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Pleurotus Ostreatus: Its Effect on Carcass, Serum Metabolites and Meat Lipoprotein Content of Broiler Chickens

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ABSTRACT

High demand by consumers for poultry meat and products devoid of residue of antibiotics and micro-organisms has been the drive for recent research into the Nigerian poultry industry. This study was carried out to investigate the potential effect of *Pleurotus ostreatus* (oyster mushroom) on carcass, blood and meat cholesterol of broiler chickens. A total of 90 two-week old broiler chickens were randomly allotted to three levels of administration of ethanolic extract of *Pleurotus ostreatus*: control (0 ppm), 7.5 ppm and 15 ppm. Data obtained on carcass characteristics, serum metabolites and meat lipid profiles were subjected to a completely randomised design. Most carcass indices considered were not influenced ($p>0.05$) except those for the legs and breast. Breast meat (%) was highest in 15 ppm group. Meat lipoprotein content was significantly ($p<0.05$) affected by oyster mushroom administration except for triglycerides and very low density lipoprotein (LDL). High density lipoprotein (HDL) was highest in the 15-ppm dosed group, while LDL was lowest in meat obtained from the 15-ppm dosed group. In conclusion, the use of oral administration of *Pleurotus ostreatus* at 15 ppm in water can increase HDL and decrease the concentrations of LDL, VLDL and triglycerides in thigh meat.

Keywords: Carcass characteristics, oyster mushroom, antibiotics, health indices, lipoproteins, growth promoters, blood serum, meat quality

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INTRODUCTION

Antibiotics are growth promoters commonly used in the poultry industry. At present the convention of using antibiotics as growth promoters in the diets of birds

has been proscribed due to fears about their possible residue/filtrate in animal tissue (Dipeolu, 2004; Simon & Baxter, 2006). The prohibition on the use of most synthetic antimicrobial growth promoters (AGP) and their relatively negative residual effects has led to preference for natural ingredients such as oyster mushroom extracts (*Pleurotus ostreatus*) over synthetic antimicrobial growth promoters (SAGP).

Mushrooms have long been cherished as a vital source of bioactive compounds of medicinal value (Breene, 1990). Fungi have a wide range of activities that have found their use in combatting outbreaks of disease (Chang & Buswell, 1996). Mushrooms and their different derivatives contain a variety of active substances like ergothioneine (Dubost et al., 2007) phenolic antioxidants, variegatic acid and dibiviquinone (Kasuga et al., 1995). These biologically active components found in mushroom possess antifungal, antibacterial, antioxidant and antiviral properties (Barros et al., 2009) and have been found admissible as insecticides and nematicides.

The quality of carcass (meat) produced by the poultry industry has a direct consequence on consumers' health. Many consumers, therefore, endeavour to eat more healthy products (Bickel & Wetscherek, 2005) with the supposition that chicken meat has low fat content compared to beef or pork. Numerous attempts have been made to produce meat, especially broilers, free of synthetic drugs, lower in cholesterol and fat content and without antibiotics. This was to prevent drug residue in meat samples and subsequent introduction of emerging antibiotic resistant strains (Saleha et al., 2009). These attempts were made with no priority given to attendant consequences on the performance of the animals.

The poultry industry in Nigeria at present has set no ban on antibiotic usage; however, researchers have taken on the challenge of seeking substitutes that can replace synthetic drugs and also satisfy consumer demand for residue-free products. Currently, plant-based products have been identified as replacements for synthetic drugs because they possess phyto-nutrients with excellent antioxidant and anti-cholesterolemic properties. Of particular interest is mushroom (*Pleurotus ostreatus*). The chemical evaluation of *Pleurotus ostreatus* revealed it to be a biological plant capable of inducing several bio-medicinal functions and activities in animals e.g. immunomodulation, anti-tumour causing, antidiabetic, antibiotic or antiparasitic, hypercholesterolemic, hepatoprotective, antipathogenic, detoxicant and antioxidant (Guo et al., 2004; Wasser, 2010). The present study was, therefore, conducted to determine the possible effect of *Pleurotus ostreatus* extract on carcass yield, serum biochemical and the carcass lipid profile of broiler chickens.

MATERIALS AND METHOD

Area Depiction

The experiment was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun

State, Nigeria located on latitude 7° 15' N, longitude 3° 26' E and 76 m above sea level (Google Earth, 2014).

Experimental Birds and Management

A total of 90 two-week old Arbor Acre broiler chicks were randomly divided into three treatment groups i.e. T1, T2, T3 containing 30 birds per treatment. T1 served as the control group, where the birds were given medication (coccidiostat and antibiotics), while T2 and T3 were administered different levels of oyster mushroom extract in water. Each group was further subdivided into 3 replicates of 10 birds.

Experimental Period

The duration of the experiment was six weeks. The study was carried out between July and September 2015.

Processing of Experimental Materials

Fresh oyster mushroom (*Pleurotus ostreatus*) was purchased and the extraction process was carried out at the Processing Laboratory, Department of Animal Production and Health, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

Procedure

A fresh sample of oyster mushroom was properly rid of dirt and was subsequently weighed using a digital scale. Both stalks and pliae were rendered into bits and soaked in ethanol to the ratio of 1:2 (w/v). The mushroom was soaked in a determined volume of ethanol for 24 h after which the extract was poured out. Subsequent soaking was done until a clear solution was obtained. The extract was then concentrated using a water bath at 50^{o}C until a semi-liquid solution was obtained.

Details. Antibiotics with scheduled medication and vaccination was given to the birds in the control experimental group (T1) while mushroom (*Pleurotus ostreatus*) extract was given in water i.e. orally to birds in the other two groups (T2, T3) at 7.5 ppm and 15 ppm twice a week throughout the duration of the study.

Diet. The birds were fed *ad lib* from the starting phase i.e. 14-28 days on a commercial starter and a finisher ration from Days 29-56.

Haematological analysis. On the 28th and 56th day of the experiment, blood samples (2 ml each) were collected from three birds per replicate into Ethylene Diamine Tetra-Acetate (EDTA) bottles for haematological analysis. A heparine tube was used for the serum constituents. The blood samples were analysed for Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC) and Haemoglobin (Hb). Standard methods were used for haematological parameters (Schalm, 1986), while serum total protein, albumin and globulin were analysed colourimetrically using a diagnostic reagent kit.

Determination of carcass yield. At the end of the experiment, four birds per replicate were randomly selected, weighed,

slaughtered and eviscerated. The dressed weight was determined while cut-up parts such as the head, neck, thigh, legs, drumstick, back, wings and breast were weighed using a digital scale. The organs such as liver, gizzard and heart were also weighed. The averages of weight were calculated and the parts mentioned were expressed as percentages of the live weight (Sogunle et. al., 2009).

Muscle pH. At 20 min post-mortem, the breast muscle pH was determined at a depth of 2.5 cm below the surface using a Model PH-211 meter equipped with a spear electrode.

Meat Cholesterol Profile

A known quantity (50 g) of meat was sampled from the thigh region of dressed carcasses and analysed to determine the amount of total cholesterol, triglyceride, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL).

Determination of Total Cholesterol

Serum cholesterol was determined spectrophotometrically according to the methods of Allain et al. (1974). The reagent was made up of three enzymes, cholesterol esterase (C, E), cholesterol oxidase (CO) and peroxidase (POD) and two substrates, 4-aminoantipyrine (4-AA) and phenol.

Procedure. Three clean test tubes labelled blank (B), standard (S) and test (T) were arranged in a test tube rack. A volume of 10ml of distilled water, standard cholesterol and serum was added to each test tube, respectively. A volume of 1ml of reagent was added to all the test tubes. The reaction mixtures were mixed and incubated for 10 min at room temperature and the absorbance of the sample was read at 550 mm wavelength against the blank.

$$\text{Cholesterol Concentration in Serum } \left(\frac{\text{mg}}{\text{dl}}\right) = \frac{(\text{Absorbance of test} \times \text{Concentration Of STD})}{\text{Absorbance of STD}}$$

Determination of HDL

Three clean test tubes marked and labelled blank (B), standard (S) and test (T) were arranged in a test tube rack. To each of these tubes were added 1.0 ml of working reagent while 0.05 ml distilled water, HDL standard and super nutrient were added to each test tube. The reaction mixtures were incubated at 37^{o}C for 5 min. The absorbance of the standard (Abs. S) and test samples (Abs. T) against the blank were measured within 60 min using a spectrophotometer. Below is the calculation for measuring HDL cholesterol in the samples.

$$\text{HDL cholesterol (mgldl)} = \frac{\text{Abs. T x con. of STD}}{\text{Abs. S}}$$

Determination of LDL-C

Three clean test tubes marked or labelled blank (B), standard (S) and test (T) were arranged in a test tube rack. A volume of 1000 ml of cholesterol reagent was added to each test tube, 50 ml of water, cholesterol stand and super nutrient were added to each test tube. The reaction mixture were incubated for 10 min at 25^{o}C, and the

absorbance of the test (Abs. T) and standard (Abs. S) samples were measured against the blank (B). Below is the calculation for measuring the concentration of cholesterol in the samples.

$$\text{Conc. of cholesterol} = \frac{\text{Abs. T x conc. of STD}}{\text{Abs. S}}$$

Determination of VLDL-Cholesterol

The concentration of very low density lipoprotein (VLDL) cholesterol was calculated by modification of Freidwald's formulae, as shown below.

$$\text{VLDL} - \text{Cholesterol} = \text{triglyceride value divide by 5}$$

Data Analysis

Data obtained were subjected to a completely randomised design and the significant differences among the treatment means were separated using the Dunnett Multiple Comparisons Test contained in the Statistical Analysis Software (SAS) (2009) package.

RESULTS AND DISCUSSION

Effects of *Pleurotus ostreatus* (Oyster Mushroom) Extract at Different Levels on Carcass Yield of Broiler Chicken

The effect of the ethanolic *Pleurotus ostreatus* (oyster mushroom) extract on carcass yield of broiler chickens is presented in Table 1. All parameters considered were not significantly ($p>0.05$) affected. However, the wings, back, thigh and drumstick were numerically higher in the 15-ppm dosed group. The drumstick, a prime cut of carcass that provides the greatest portion of edible meat in broilers (Sogunle et al., 2009), was highest in the 15-ppm group compared to the other treatment groups. This confirmed the report of Guo et al (2003) and Giannenas et al. (2010) that mushroom was capable of enhancing the growth performance of birds, a phenomenon that was reflected in the carcass composition, especially in the meaty cut-parts.

Table 1
Effects of Pleurotus ostreatus *(oyster mushroom) extract on carcass yield of broiler chickens*

Parameter	0 ppm	7.5 ppm	15 ppm	SEM
Live weight (g)	1566.70	1433.30	1466.70	44.44
Carcass (g)	1360.00	1233.30	1363.30	50.16
[1]Cut-up parts (%)				
Head	2.81	2.94	2.92	2.26
Neck	5.02	4.33	4.91	0.77
Wings	8.02	8.26	8.14	8.29
Back	14.36	13.58	14.98	13.65
Legs	5.11[a]	4.40[b]	4.43[b]	0.17
Thigh	9.28	9.21	9.61	0.81
Drumstick	10.64	10.18	10.73	0.28
Breast	17.43[ab]	15.90[b]	18.69[a]	0.58
PH	6.58[b]	6.73[a]	6.60[ab]	0.15
[2]Organ (%)				
Gizzard	2.18	2.57	2.03	0.07
Liver	1.62	1.66	1.78	1.51
Abdominal Fat	0.00	0.00	0.00	0.00
Heart	0.41	0.44	0.45	0.33

[a,b]: means in the same row with different superscript differ significantly ($p<0.05$)
[1,2]: values are expressed as percentage of live weight.

The non-significant effect of *Pleurotus ostreatus* (oyster mushroom) extract on organs of birds in the present study follows asimilar trend of occurrence as found by Guo et al. (2004), who used fungus (Mushroom: *Lentinus edodes* and *Tremella fuciformis*) as alternatives for antibiotics in broilers. The extract exhibited growth promoting capacity as did the antimicrobial growth promoters used in the control group. Alternative growth promoting options of plant origin like turmeric rhizome (Emadi & Kermanshahi, 2006) have been documented to improve carcass quality and cut prime, decrease the abdominal fat pad and increase liver weight and whole goblet weight (Durrani et al., 2006; Dhama et al., 2015). The insignificant effect of oyster mushroom extract on relative weight of organs also aligns with the reports of Denli et al. (2003) and Daneshmad et al. (2012), who used combinations of oyster mushroom and other phytobiotic options.

Effects of *Pleurotus ostreatus* (Oyster Mushroom) Extract at Different Levels on Meat Cholesterol of Broiler Chicken

The effect of *Pleurotus ostreatus* (Oyster mushroom) extracts at different levels of inclusion on the cholesterol of broiler meat is shown in Table 2. All parameters considered were influenced ($p<0.05$) by oyster mushroom administration except triglyceride and VLDL-cholesterol. Total cholesterol was highest ($p<0.05$) in the 15-ppm group, while statistically lower and similar values were obtained in the control and the 7.5-ppm group.

Table 2
Effects of Pleurotus ostreatus *(oyster mushroom) extract at different levels on meat cholesterol of broiler chickens*

Parameter	Control	7.5 ppm	15 ppm	SEM
Total cholesterol (mg/dl)	64.50^{b}	61.00^{b}	74.50^{a}	2.33
Triglyceride (mg/dl)	101.00	112.50	92.50	13.67
High density lipoprotein (mg/dl)	30.30^{b}	27.50^{b}	46.50^{a}	3.01
Low density lipoprotein (mg/dl)	14.25^{a}	11.00^{b}	9.50^{c}	1.40
Very low density lipoprotein (mg/dl)	20.20	22.50	18.50	2.73

^{abc}: means in the same row with different superscript differ significantly ($p<0.05$)

The HDL cholesterol concentration of meat increased as the level of administration of oyster mushroom increased, with the highest value obtained in the 15-ppm group. This increase indicated the ability of oyster mushroom extract in aggregation HDL-c components in meat, favouring its consumption by people with no predisposition to adverse disease conditions, thereby meeting the increasing demand for organically produced food.

The LDL-cholesterol content of meat sampled was lowest in the 15-ppm dosed group, with the highest value obtained in

the control group. The VLDL-cholesterol was numerically lowest in the 15-ppm group although statistically similar to the other treatment groups. This confirms the ability of plant extracts to influence the post-mortem quality of meat, particularly its cholesterol concentration (Wallace et al., 2010).

Effects of *Pleurotus ostreatus* (Oyster Mushroom) Extract on Some Serum Metabolites of Broiler Chickens

All serum metabolites determined presented in Table 3 were not significantly ($p>0.05$) affected except for triglyceride at 28 days of age. There were no marked changes in the concentration of serum for total protein, albumin and globulin for all the groups; however, the percentage increase in albumin was greatest in the control group. Mushroom contains basic antioxidant compounds, namely ascorbic acid, Vitamin C, Vitamin E, β carotene and phenolic compounds (Yang et al., 2002), therefore its usage will enhance the immunity of the birds.

Table 3
Effects of Pleurotus ostreatus *(oyster mushroom) extract on some serum metabolites of broiler chickens*

Parameter		Control	7.5 ppm	15 ppm	SEM
Total Protein (g/dl)	**56 days**	4.95	5.20	5.40	0.13
	28 days	4.60	4.90	5.10	0.21
Albumin (g/dl)	**56 days**	2.95	2.85	2.95	0.12
	28 days	2.30	2.80	2.85	0.22
Globulin (g/dl)	**56 days**	2.00	2.35	2.45	0.11
	28 days	2.30	2.10	2.25	0.07
Cholesterol (mg/dl)	**56 days**	92.00	85.50	82.00	4.10
	28 days	83.50	80.00	85.50	2.88
Triglyceride (mg/dl)	**56 days**	94.00	84.00	87.50	2.57
	28 days	96.00[a]	87.50[c]	107.50[a]	4.03

[abc]: means in the same row with different superscript differ significantly ($p<0.05$)

There was an increase in the serum cholesterol of the control and the 7.5-ppm dosed group (10.18 and 6.43 %, respectively), while a 4.01% reduction was observed in birds administered the highest dosage of 15 ppm in water. Plants and phytochemicals alter cholesterol metabolism in animals (Wallace et al., 2010). *Pleurotus ostreatus* (oyster mushroom) contains phytogenic

substances, the most prominent being phenolic compounds which have hypo-cholesterolemic effect (Ikeda et al., 1992; Daneshyar et al., 2011) and are positively responsible for the reduction observed in serum total cholesterol. The possible pathway to this reduction may be the ability of the extract to inhibit the activity of HMG-CoA reductase in the liver (enzyme responsible for cholesterol synthesis) at the highest level of administration due to lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme, a reductase (HMG-CoA reductase) that interferes with mevalonate production. The concentration of lovastatin was high enough to bind with HMG-KoA reductase; this prevents mevalonat acid, a compound of cholesterol biosynthesis, from forming, an action that inhibits the formation of cholesterol (Cheung et al., 1993).

Triglyceride value (on Day 28) was statistically highest at 15 ppm, which was the least value obtained in the 7.5-ppm group. In addition, the percentage reduction in triglyceride value from Day 28 to 56 was greatest in the 15-ppm group compared to the control and the 7.5-ppm treatment. The depression in serum triglyceride may be due to altering and lowering of the hepatic lipogenesis effect of oyster mushroom extract since it is produced in the liver. It affected the triglyceride aggregation (Daneshyar et al., 2011) in meat sampled from the three treatment groups with least effect (numerically) in the 15-ppm dosed group. Since lipid content and composition of meat can greatly influence its dietetic, sensory and storage attributes (Chartrin et al., 2005), meat samples higher in HDL and lower in both LDL and VLDL are suitable for consumption with no possible predisposition to disease conditions.

CONCLUSION

The results of the study showed that application of *Pleurotus ostreatus* (oyster mushroom) (applied via drinking water at 15 ppm) could be considered a potential natural growth promoter and a cholesterol-lowering agent in producing organically acceptable meat to the populace without adverse effects on the health of people and the health indices of birds.

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REFERENCES

Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W., & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry, 20*(4), 470-475.

Barros, L., Venturini, B. A., Baptista, P., Estevinho, L. M., & Ferreira, C. F. R. (2008). Chemical composition and biological properties of Portuguese wild mushrooms: A comprehensive study. *Journal of Agriculture and Food Chemistry, 56*(10), 3856–3862.

Bickel, S., & Wetscherek, W. (2005). Influence of fat source on the performance of broilers and on relevant carcass characteristics for consumers (3rd report): Effects of the use of rape seed oil ad animal fat in poultry fattening on fatty acid quality of grilled broiler meat. *Bodenkultur, 56*(1/4), 39–45.

Breene, W. M. (1990). Nutritional and medicinal value of specialty mushrooms. *Journal of Food Protection, 53*(10), 883–894.

Chang, S. T., & Buswell, J. A. (1996). Mushroom nutriceuticals. *World Journal of Microbiology Biotechnology, 12*(5), 473–476.

Chartrin, P., Schiavone, A., Bernadet, M. D., Guy, G., Mourot, J., Duclos, M. J., & Baeza, E. (2005). Effect of genotype and overfeeding on lipid deposition in myofibres and intramuscular adipocytes of breast and thigh muscles of ducks. *Reproduction Nutrition Development, 45*(1), 87–99.

Daneshmand, A., Sadeghi, G. H., & Karimi, A. (2012). The effects of a combination of garlic, oyster mushroom and propolis extract in comparison to antibiotic on growth performance, some blood parameters and nutrient digestibility of male broilers. *Brazilian Journal of Poultry Science, 14*(2), 141–147.

Denli, M., Okan, F., & Celik, K. (2003). Effect of dietary probiotic, organic acid and antibiotic supplementation. *Pakistan Journal Nutrition, 2*(2), 89–91.

Dharma, K., Latheef, S. K., Mani, S., Samad, H. A., Karthik, K., Tiwari, R., … & Tufarelli, V. (2015). Multiple beneficial applications and modes of action of herbs in poultry health and production. *International Journal of Pharmacology, 11*(3), 152–176.

Dipeolu, M. A. (2004). Problems and prospects of antibiotics residues in meat products in Nigeria. *Vom Journal Veterinary Science, 1*(1), 63–67.

Dubost, N. J., Ou, B., & Beelman, R. B. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry, 105*(2), 727–735.

Durrani, F. R., Ismail, M., Sultan, A., Suhail, S. M., Chand, N., & Durrani, Z. (2006). Effect of different levels of feed added turmeric (Curcuma longa) on the performance of broiler. *American Journal of Agriculture and Biological Sciences, 1*(2), 9–11.

Emadi, M., & Kermanshahi, H. (2006). Effect of turmeric rhizome powder on performance and carcass characteristics of broiler chickens. *International Journal of Poultry Science, 5*(11), 1069–1072.

Giannenas, I., Pappas, I. S., Mavridis, S., Kontopidis, G., Skoufos, J., & Kyriazakis, I. (2010). Performance and antioxidant status of broiler chickens supplemented with dried mushrooms (*Agaricus bisporus*) in their diet. *Poultry Science, 89*(2), 303–311.

Google Maps. (2014). *Federal University of Agriculture*.Retrieved from https://earth.google.com/web/@7.22330744,3.44033719,137.84884575a,1046.69760578d,35y,100.57030218h,44.99999706t,-0r/data=Cm4abBJmCiUweDEwM2EzN2MwMDA4ZDY4MDk6MHg2Mjc3MWVhYzUyMmQyNzM0GWy0vTOq5BxAIalRGZXRhQtAKitGZWRlcmFsIFVuaXZlcnNpdHkgb2YKQWdyaWN1bHR1cmUsIEFiZW9rdXRhGAIgAQ.

Guo, F. C., Savelkoul, H. F. J., Kwakkel, R. P., Williams, B. A., & Verstegen, M. W. A. (2003). Immunoactive, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. *World's Poultry Science Journal, 59*(4), 427–440.

Guo, F. C., Williams, B. A., Kwakkel, R. P., Li, H. S., Li, X. P., Luo, J. Y., … & Verstegen, M. W. A. (2004). Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Science, 83*(2), 175–182.

Ikeda, I., Imasato, Y., Sasaki, E., Nakayama, M., Nagao, H., Takeo, T., … & Sugano, M. (1992). Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochimica et Biophysica Acta, 1127*(2), 141–146.

Kasuga, A., Aoyagi, Y., & Sugahara, T. (1995). Antioxidant activity of fungus Suihs bovinus (L: Fr.) O. Kuntze. *Journal of Food Science, 60*(5), 1113–1115.

Saleha, A. A., Myaing, T. T., Ganapathy, K. K., Zulkifli, I., Raha, R., & Arifah K. (2009). Possible effect of antibiotic-supplemented feed and environment on the occurrence of multiple antibiotic resistant Escherichia coli in chickens. *International Journal Poultry Science, 8*(1), 28–31.

SAS Institute Inc. (2009). *SAS/STAT® 9.2. User's Guide* (2nd Ed.). Cary, North Carolina: SAS Institute Inc.

Simon, A. H., & Baxter, G. A. (2006). Biosensor screening for veterinary drug residues in food stuffs. *Journal of AOAC International, 89*(3), 862–867.

Sogunle, O. M., Ekunseitan, D. A., Adeoti, M. T., Iyanda, A. I., & Fanimo, A. O. (2009). Performance and carcass characteristics of two strains of broiler chickens fed three different commercial feeds. *Journal of Applied Agricultural Research, 1*, 53–59.

Wallace, R. J., Oleszek, W., Franz, C., Hahn, I., Baser, K. H. C., Mathe, A., & Teichmann, K. (2010). Dietary plant bioactives for poultry health and productivity. *British Poultry Science 51*(4), 461–487.

Wasser, S. P. (2010). Current findings, future trends and unsolved problems in studies of medical mushrooms. *Applied Microbiology and Biotechnology, 89*(5), 451–58.

Weiss, D. J., & Wardrop, K. J. (Eds.). (1986). *Schalm's Veterinary Hematology* (6th Ed.). United States of America, USA: John Wiley & Sons.

Yang, J. H., Lin, H. C., & Mau, J. L. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry, 77*(2), 229–235.