radicals and metabolic substances, are potentially toxic, highly reactive and can modify several biological macromolecules such as nucleic acids, proteins, and lipids (Davies, 1995). The use of anti-stress compounds and antioxidants of plant sources that can ameliorate the stress and significantly prevent or delay oxidation of biological macromolecules are becoming popular (Vara Prasad Reddy *et al*., 2009; Jimoh *et al*., 2016).

Pawpaw plant is among the numerous plants that possess antioxidant properties. Maisarah *et al.* (2014) reported the antioxidant activities of leaves and papaya seeds; while Oloruntola *et al*. (2016a) reported immuno-stimulatory properties of papaya leaf meal. The main constituents of pawpaw leaf meal and seed are β-Carotene, total phenolic content, ascorbic acid, vitamin E and minerals such as Ca, Mg, P, and Na among others (Maisarah *et al.,* 2014). Previous reports shows that papaya contains bioactive compounds that are capable of acting through multiple mechanism such as proliferation apostosis, proliferation, cancer cell signalling and carcinogen elimination to exhibit *in vitro* anticancer activities (Wahl *et al*., 2010). Papaya also possesses antibacterial, antiviral, anti-inflammatory, anti-tumor and free-radical scavenging activities (Maisarah *et al.,* 2014). However, there exists variability in the bioactive compounds of processed pawpaw plant parts (leaf meal and seed meal). Combining these pawpaw parts in ratio to form composite mix could produce interactive effects when supplemented in poultry diet.

Therefore, this study was conducted to study the role of dietary supplementation of pawpaw leaf and seed meal composite mix on antioxidant enzyme status in broiler.

Materials and Methods

Pawpaw leaves were harvested within the Federal Polytechnic, Ado-Ekiti, Nigeria. Thereafter, the leaves were chopped into smaller pieces and spread lightly on a clean tarpaulin to air-dry for about 14 days and milled to powdery form to produce pawpaw leaf meal (PLM).Seeds of ripe pawpaw fruits were also collected, spread lightly on tarpaulin to air-dry for 20 days and ground to powdery form to produce pawpaw seed meal (PSM). The PLM and PSM were mixed together in ratio 3:1 to form a pawpaw leaf and seed meal composite mix (PLSMM) and thereafter analyzed (Table 1) for tannin (Aina *et al*., 2012), terpenoid (Sofowora, 1993), cardiac glycoside (Sofowora, 1993), saponin (Brunner, 1984), steroid (Madhu *et al*., 2016), ferric reducing antioxidant property (Pulido *et al*., 2002), 2,2-diphenyl-1-piccrlhydrazyhydrate (Gyamfi *et al*., 1999) and vitamin C (Benderitter *et al*., 1998). All determinations were done in triplicates.

Two basal diets were formulated for the starter (0 to 21 days of age) and grower/finisher phase (22 to 42 days of age) to meet the NRC (1994) requirements for broilers. Thereafter, basal diets were divided into six (6) portions. Portion one (Diet 1), contained 0g/kg PLSMM supplementation, portion two (Diet 2), was supplemented with 2 g/kg PLSMM, while portions three (Diet 3), four (Diet 4), five (Diet 5) and six (Diet 6) were supplemented with 4 g/kg, 6 g/kg, 8 g/kg and 10 g/kg respectively. The six diets were analyzed for their crude protein and crude fiber composition (AOAC, 1995).

The experimental site geographical coordinates are latitudes of 7°37´N and 7°12´N and longitudes 5°11´E and 5°31´E, having mean annual rainfall and mean annual temperature of 1247 mm and 26.2 °C respectively (Oloruntola *et al*., 2016b). Three hundred and ninety-six (396), 1-day old Arbor Acres broiler chicks were randomly distributed to six dietary treatments (11 birds/replicate; 66 birds/treatment) in a completely randomized design (CRD). Eleven (11) birds were housed in their respective pen (200 x 110 cm).Light was provided 23 hours/day while birds were fed*ad libitum* throughout the period of the experiment.

On day 42 of the experiment, 3 birds per replicate were randomly selected and sacrificed. Blood were allowed to flow into a plain bottle for antioxidant enzyme determination. The glutathione peroxidase (GPx)(Rotruck *et al*., 1973), superoxide dismutase (SOD) (Misra and Fridovich, 1972) and catalase (CAT)(Aebi, 1974) were determined.

Completely randomized design was used in this experiment. All data collected were subjected to analysis of variance using SPSS version 20. The differences between treatment means were examined by Duncan multiple range test.

Results and Discussion

The phytochemical constituents and antioxidant properties of PLSMM are presented in Table 1. The PLSMM contains some phytochemicals of pharmacological importance. Phytochemicals present biological properties such as antimicrobial, antioxidant and anti-stress (Valenzuela-Grijalva *et al*., 2017). The PLSMM also contained appreciable amount of Vitamin C, 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and ferric reducing antioxidant properties (FRAP). The abstraction of electrons by oxidized molecules from other molecules, when not controlled results in chain reaction which can cause extensive tissue damage (Akbarian *et al.,* 2016). However, the cells have evolved defence systems to control the production of reactive species (RS) among which is an enzymatic high molecular weight (e.g glutathione peroxidase, arylesterase and superoxide dismutase) compounds. These compounds protect cells from oxidation damage by limiting the oxidation rate and progression (Akbarian *et al.,* 2016).

Table 1: Phytochemical constituents and antioxidant properties of pawpaw leaf and seed meal composite mix (PLSMM)

Phytochemical constituents (%)		Antioxidant properties	
Terpenoid	12.82±0.00	DPPH (%)	48.09±0.17
Tannin	6.17±0.01	FRAP (mg/g)	128.53±0.33
Cardiac glycoside	18.59±0.00	Vitamin C (mg/g)	21.62±0.01
Saponin	4.72±0.11		
Steroid	5.84±0.01		

FRAP: Ferric reducing antioxidant property; DPPH: 2, 2-diphenyl-1-piccrlhydrazyl hydrate

In this study, the dietary PLSMM supplementation improved the defence system of the birds against the RS production. The superoxide dismutase (SOD) significantly ($p<0.05$) increased in the broilers fed diets supplemented with 6, 8 and 10g/kg PLSMM. This implies PLSMM dietary supplementations at these levels

improved/increased (SOD) activity. This result agreed with report of Vara Prasad Reddy (2009) who recorded enhanced SOD activity in broilers due to 0.5% *Ocimum sanctum* dietary supplementation. Glutathione peroxidase (GPx) main biological role is to protect the organism from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. In this study, significantly ($p<0.05$) increased glutathione peroxidase (GPx) activity was recorded at 4, 6, 8 and 10g/kg PLSMM dietary supplementation when compared to 0 and 2g/kg PLSMM dietary supplementation.

Catalase plays vital role in protecting cell from oxidative damage by catalysing the decomposition of hydrogen peroxide to water and oxygen. Catalase activity significantly ($p<0.05$) increased in birds fed 8g/kg PLSMM supplemented diets when compared to the other diets. The improvement in antioxidant enzymes (SOD, GPx and CAT) activity by PLSMM dietary supplementation in this study supports the earlier reports that pawpaw plant parts (leaves, fruits, seeds) are good sources of natural potent antioxidants (Zho *et al.,* 2011; Maisarah *et al.,* 2014). In addition, the positive effects of antioxidants properties of phytochemicals to poultry on oxidative status parameters was summarized and reported by Akbarian *et al.*(2016).

Table 2: Effects of pawpaw leaf and seed meal mix (PLSMM) supplementation on anti-oxidative status of broiler chickens

Parameters	D1	D2	D3	D4	D5	D6	SEM	P value
SOD	92.00^{d}	93.50^{c}	95.00^{b}	98.00^{a}	97.50^{a}	97.00^{a}	0.55	0.01
GPX	121.69^{c}	145.52^{b}	164.12^{a}	177.91^{a}	182.76^{a}	170.62^{a}	5.48	0.01
CAT	416.31^{c}	444.45^{bc}	455.11^{ab}	464.81^{ab}	481.02^{a}	458.58^{ab}	6.04	0.02

Means within a row with different letters are significantly different ($P<0.05$); SEM : Standard error of mean; SOD: Superoxide dismutase (m/mg); GPx: Glutathione peroxidase (mg protein); CAT: Catalase (mg/g); D1: 0 g/kg PLSMM (control); D2: 2 g/kg PLSMM, D3: 4 g/kg PLSMM; D4: 6 g/kg PLSMM; D5: 8 g/kg; D6: 10 g/kg PLSMM.

Conclusion and Recommendation

It can be concluded that PLSMM has phytochemicals of pharmacological importance and also possesses antioxidant properties. PLSMM, when used as supplementsin broiler diets at a rate of 8g/kg can combat oxidative stress by enhancing the SOD, GPx and CAT activities.

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