



Does *Pleurotus ostreatus* influence health status and meat quality attributes of broiler chickens?

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ABSTRACT

An 8-week study was piloted to establish the effect of *Pleurotus ostreatus* (PO) extract (Oyster mushroom) on health status and quality attributes of meat. One hundred and eighty (180) day old Cobb chicks were allotted into three treatments and orally administered extract of *Pleurotus ostreatus* at 0 (control), 2000 and 4000 mg/L. Haematological indices, serum metabolites, quality attributes of meat and oxidative stability of meat were determined. Oral administration of PO had no effect on haematological indices and some serum metabolites except serum albumin and calcium. Thiobarbituric acid (TBARS) in blood was lowered in 4000 mg/L group at week 4 and 8 of growth. Crude protein and water holding capacity (WHC) increased as level of oral administration of PO increased with the highest values in 2000 mg/l and 4000 mg/L groups. Glutathione Peroxidase (GSH-Px) activity in meat tissue was highest in the 4000 mg/L treatment while Thiobarbituric Acid Reactive Substances was statistically lowest and similar in the control and 4000 mg/L groups. It is concluded that *Pleurotus ostreatus* extract can be orally administered up to 4000 mg/L as an antioxidant in birds in order to decrease lipid peroxidation in birds and improve meat quality and oxidative stability.

Keywords: Blood indices, Meat oxidative stability, Nutraceuticals, Oxidative stress, Stress biomarkers

The foremost goal in a poultry enterprise is producing meat that is safe for consumption likewise taking into cognizance the welfare of birds and the environment (Ekunseitán *et al.* 2021). Consumers are more conscious informed of the nutritive and quality attributes of their food (Lee *et al.* 2012) as it has a direct effect on their health (Montes *et al.* 2020). The rich chemical composition of meat predisposes it and its' products to quality deterioration resulting in chemical and microbial changes within the tissue. Lipid oxidation is one of the foremost chemical reactions that results into chemical degradation of meat and its product. The use of materials capable of improving the oxidative status of animals and subsequently on its product becomes necessary since growth promoters and synthetic compounds are banned as a result of their residual and negative effect in humans (Mahfuz *et al.* 2020a)

Researchers have sought options after the ban on usage of synthetic growth promoters (Simon 2005) and synthetic antioxidants (Mahfuz and Piao, 2019) due to escalating concerns of residues in animal products and emerging trend of antibiotic resistant strains of microorganisms (Saleha *et al.* 2009). The use of nutraceuticals has been found to be a better replacement and greater option in diets of animals.

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Hence the need to source for options in the control of lipid oxidation in livestock nutrition and products by using antioxidant compounds from natural sources (Novakoviã *et al.* 2020).

Mushrooms has been established to exhibit health-promoting benefits in both humans and animals, this is due to its antioxidants, phenolic compounds, tocopherols, carotenoids, and antibacterial compounds (Zhou *et al.* 2010). Studies conducted in poultry birds have shown *Pleurotus* positive influence on growth, gut health and immune responses, health status and activities against pathogenic organisms (Giannenas *et al.* 2010a, Ekunseitán *et al.* 2017, 2018, Ademola *et al.* 2019). However, there is limited information of its effect on health and quality meat attributes of birds raised in tropical areas with stressors especially via oral route. Therefore, the present study was to investigate the effects of *Pleurotus ostreatus* on health indices and quality attributes of meat.

MATERIALS AND METHODS

The experiment was conducted at the Poultry unit of the Teaching and Research Farms, Federal university of Agriculture, Abeokuta, Ogun State, Nigeria positioned on latitude 7°15' N, longitude 3°26' E and 76 m above sea level.

Pleurotus ostreatus was obtained from a commercial company for uniformity of test ingredient. One kg of fresh

Pleurotus ostreatus was mixed with 2 litres of ethanol in the ratio of 1:2 and was infused for 3 days (72 hours) for extraction. The liquid was poured off after 72 hours while fresh ethanol was added until extraction was fully done. After extraction, the extract was sieved out using a muslin cloth. Extract was clarified by filtration through celite on water pump and then concentrated into a paste using a rotation evaporator.

One hundred and eighty (180) day-old cobb chicks were purchased and randomly allotted to three (3) treatment groups: control (0), 2000 and 4000 mg/1000 ml of water). Each treatment group of 60 birds were further divided into four replicates of 15 birds each. The extract of *Pleurotus ostreatus* was administered orally via water once a week for 8-weeks duration of the experiment. Vaccination was given to all treatment groups while medication (antibiotics and coccidiostat) was administered only to the control group. A commercial starter diet (Crude protein: 22%, fat: 6%, fibre: 3.5%, Metabolisable Energy: 3000 Kcal/kg) was given from day-old to 4 weeks of age while finisher diet (Crude protein: 17%, fat: 4%, fibre: 3.5%, Metabolisable Energy: 2900 Kcal/kg) from 5 to 8 weeks of age.

All procedures carried out were in agreement with ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This experiment conformed to the ethical standards of the College of Animal Science and Livestock Production (COLANIM) Committee on Animal Experimentation, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria. The experiment begun after the proposal was ratified by the animal welfare expert in the college (ethical clearance number COLANIM/APH/UG/ 20120685).

Three millilitres (3 ml) of blood were collected via the jugular vein into from six birds per replicate into sample bottles containing ethyl dimethyl tetra acetic acid (EDTA) for determination of haematological parameters. Blood was analyzed for packed cell volume (PCV) and haemoglobin (Hb) concentration (Jain 1993). Red blood cells (RBC), white blood cell (WBC) (Jain 1986) and WBC differential counts (Ritchie *et al.* 1994).

Approximately 4 ml of blood was dispensed into sample bottle. Blood was centrifuged and the sera obtained were analyzed for total protein, albumin, globulin, calcium, potassium, Aspartate Aminotransferase, Alanine Aminotransferase and Alkaline Phosphate. Total protein and albumin (Tietz 1995), serum calcium and potassium were detected Auto Biochem Analyzer using the Randox standard kit. Aspartate Aminotransferase, Alanine Aminotransferase and Alkaline Phosphate were also analysed (Rietman and Frankel 1957).

Oxidative stress biomarker determination was carried out at day 28 (week 4) and day 56 (week 8). About 4 ml of blood was collected from tagged birds in each replicate via jugular vein and dispensed into lithium heparin tubes. The blood was analyzed for thiobarbituric acid reactive substance (TBARS) (Rael *et al.* 2004)

Meat quality measurement: At the 56th day, four birds closest to average body weight of in each replicate were selected for meat quality evaluation. Birds were starved for 12 hours but allowed access to fresh water. Selected birds were slaughtered, bled appropriately, plucked and eviscerated. Fresh portions of meat tissues were excised from the breast and placed in a sample tube for further quality analysis. Water-holding capacity (WHC) was estimated using a modification of the filter paper press method (Wierbicki and Deatherage 1958).

Proximate composition (Moisture, crude protein, and crude fat contents) of meat tissue were carried out immediately according to the methods of AOAC (2000).

Biomarkers determinations: The activities of Glutathione peroxidase (GSH-Px) in meat tissues were assayed using spectrophotometric method (Ohkawa *et al.* 1979) while degree of lipid oxidation (MDA) level was determined with thiobarbituric acid (TBARS) using spectrophotometric method (Giannenas *et al.* 2011).

Data generated were arranged in a one-way analysis of variance and analyzed using the GLM procedure of the SAS/STAT module (SAS 9.3). Significance differences amongst treatment means were determined at $P = 0.05$.

RESULTS AND DISCUSSION

The influence of *Pleurotus ostreatus* administration on blood Thiobarbituric acids (TBARS) at day 28 and 56 is presented in figure 1. TBARS was significantly influenced ($P < 0.05$) at both starter and finisher phases of growth with the value greatly lowered in birds in 4000 mg/L group. A decrease in oxidative stress is characterized by reduction in MDA levels. The antioxidant activity of mushrooms has been documented as a radical activity scavenger and likewise exhibiting cellular protection against oxidative damage (Akanmu *et al.* 1999, Yang *et al.* 2002). Attributes of PO has been researched and posited to contain high molecular weight phenolics (Yim *et al.* 2010) contributing to its radical cation scavenging ability, Mahfuz *et al.* (2020b) ascribed the increased the value of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (CAT) and lowering malondialdehyde (MDA) values in serum, muscle and liver sample of broilers to different bioactive components especially phenolic compound in mushroom. Mushroom family has been found to contain vitamin C and selenium that plays important role in antioxidant functions (Bederska-Łojewska *et al.* 2017, Mahfuz and Piao 2019). This observation was contradictory to that reported by Yunita *et al.* (2020) who observed an increased in serum MDA levels when β -glucan extract of oyster mushroom was used. Therefore, PO extract route of administration in the present study is effective and will allow ease of absorption of these polyphenolic compounds (Qing *et al.* 2020) by the intestine thereby reaching the plasma and marked organs (Giannenas *et al.* 2011) easily to exhibit its antioxidant effect. The intensive activity of extracts observed in the experimental animals could be as a result of bioavailability of antioxidant compounds from the extract

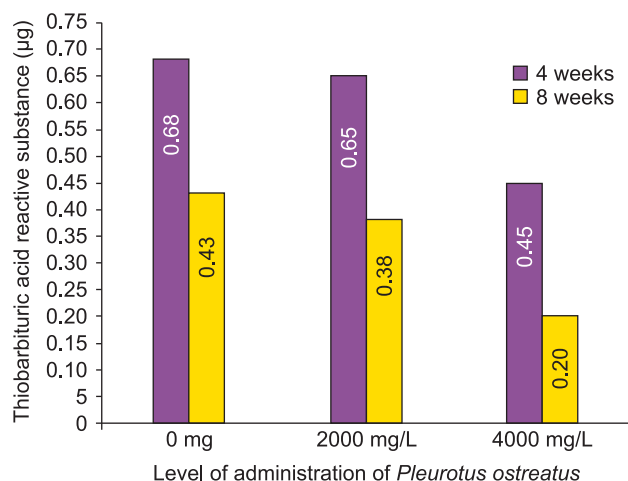


Fig. 1. Effect of administration of *Pleurotus ostreatus* on blood antioxidants of broiler birds at day 28 and 56.

than if the powdery form is used since presence of fibre will impair the rate of absorption of the active compounds. *Pleurotus eryngii* has been documented to exhibit higher antioxidant enzyme activities and lower egg yolk and serum cholesterol when used in laying birds (Lee *et al.* 2015, Mahfuz and Piao 2019). The attendant effect of environmental temperature changes especially in the tropics like Nigeria predisposes animals to stress, a dietary approach via oral administration of nutraceuticals (mushroom) will help alleviate the challenge resulting in improvement of efficiency of broiler production under various stress conditions. As the problem of global warming has created an enormous challenge for the livestock industry, especially in tropical and subtropical zones.

All parameters measured were not significantly ($P>0.05$) affected by the oral administration of *Pleurotus ostreatus* extract except the serum calcium and potassium at day 28 and albumin and calcium at day 56 (Table 1 and 2). Serum potassium was lowest in the *Pleurotus* treated groups (2000 and 4000 mg/L) at day 28 but numerically higher than the control group at day 56. This is an indication that there was no nephrotoxic potential by the extract to result in inability of kidney to concentrate electrolytes. The increased serum albumin content observed at day 56 may be related to direct effect of the antioxidant compounds such as phenols, ascorbic acid, vitamin E and β -carotene (Yang *et al.* 2002, Ekunseitan *et al.* 2017) contained in *Pleurotus ostreatus* capable of positively influencing the immune status of the birds, cellular response, maintenance of oncotic pressure and balance (Melilo 2013, de Lima *et al.* 2020). This outcome of the current study contradicts Hassan *et al.* (2020) who observed no effect dietary supplementation of oyster mushroom blood total protein, total albumin, globulin, or urea nitrogen concentrations between the treatment groups and control.

Increased serum calcium observed in the study was dose-dependent with the highest value observed in 4000 mg/L treatment group. Serum calcium is a basic essential component in animal systems for normal bone and body

Table 1. Effect of oral administration of *Pleurotus ostreatus* on haematological parameters and some serum metabolites of broiler chickens (day 28)

Parameter	<i>Pleurotus ostreatus</i>		
	0 mg/L	2000 mg/L	4000 mg/L
<i>Haematological indices</i>			
Packed cell volume (%)	39.75±3.22	37.75±1.38	34.00±0.71
Haemoglobin (g/dl)	13.20±1.08	12.55±0.46	11.50±0.22
Red blood cell ($\times 10^{12}/L$)	1.93±0.55	1.40±0.22	1.55±0.21
White blood cell ($\times 10^9/L$)	10.36±0.59	11.75±0.82	12.18±0.84
Heterophils (%)	30.50±9.54	34.75±9.39	19.75±3.75
Lymphocytes (%)	68.50±10.51	64.25±9.24	78.75±3.50
Eosinophils (%)	1.00±1.00	0.50±0.29	0.75±0.48
Basophils (%)	0.00±0.00	0.00±0.00	0.25±0.25
Monocytes (%)	0.00±0.00	0.50±0.29	0.50±0.29
<i>Serum metabolites</i>			
Total protein (g/dl)	3.90±0.19	3.80±0.15	3.89±0.36
Albumin (g/dl)	1.65±0.25	1.83±0.25	1.58±0.13
Globulin (g/dl)	2.25±0.10	1.98±0.21	2.30±0.25
Calcium (mg/dl)	8.93±0.39 ^{ab}	8.38±0.16 ^b	9.58±0.31 ^a
Potassium (mEq/l)	1.49±0.34 ^a	0.61±0.20 ^b	0.64±0.05 ^b
Aspartate aminotransferase (U/L)	66.75±4.31	70.75±5.19	67.50±2.75
Alanine aminotransferase (U/L)	37.00±1.47	35.50±2.02	38.50±1.55
Alkaline phosphate (U/L)	29.75±3.20	33.00±1.91	29.00±1.22

^{a,b}Means not followed by the same superscript are significantly different ($P<0.05$) along the row. Mean values±SEM.

system homeostasis (Stanford 2006), muscle and nerve conduction for effective transfer of nerve impulses blood (Guyton and Hall 2000), blood clotting and the control of some hormone secretion. The improvement and the stability of systems (calcium homeostasis) is as a result of various polyphenolic compounds inherent in *Pleurotus ostreatus* with the ability to scavenge free radicals. The positive effect on bird's serum calcium exhibited by *Pleurotus ostreatus* may solve the problem of malabsorption occurrence which interfere with intestinal and uterine absorption of calcium across the intestinal and uterine wall during bone and eggshell formation (Igwe *et al.* 2017) when serum calcium is lowered in laying birds, as overall skeletal system provides information or hinged on calcium homeostasis.

Table 2. Effect of oral administration of *Pleurotus ostreatus* on haematological parameters and some serum metabolites of broiler chickens (day 56)

Parameter	<i>Pleurotus ostreatus</i>		
	0 mg/L	2000 mg/L	4000 mg/L
<i>Haematological indices</i>			
Packed cell volume (%)	37.00±0.91	36.75±1.32	34.50±1.44
Haemoglobin (g/dl)	11.58±0.51	12.62±0.72	11.10±0.73
Red blood cell (×10 ¹² /L)	2.85±0.19	3.33±0.34	2.55±0.25
White blood cell (×10 ⁹ /L)	10.90±0.53	10.63±1.03	11.05±1.21
Heterophils (%)	33.00±2.52	32.50±1.32	30.75±1.43
Lymphocytes (%)	64.75±2.50	66.00±2.04	67.25±1.32
Eosinophils (%)	0.50±0.29	0.25±0.25	0.50±0.29
Basophils (%)	0.50±0.29	0.25±0.25	0.25±0.25
Monocytes (%)	1.25±0.25	1.00±0.71	1.25±0.48
<i>Serum metabolites</i>			
Total protein (g/dl)	4.35±0.31	3.85±0.47	4.43±0.48
Albumin (g/dl)	1.70±0.07 ^b	1.80±0.00 ^b	2.33±0.25 ^a
Globulin (g/dl)	2.65±0.28	2.05±0.47	2.13±0.46
Calcium (mg/dl)	8.80±0.48 ^b	14.20±3.04 ^{ab}	16.3±31.28 ^a
Potassium (mEq/l)	5.18±1.39	6.28±1.22	6.68±1.28
Aspartate aminotransferase (U/L)	50.50±1.66	53.25±4.55	47.75±1.31
Alanine aminotransferase (U/L)	28.50±2.10	28.75±2.32	28.50±1.85
Alkaline phosphate (U/L)	44.25±5.31	40.25±2.95	42.00±1.87

^{a,b,c}Means not followed by the same superscript are significantly different (P<0.05) along the row. Mean values±SEM.

The non-significance influence on haematological indices observed in the current study at both day 28 and 56 aligns with report of Toghiani *et al.* (2012) that intrinsic substances in mushroom do not positively stimulates the process of haematogenesis but contradicts reports of de Lima *et al.* (2020) who observed a significant increase in haemoglobin concentration and mean corpuscular volume (MCV).

The effect of *Pleurotus ostreatus* on the antioxidant status of meat is presented in Table 3. The highest (P<0.05) glutathione peroxidase (GSH-Px) was observed in 4000 mg/l group while least and comparable values was obtained in the control and 2000 mg/l group. The genetic selection process for recent strains of broiler for broader muscles

Table 3. Effect of *Pleurotus ostreatus* on the antioxidant status of breast meat from broiler chicken

Parameter	0 (mg/l)	2000 (mg/l)	4000 (mg/l)
Glutathione peroxidase (µmg)	1.60±0.44 ^b	1.43±0.05 ^b	2.15±0.16 ^a
Thiobarbituric acid reactive substances (µmg)	0.23±0.05 ^b	0.90±0.17 ^a	0.44±0.03 ^b

^{a,b}values in a row not sharing a common alphabet are significantly different (P>0.05). Mean values±SEM.

makes their meat tissue and products susceptible to oxidative instability or damage due to the presence of unsaturated lipids (Est´evez 2015, Dominguez 2019) and likewise variety of oxidizable functional groups of the amino acids it contains. GSH-Px activity was linearly related to the concentration of the level of administration of *Pleurotus ostreatus*. *Pleurotus* species has been documented to elevate peroxidase, superoxide dismutase (SOD) and catalase activity *in vitro* (Khatun *et al.* 2009). Its positive effect on GSH-Px may be hinged on its antioxidant property due to the presence of phenolic compounds capable of neutralizing free radicals in the meat samples (Rodriguez-Carpena *et al.* 2011, Amarowicz and Pegg 2019) and Selenium, a mineral positively involved in GSH-Px activity. Inclusion of dried mushroom in diets has been reported to increase synthesis of superoxide dismutase (SOD), catalase, glutathione, glutathione reductase, glutathione peroxidase (GSH-Px) and glutathione S-transferase production in meat (Giannenas *et al.* 2010, Mahfuz *et al.* 2020b, Mahfuz *et al.* 2020a), thereby affirming the increased enzyme activity in the present study. GSH-Px activity has been discovered to decreased after antibiotics treatment, therefore the positive effect exhibited by *Pleurotus ostreatus* affirms reports of studies of its strong evidence of antioxidant activity *in vitro* (Hassan *et al.* 2020). Thiobarbituric Acid Reactive substances was highest at 2000 mg/l but least in meat obtained from the control group and 4000 mg/l. The antioxidant protective influence was distinct and pronounced at the highest level of administration of oyster mushroom. Oyster mushroom contains a significant amount of minerals notably selenium (Se) (Alam *et al.* 2008, Ramos *et al.* 2019) which is principal component of GSH-Px (Se-containing enzyme). The preeminent enzyme-activity could be due to active initiation process of glutathione (GSH) enzyme due to selenium uptake (Giannenas *et al.* 2010b) from extract. The higher activity may be a direct response to the TBARs values being significantly lowered as elevated GSH-Px is majorly due to glutathione enzyme initiation passive sparing of glutathione through reduction of oxidative stress load in the meat sample. Plant-derived antioxidants such as ascorbic acid which is present in *Pleurotus ostreatus* (Ekunseitan *et al.* 2017, Ramos *et al.* 2019, Montes *et al.* 2020), has been shown to reduce the production of TBARs and drip loss in chickens' pectoralis muscles, thus improving the oxidative status. These

Table 4. Effect of oral administration of *Pleurotus ostreatus* (oyster mushroom) on some quality attributes of breast meat of broiler chickens

Parameter	0 mg/l	2000 mg/l	4000 mg/l
Moisture (%)	72.38±0.55	71.35±0.24	71.30±0.57
Crude protein (%)	23.35±1.20 ^b	24.72±0.74 ^a	24.35±0.22 ^a
Fat (%)	2.32±0.06	2.33±0.08	2.33±0.06
Water absorption capacity (%)	13.93±2.43	16.32±2.43	15.46±3.17
Water holding capacity (%)	39.16±0.67 ^c	42.63±0.62 ^b	45.43±0.25 ^a

^{a,b}Means not followed by the same superscript are significantly different ($P < 0.05$) along the row. Mean values±SEM.

sequence of process of positive effect will concomitantly result in stabilizing the meat tissue against lipid oxidation thereby prolonging the quality of meat and shelf life as use of mushrooms and plant materials containing phenolic antioxidant in poultry delayed lipid and protein oxidation in meat and poultry products (Shah *et al.* 2014) and improved growth.

The effect of oral administration of *Pleurotus ostreatus* on quality attributes of breast meat of broiler chickens is shown in Table 4. The results revealed that *Pleurotus ostreatus* had influence ($P < 0.05$) on crude protein and WHC of the meat. The improvement in the crude protein content contradicts the report of Lee *et al.* (2012) who observed no effect on crude protein content of meat but rather a reduction in fat component only in meat samples from birds with access to mushroom. This increase in protein translates to improved meat quality as chemical composition of meat muscles is a vital quality measurement of broiler meat (Grashorn *et al.* 2002). The increase in the WHC indicates a higher ability of the meat tissue to retain intrinsic water within as meat with reduced WHC allows the seepage of proteins off the meat. Protein and fibre component in mushrooms has been reported (Wang *et al.* 2019) to increase retention of water and moisture when used as replacer in meat formulation during process. Higher drip loss or reduced WHC results in liquid leakage and loss of soluble intrinsic nutrients resulting in the development of hardened, tasteless muscles, off-flavours and decreased quality attributes. The reduction of MDA production in the meat muscles in the present study by oral administration of *Pleurotus ostreatus* could be directly linked with the higher WHC because improved oxidative status provides better protection against stress-induced increase in lipid oxidation. Antioxidant protective effect was more pronounced at the highest dosage of the extract.

It can be concluded that oral administration of *Pleurotus ostreatus* up to 4000 mg/L can be used as natural antioxidants in alleviating stress, maintaining homeostasis in poultry birds and improving the oxidative stability of meat in broiler chickens.

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