White Blood Cell and Serum Biochemistry of Rabbits on Varying Levels of Oyster Mushroom (*Pleurotus Ostreatus*) Extract under Two Housing Types

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Abstract

A total of ninety-six, six weeks old rabbits were grouped into two housing types (steel and wooden hutches), sex (male and female) and four inclusion levels (0, 5, 10 and 15 ml) of oyster mushroom extract per litre of water in a 2 × 2 × 4 factorial experimental layout. One rabbit each was selected from each replicate at end of the 10th week of the experiment for white blood cell and serum biochemistry analysis. Results showed that growing rabbits on 5 ml of mushroom (*Pleurotus ostreatus*) extract per litre of drinking water had the lowest ($P < 0.05$) neutrophil (31.00 %) and the highest ($P < 0.05$) lymphocyte (67.25 %) in the white blood cell. Male rabbits in steel hutch have a higher ($P < 0.05$) eosinophil (0.75 %) in the white blood cell than the female rabbits in steel hutch (0.00 %). Rabbits in steel hutch had a lower ($P < 0.05$) low density lipoproteins (13.89 mg/dl) in the serum than rabbits housed in wooden hutch (16.02 mg/dl). It was concluded that housing rabbits in wooden hutch and inclusion of oyster mushroom up to 15 ml as phytobiotic for rabbits would optimize their health status.

Keywords: housing types; steel hutch; wooden hutch; oyster mushroom extract; white blood cell; serum biochemistry; rabbits; phytobiotic.

Introduction

Housing constitutes an important factor in rabbit production (Mailafia *et al.*, 2010). The main purpose of housing is to protect the rabbits against adverse climatic conditions, predators, ectoparasites and endoparasites (Hoy and Verga, 2006). The main welfare indicators to assess suitability of rabbit housing are mortality, morbidity, physiological parameters, rabbit behaviour and production performance (Hoy,

2008). Steel hutches can be easily cleaned, disinfected and also help to prevent unsanitary conditions and inadequate ventilation, that aid and promote spread of bacterial diseases which wooden hutches are more prone to because wood is very hard to clean and in turn become sources of contamination (Lebas, 1997; Shaeffer and Kime, 2008), however, greater incidence of diseases especially respiratory problems such as pneumonia and chronic respiratory disease

might be prominent with the use of steel hutches due to uncontrolled air flow. The treatment and control of most of these bacterial diseases is through administration of antibiotic drugs. However, livestock farmers are increasing efforts to eliminate the usage of antibiotics and attempts have been made to replace chemical compounds from conventional multivitamins and antibiotics with natural plant products as active substances to improve on production results, health condition of rabbits and to produce organic rabbit meat (Gugolek *et al.*, 2011). This thus, necessitated the need to search for an organic agriculture friendly and cheaper alternative to the usage of conventional drugs such as the application of mushrooms.

Mushrooms have been widely used as food and food supplements from ancient times. Their usage is being increased day by day for their role as an important food item in human health, nutrition and disease prevention (Chang, 1996; Khan *et al.*, 2008). Dietary mushrooms provide a wide variety of medicinal properties and they are effective against certain life-threatening diseases. These medicinal properties might be due to the presence of some important substance in dietary mushrooms (Alam *et al.*, 2008). *Pleurotus* species are of highly medicinal importance and values, and this is due to the nutritional and chemical composition of these mushrooms. *Pleurotus* as health promoter is gaining more importance as compared to other medicinal mushrooms resulting in an upsurge in their research activities during the past decades (Patel *et al.*, 2012). Major medicinal properties attributed to oyster mushrooms include antibiotic, immunity and blood lipid lowering effects (Alam *et al.*, 2008). *Pleurotus* species of mushroom have some bacteria fighting substances such as pleuromutillin, an antibiotic which can kill various bacteria such as *Enterococcus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermis*, *Staphylococcus aureus*, *Streptococcus* (Morschhauser *et al.*, 2000; Sandven, 2000; Thomson and Moland, 2000; Stamets, 2005; Nithya and Ragunathan, 2009; Akyuz *et al.*, 2010). *Pleurotus* species have been reported to possess therapeutic properties like anti-oxidant, anti-inflammatory, immunostimulatory and immunomodulatory (Shamtsyan *et al.*, 2007; Elmastas *et al.*, 2007), and ribonuclease activity (Wang and Ng, 2000). *P. ostreatus* are very

effective in reducing the total plasma cholesterol and triglyceride level (Alam *et al.*, 2007) and thus reduce the chance of atherosclerosis and other cardiovascular and artery related disorders. *P. ostreatus* has been explored to combat simple and multiple drug resistant isolates of *E. coli*, *Staphylococcus epidermis*, *S. aureus* (Akyuz *et al.*, 2010) and species of *Candida* (Wolff *et al.*, 2008), *Streptococcus*, *Enterococcus* (Morschhauser *et al.*, 2000; Sandven, 2000; Thomson and Moland, 2000). Hence, this research studied the white blood cell and serum biochemistry of rabbits on varying levels of Oyster mushroom (*Pleurotus ostreatus*) extract under two housing types.

Materials and Methods

The experiment was carried out at the Rabbitry Unit of the Directorate of University Farms, Federal University of Agriculture (FUNAAB), Abeokuta, Ogun State, Nigeria. The site is located in the rain forest vegetation zone of South-Western Nigeria on latitude 7° 13' 49.46" N, longitude 3° 26' 11.98" E and altitude 76m above sea level. The climate is humid with a mean annual rainfall of 1037mm and mean temperature range of 31.9 to 34.7 °C and humidity from 79.7 to 90.1 %, respectively.

Ninety-six (96) rabbits consisting of forty-eight (48), six (6) weeks old male rabbits and forty-eight (48) six (6) weeks old female rabbits of mixed breeds (Chincilla, Dutch and New Zealand White) with initial live weight range of 600-650 g were used for the study. The rabbits were allotted on weight equalization basis into two different housing types of steel and wooden hutches of forty-eight (48) rabbits each. The housing types of steel and wooden hutches are further divided into male and female groups of twenty-four (24) rabbits each to check for the effect of sex and each group composed of four (4) varying levels (0, 5, 10 and 15 ml) of oyster mushroom (*Pleurotus ostreatus*) extract per litre of water. Six (6) rabbits were assigned to each treatment group and each group was replicated thrice with two (2) rabbits per replicate. The rabbits were acclimatized for a period of two (2) weeks prior to the start of the experiment. The experiment was carried out for a period of ten (10) weeks. The rabbits were maintained on concentrate diets and a uniform feed composition was fed to the rabbits as shown on Table 1. Antibiotics and multi-vitamins were administered orally in the course of the experiment

only to rabbits with no level of inclusion of oyster mushroom (*Pleurotus ostreatus*) extract. The multi-vitamins and antibiotics used was Neotreat WSP® from Kepro, Netherlands. The oyster mushroom (*Pleurotus ostreatus*) extract levels was administered to the rabbits thrice per week and at a day interval within each week. The oyster mushroom (*Pleurotus ostreatus*) extract levels was administered to the rabbits for eight (8) weeks and withdrawn for the last two (2) weeks of the experiment. The rabbits were housed individually in the steel and wooden hutch with each cell having dimension of 60 cm x 60 cm x 50 cm. Feed and water troughs were provided in the hutches with unrestricted access to feeds and fresh clean drinking water. The rabbits were raised under ambient temperature and photoperiod. Daily routine management procedure was adhered to.

Oyster mushroom (*Pleurotus ostreatus*) extract was prepared with the use of hot water extraction procedure by extending the boiling process of the mushrooms so as to fully extract the polysaccharides which is considered to be medicinal out of the mushroom cell wall (Sogunle *et al.*, 2016). The Oyster mushroom was poured in the pot at the rate of 500 g of Oyster mushroom to 1 litre of water and cooked at 57.2 °C for twenty (20) minutes. The newly formed extracts were then cooled and strain-off the mushrooms with the aid of a sieve. The extracts were kept in a dark-coloured recipient (to prevent photolysis due to light penetration) and then stored in the refrigerator (in the lower compartment of the refrigerator) until needed.

100 ml of the sample (Oyster mushroom extract) was mixed with 50 ml absolute methanol by soaking in a well cleaned bottle for a week. The mixture was shaken vigorously and allowed to stand in order to form a suspension. The organic solvent with extract was collected by filtering into a quartz beaker; the process was repeatedly carried out for two more consecutive times. The aliquot collected were combined and concentrated on a steam bath to about 5ml. This was purified by passing through a pasture pipette packed with anhydrous sodium sulphate on a membrane and air dried to about 2 ml for gas chromatographic analysis. The extract of the sample was subjected to Gas Chromatographic and Mass Spectroscopy (GC/MS) analysis, this group of powerful instruments interfaced helped to characterize the various compositions. The gas

chromatographic Model: 7890A (GC) analysis was performed on an Agilent Technologies interfaced with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70 v with an ion source temperature at 250 °C. Highly pure helium gas (99.9 % purity) was used as carrier gas, while HP-5ms (30 mm X 0.25 mm X 0.320 µm) was used as the stationary phase. The oven temperature was at 80 °C held for 5 minutes and increased to 250 °C while holding for 16 minute at the rate of 4 degrees/minute 1 µ / l was auto injected.

One (1) rabbit each was selected from each replicate at end of the 10th week of the experiment for white blood cell and serum biochemistry analysis. Blood sample of 3ml of blood was withdrawn from the ear vein of each rabbit by means of sterile hypodermic needle and syringe in the morning between 7:00 and 9:00. About 0.5 ml was used for white blood cell differential and stored in Bijou bottles with ethylene diamine tetra acetate (EDTA) as anticoagulant while the other 2.5 ml was stored without coagulant for serum biochemistry analysis. Sample bottles containing blood samples were placed on ice packs to maintain a cool and stable temperature and immediately sent for laboratory analysis.

The estimate of the total number of white blood cells was carried out immediately after collection of blood sample from the rabbits using Neubauer haemocytometer counting chamber (Jain, 1986). About 0.2 ml of blood sample was pipetted and mixed with 4 ml of WBC diluting fluid (WBC fluid made up of 3% aqueous solution of acetic acid and 1% gentian violet). The sample was then put into the haemocytometer and cell counted and expressed as 10⁹ WBC per litre of blood. The values for neutrophils, lymphocytes, monocytes, eosinophils and basophils were determined from the white blood cell count.

The serum biochemical measurements determined are total serum protein, serum globulin, serum albumin, serum glucose, serum urea, serum creatinine, serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides.

Total serum protein was prepared according to Kaneko (1989) using two solution. The first solution consists of 45g of sodium potassium tetrates (Rochelle salt) dissolved in 400 ml of 0.2N NaOH in a beaker. 1.5g of CuSO₄.5H₂O and 5g of potassium iodide was added and was dissolved

completely by stirring. The mixture was rinsed with 0.2N NaOH and poured into a flask. The solution was made up to a litre with 0.2N NaOH. The second solution consists of 0.5% potassium iodide (KI) in 0.2N NaOH working Biuret reagent. An aliquot of first solution was diluted to 250 ml with second solution. An aliquot of 0.1 ml was pipetted into a test tube and 2.9 ml of water was added. The blank consisting of 3.0 ml distilled water was pipetted into the test tube. To each test tube, 3.0 ml of working biuret reagent was added and all the tubes were incubated in 37°C water bath for 10 minutes. Readings were taken at 540 nm after setting the instruments to zero with the blank solution. Total serum protein of each sample was calculated from the formula below:

Total Serum Protein (g/100ml) =

$$\frac{\text{Optical density of test} \times \text{Concentration of standard}}{\text{Optical density of standard}}$$

Serum albumin was determined using the bromocresol purple method. The bromocresol purple is a stable complex with absorbance maximum at 600 nm. The intensity of the colour produced is directly proportional to the albumin concentration of the sample. 4 ml of bromocresol purple was added to 0.2 ml of each of the serum samples. Bromocresol purple solution (4 ml) was used as a blank. The content of each tube was mixed and left at room temperature for 10 minutes at pH 4.2 ± 0.05. After 10 minutes, the test solution was read at a wavelength of 640 nm in a spectrophotometer set to zero with the blank solution. Values of the samples were calculated using the formula:

Serum albumin (g/100 ml) =

$$\frac{\text{Optical density of test} \times \text{Concentration of standard}}{\text{Optical density of standard}}$$

Serum globulin was calculated as follows:

Serum globulin (g) =

Total Serum Protein (g) – Serum albumin (g)

Serum urea was determined using a kit (Quinica clinical spam) having a linear measurement of about 566.6 ml per litre of urea concentration. The serum urea was determined calorimetrically. The spectrophotometer (Model SP6-400 Ur Pyeunicam) was set at 600 nm wavelength and equivalent wavelength of sample read. Serum urea was calculated as:

Urea (mg) =

$$\frac{\text{Sample Optical Density} \times 40}{\text{Optical density of standard}}$$

Serum creatinine was analysed using calorimetric method. 1.0 ml of trichloroacetic acid and 1.0 ml of serum was mixed and centrifuged at 250 rpm for 10 minutes and the supernatant poured off. The mixture was allowed to cool for 20 minutes at a temperature of 25°C. The absorbance of the sample and standard was measured against the blank.

Concentration of Creatinine (mg/dL) =

$$\frac{\text{Absorbance of sample} \times 2}{\text{Absorbance of standard}}$$

Absorbance of standard

The serum cholesterol was determined using enzymatic endpoint method as described by Roeschlau *et al.* (1974). The reading taken at wavelength 520 nm.

High density lipoproteins (HDL) was determined as follows. About 200 µl of test sample was precipitated and left for 10 minutes. The supernatant was centrifuged at 4000 rpm for 10 minutes. Sample supernatant of 100 µl was added to 1000 µl cholesterol reagent, mixed well and incubated for 10 minutes at 37°C. The absorbance was read against blank on a spectrophotometer at 505 nm (Randox, 2012).

Concentration of HDL (mg/dL) =

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Absorbance of standard

Low density lipoproteins (LDL) was determined as follows:

About 10 µl of sample test was mixed well with 1000 µl of LDL cholesterol reagent and incubate for 10 minutes at 37°C with the standard. Its absorbance was read against blank on a spectrophotometer at 505 nm (Randox, 2012).

Concentration of LDL (mg/dL) =

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Absorbance of standard

Serum triglyceride was analysed using a spectrophotometer by measuring the absorbance of alkali hydrolysis forming glycerol which is oxidised to form periodate of test sample after incubation for 10 minutes at 37°C and read against the blank at 505 nm and standard reagents (Randox, 2012).

Concentration of triglyceride (mg/dL) =

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Absorbance of standard

The experiment layout was a 2 × 2 × 4 factorial arrangement and the data generated were subjected to a Completely Randomized Design. The rabbits were grouped into two housing types

Table 1: Gross composition of concentrate diet for the rabbits

Ingredients	Composition (%)
Maize (Yellow)	35.00
Soya bean meal	10.00
Wheat offal	20.00
Rice bran	17.00
Palm kernel cake	12.00
Fish meal (65%)	2.00
Bone meal	2.00
Limestone	1.00
Premix (Growers)	0.50
Salt (NaCl)	0.50
Total	100.00
Calculated Analysis	
ME (MJ/kg)	10.53
Crude protein (%)	16.64
Crude fibre (%)	6.68
Ether extract (%)	5.99

(steel and wooden hutch), sex (male and female) and of four inclusion levels (0 ml, 5 ml, 10 ml and 15 ml) of Oyster mushroom (*Pleurotus ostreatus*) extract per litre of drinking water. Significantly ($P < 0.05$) different means were separated using Tukey Test as contained in Minitab® 17.1.0 (2013). The data generated were analysed with the use of General Linear Model (GML) of the same package.

Results and Discussion

The results of the phytochemicals of Oyster mushroom (*Pleurotus ostreatus*) extract are presented in Table 2. The results are classified into the active compounds, quality, percentage composition of each compound and their bioactivity. The active compounds of 9-Octadecenoic acid (Z), methyl ester (oleic acid); Methyl octadeca-10, 13-dienoate; and 9, 12-Octadecadienoic acid (Z, Z), methyl ester (linoleic acid) in the Oyster mushroom (*Pleurotus ostreatus*) are of the highest quality while the least of quality was 1,6-Octadiene, 3, 7-dimethyl-, (S)- (linalool). The most abundant of these compounds was 9, 12-Octadecadienoic acid (Z,Z)- methyl ester which is linoleic acid; a polyunsaturated fatty acid with a constituent of 30.10 % while the least compounds contained in Oyster mushroom extract were 2H-Pyran, 3, 4-dihydro and Methyl 20-methyl-heneicosanoate with 1.94 %, respectively. The phytochemical analysis of Oyster mushroom extract in this study disclosed the presence of phyto-constituents. The presence of Benzene acetaldehyde; a

flavonoid compound which has antimicrobial properties was observed. The odor from *Pleurotus* mushroom called Anise could be attributed to the presence of benzaldehyde which smells more like almonds. Eman Mostafa and Farghaly (2014) observed the presence of benzene, 1-chloro-4-methoxy- (0.8 mass fractions) and benzene acetic acid (0.3 mass fractions) in Oyster mushroom with Benzene acetic acid being an active plant hormone, possessing a honey-like odor in low concentrations. The presence of Hexadecanoic acid, methyl (methyl palmitate) which also has antimicrobial and antioxidant properties was observed. This supports Wasser (2002) and Elmastas *et al.* (2007) who reported the presence of antioxidant activity and antioxidant compounds in edible Oyster mushrooms.

Table 3 shows the effects of housing types, sex and inclusion levels of Oyster mushroom on white blood cell count and white blood cell differential of rabbits. The housing types and the sex did not significantly ($P > 0.05$) influence white blood cell, neutrophil, lymphocyte, eosinophil, basophil and monocyte of the rabbits. However, the inclusion levels of Oyster mushroom had significant ($P < 0.05$) differences on neutrophil, lymphocyte of the rabbits. The rabbits on 0 ml (antibiotics and multivitamins) had the highest neutrophil (36.13 %) from the white blood cell while the rabbits on 5 ml of mushroom extract in drinking water had the lowest (31.00 %). The rabbits on 5 ml of mushroom extract in drinking water had the

Table 2: Phytochemicals of Oyster mushroom (*Pleurotus ostreatus*) extract

Active Compounds	Quality	Percentage	Bioactivity	References
2H-Pyran, 3, 4-dihydro-	53	1.94 %	Install tetrahydropyranyl (THP) protecting groups on alcohol and amines	Eman Mostafa and Farghaly, 2014; Jadhav <i>et al.</i> , 2014
Benzene acetaldehyde	90	2.76 %	Antimicrobial	Jadhav <i>et al.</i> , 2014; Joseph and Priya, 2011; Patel <i>et al.</i> , 2012; Golak-Siwulska <i>et al.</i> , 2018
2-Pyrrolidinone	80	5.02 %	e.g. Doxapram – stimulates chemoreceptors in the carotid arteries which in turn, stimulates the respiratory centre in the brain stem.	Eman Mostafa and Farghaly, 2014; Golak-Siwulska <i>et al.</i> , 2018
Methenamine	91	8.14 %	Used medically as Mandelic acid salt for the treatment of urinary tract infection (bacteriocidal)	Jadhav <i>et al.</i> , 2014; Joseph and Priya, 2011; Golak-Siwulska <i>et al.</i> , 2018
Hexadecanoic acid, methyl ester (Methyl palmitate)	97	10.22 %	Is the most common saturated fatty acid in animals, plants and micro-organisms with antioxidant and antimicrobial activities	Eman Mostafa and Farghaly, 2014; Jadhav <i>et al.</i> , 2014; Joseph and Priya, 2011; Patel <i>et al.</i> , 2012; Golak-Siwulska <i>et al.</i> , 2018
1, 6-Octadiene, 3, 7-dimethyl-, (S)- (linalool)	15	2.20 %	Anti-stress	Patel <i>et al.</i> , 2012; Golak-Siwulska <i>et al.</i> , 2018
9-Octadecenoic acid (Z)-, methyl ester (oleic acid)	99	17.99 %	Mono-unsaturated fatty acid associated with decreased LDL and possibly increased HDL	Fogarisa <i>et al.</i> , 2018; Eman Mostafa and Farghaly, 2014; Jadhav <i>et al.</i> , 2014; Golak-Siwulska <i>et al.</i> , 2018
10,13-Octadecadienoic acid, methyl ester	99	4.25 %	Unsaturated fatty acid	Fogarisa <i>et al.</i> , 2018; Golak-Siwulska <i>et al.</i> , 2018
Octadecanoic acid, methyl ester	98	10.18 %	Saturated fatty acid has antimicrobial activity	Fogarisa <i>et al.</i> , 2018; Patel <i>et al.</i> , 2012; Golak-Siwulska <i>et al.</i> , 2018
9, 12-Octadecadienoic acid (Z,Z)- methyl ester (linoleic acid)	99	30.10 %	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge; It has Omega 6 fatty acid	Alam <i>et al.</i> , 2007; Jadhav <i>et al.</i> , 2014; Joseph and Priya, 2011; Patel <i>et al.</i> , 2012; Sarangi <i>et al.</i> , 2006; Golak-Siwulska <i>et al.</i> , 2018
Benzamide, 4-ethyl-N-methyl-	35	2.36 %	Cell differentiation inducer: A method of treating a malignant tumor selected from the group consisting of leukemia, colorectal cancer, ovarian cancer, oral cancer, lung carcinoma, breast carcinoma, prostate carcinoma, and melanoma	Joseph and Priya, 2011; Patel <i>et al.</i> , 2012; Sarangi <i>et al.</i> , 2006; Golak-Siwulska <i>et al.</i> , 2018
Methyl 20-methyl-heneicosanoate	95	1.94 %	Silicone elastomers in cosmetic esters: teeth impression molds	Jadhav <i>et al.</i> , 2014; Golak-Siwulska <i>et al.</i> , 2018
1, 2-Benzenedicarboxylic acid, diisooctyl ester	64	2.90 %	Plasticizer compound: antimicrobial, antifouling	Jadhav <i>et al.</i> , 2014; Eman Mostafa and Farghaly, 2014; Joseph and Priya, 2011; Patel <i>et al.</i> , 2012 Golak-Siwulska <i>et al.</i> , 2018;
		100.00 %		

WBC= White blood cell

Table 3. Effects of housing types, sex and Oyster mushroom (*Pleurotus ostreatus*) extract levels on white blood cell count and white blood cell differential of rabbits

Parameters	Housing types			Sex			Mushroom extract levels				
	Steel hutch	Wooden hutch	SEM	Male	Female	SEM	0 ml	5 ml	10 ml	15 ml	SEM
WBC ($\times 10^9/L$)	7.69	7.29	0.42	7.30	7.65	0.40	6.90	7.48	7.41	8.13	0.57
Neutrophil (%)	33.40	33.94	1.02	34.00	33.37	0.96	36.13 ^a	31.00 ^b	34.25 ^{ab}	33.38 ^{ab}	1.35
Lymphocyte (%)	65.07	64.35	0.95	64.31	65.06	0.89	62.75 ^b	67.25 ^a	64.00 ^{ab}	64.75 ^{ab}	1.26
Eosinophil (%)	0.40	0.29	0.13	0.50	0.19	0.12	0.13	0.38	0.38	0.50	0.17
Basophil (%)	0.33	0.47	0.21	0.25	0.56	0.19	0.50	0.25	0.25	0.63	0.28
Monocyte (%)	0.80	1.00	0.30	1.00	0.81	0.28	0.50	1.13	1.38	0.63	0.40

^{a, b}: Means in the same row with different superscripts differ significantly ($P < 0.05$)

SEM= Standard Error of Means

highest lymphocyte (67.25 %) from the white blood cell while the rabbits on 0 ml (antibiotics and multivitamins) had the lowest (62.75 %). The study showed that rabbits kept under different housing types of steel hutch and wooden hutch, have similar white blood cell counts and white blood cell differential. Furthermore, the similar values of white blood cell counts and white blood cell differential of the rabbits housed in steel hutch and wooden hutch to the normal range of haematological value indices recommended for healthy rabbits as reported by Archetti *et al.* (2008) and Research Animal Resource (2009) indicates that housing rabbits in either steel hutch or wooden hutch would not affect negatively the body defense mechanism. The study revealed that sex did not influence the white blood cell and white blood cell differential of the rabbits. The result corroborates the report of Chineke *et al.* (2006) and Ozkan *et al.* (2012) who recorded non-significant difference of white blood cell of male and female rabbits. The study on the levels of administration of mushroom (*Pleurotus ostreatus*) extract in water revealed a significant effect on the white blood cell differential of the rabbits and this support Sogunle *et al.* (2016) who suggested that Oyster mushroom extract can replace antibiotics inclusion in drinking water of broiler chicken. The lower neutrophil value from rabbits on 5ml of Oyster mushroom (*Pleurotus ostreatus*) extract per litre of water as compared to the control and other mushroom extract levels implies that the immediate response mechanism of the rabbits in this group to protect against infection is the poorest (Harper *et al.*, 1979; Ogbuwu, 2008). Furthermore, the higher lymphocyte values recorded from rabbits on Oyster mushroom (*Pleurotus ostreatus*) extract in drinking water

may have been due to bacterial infections which would led to the production of more antibodies by the B lymphocytes to directly attack various infected cells. The lower percentage of lymphocyte from the rabbits on conventional antibiotics and multivitamins implies that the rabbits in this group are prone to be more susceptible to secondary and opportunistic infections (Ihedioha, 2008) while the higher values of the lymphocyte from rabbits on Oyster mushroom (*Pleurotus ostreatus*) extract indicates a more effective antibody production (Frandsen, 2003). The study thus, indicates that administration of Oyster mushroom (*Pleurotus ostreatus*) extract as replacement for conventional multivitamins and antibiotics for rabbits can enhance the immediate response mechanism and production of antibodies to directly attack various infected cells mostly by bacterial infections and this confirm the reports of Morschhauser *et al.* (2000); Sandven (2000); Thomsen and Moland (2000); Stamets (2005); Nithya and Ragunathan (2009) and Akyuz *et al.* (2010) that Oyster mushroom possess antibiotic and immune-boosting properties that can fight against bacterial diseases and infection.

In Table 4, the interactive effects between housing types and sex on white blood cell count and white blood cell differential of rabbits are shown. Significant ($P < 0.05$) differences were obtained on eosinophil. The male rabbits housed in wooden hutch had the highest eosinophil (0.75 %) from the white blood cell while the female rabbits housed in wooden hutch had the lowest (0.00 %). The result of white blood cell count and white blood cell differential in the interaction between housing types and sex revealed that the sex of the rabbit will not affect its body defense mechanism when housed in either steel hutch

Table 4. Interactive effects between housing types and sex on white blood cell count and white blood cell differential of rabbits

Parameters	Steel hutch		Wooden hutch		SEM
	Male	Female	Male	Female	
WBC ($\times 10^9/L$)	7.40	8.01	7.20	7.37	0.57
Neutrophil (%)	32.13	34.86	35.88	32.22	1.35
Lymphocyte (%)	66.25	63.71	62.38	66.11	1.26
Eosinophil (%)	0.75 ^a	0.00 ^b	0.25 ^{ab}	0.33 ^{ab}	0.17
Basophil (%)	0.00	0.71	0.50	0.44	0.28
Monocyte (%)	0.88	0.71	1.12	0.88	0.40

^{a, b}: Means in the same row with different superscripts differ significantly ($P < 0.05$)

SEM= Standard Error of Means WBC= White blood cell

Table 5. Effects of housing types, sex and Oyster mushroom (*Pleurotus ostreatus*) extract levels on serum biochemistry of rabbits

Parameters	Housing types			Sex			Mushroom extract levels				
	Steel hutch	Wooden hutch	SEM	Male	Female	SEM	0 ml	5 ml	10 ml	15 ml	SEM
Protein (g/dl)	6.82	7.23	0.27	7.00	7.04	0.27	7.04	7.29	6.75	7.01	0.38
Albumin (g/dl)	3.56	3.83	0.14	3.76	3.63	0.14	3.68	3.95	3.60	3.55	0.20
Globulin (g/dl)	3.26	3.40	0.16	3.24	3.41	0.16	3.36	3.34	3.15	3.46	0.23
Cholesterol (mg/dl)	58.44	59.69	1.57	58.88	59.25	1.57	62.50	58.50	56.75	58.50	2.23
Triglycerides (mg/dl)	66.94	63.13	1.67	65.94	64.13	1.67	65.00	68.00	61.88	65.25	2.36
HDL (mg/dl)	31.16	31.04	1.22	31.11	31.09	1.22	33.30	30.29	29.38	31.44	1.73
LDL (mg/dl)	13.89 ^b	16.02 ^a	0.75	14.58	15.34	0.75	16.20	14.61	15.00	14.01	1.06
Creatinine (mg/dl)	1.34	1.07	0.36	1.39	1.01	0.36	1.94	1.00	1.03	0.85	0.51
Urea (mg/dl)	10.34	10.24	0.52	10.58	10.01	0.52	9.29	10.96	10.39	10.54	0.73
Glucose (mg/dl)	105.44	111.56	4.13	108.31	108.69	4.13	109.63	111.63	104.88	107.88	5.84

^{a, b}: Means in the same row with different superscripts differ significantly ($P < 0.05$)

SEM= Standard Error of Means HDL= High Density Lipoproteins

LDL= Low Density Lipoproteins

or wooden hutch. However, the difference in eosinophil values from the rabbits housed in steel hutch with the females having no presence of eosinophil in the white blood cell might imply that male rabbits have a higher chance of getting sick when housed in steel hutch and this supports the report of Robert et al. (2003) who suggested that absence of eosinophil in rabbit's white blood cell is an indication that the rabbits had not suffered from any allergic reaction and parasitic infection.

The effects of housing types, sex and varying levels of Oyster mushroom extract on serum biochemistry of rabbits are presented on Table 5. There was significant ($P < 0.05$) effect of housing types on low density lipoproteins. The rabbits housed in wooden hutch had higher low density lipoproteins (16.02 mg/dl) than the rabbits housed in steel hutch (13.89 mg/dl). However, sex and the varying levels of Oyster mushroom did not significantly ($P > 0.05$) influence the

serum biochemistry of the rabbits. The study on serum biochemistry of rabbits kept in steel hutch and wooden hutch revealed housing types only affected low density lipoprotein in the serum of the rabbits and this support Salama *et al.* (2015) who reported no significant effect due to housing model on all studied biochemical parameters. The lower value of the low density lipoprotein (LDL) in the serum of the rabbits housed in steel hutch indicates lesser amount of non-beneficial cholesterol in the blood serum as compared to rabbits housed in wooden hutch and the higher LDL in rabbits kept in wooden hutch might be due to stress from single housing with no visual or physical contact with other rabbits (Dontas *et al.*, 2011). This support Serra *et al.* (2004) and Weiss *et al.* (2004) which stated that stress of isolation of rabbits is a potent stressor. The sex of the rabbits did not influence the serum biochemistry, affirming Yamada *et al.* (2004) who reported no statistically

significant difference for serum glucose levels of male and female rabbits; Szabo and Milisits, 2007; Mahesh *et al.* (2008) who observed no sex related difference in serum creatinine levels and no indication for sex differences in nitrogen metabolism, growth and muscle mass. The study on the levels of administration of Oyster mushroom (*Pleurotus ostreatus*) extract in water revealed non-significant difference on the blood serum biochemistry of the rabbits and this implies that Oyster mushroom (*Pleurotus ostreatus*) extract can be used as substitute for conventional multivitamins and antibiotics in drinking water without any deleterious effect on the blood serum profile. This result support the findings of Alam *et al.* (2011) who reported no adverse effects on total protein, creatinine, blood urea nitrogen, uric acid, glucose from the serum of rats fed a diet containing a 5 % powder of *Pleurotus ostreatus* fruiting bodies.

Conclusion

It was concluded that housing rabbits in wooden hutch and replacement of conventional antibiotics and multivitamins with oyster mushroom (*Pleurotus ostreatus*) extract as phytobiotic for rabbits would optimize their health status.

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