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Growth performance and blood profile of rabbit bucks in two housing types on aqueous extract of oyster mushroom (*Pleurotus ostreatus* Jacq ex fr.)

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Abstract

The growth performance and blood profile of forty-eight (48), six (6) weeks old rabbit bucks of cross (Chinchilla and New Zealand White) were assessed for 10 weeks. The bucks were allotted on weight equalization basis in a 2 x 4 factorial experimental layout composed of 24 rabbits into steel hutches and wooden hutches each and on four (4) varying levels (0, 5, 10 and 15 mL/litre of water, respectively) of oyster mushroom (Pleurotus ostreatus Jacq ex fr.) extract. Six (6) bucks were assigned to each treatment group and replicated three times with two (2) bucks per replicate in a Completely Randomized Design. The phytochemicals of the oyster mushroom extract showed that 9,12-Octadecadienoic acid (Z, Z)- methyl ester was the most abundant. Results showed that the feed conversion ratio was significantly (P<0.05) best (6.08) in bucks reared in wooden cage and administered 15 mL oyster mushroom extract. Significantly (P<0.05) highest triglycerides (81.00 mg/dL) was obtained in bucks on steel hutch and on 5 mL oyster mushroom extract and lowest (55.00 mg/dL) in bucks on steel hutch and on 10 mL oyster mushroom extract. The lowest (P<0.05) lymphocytes (59.00%) were recorded in bucks on wooden cage and administered 10 mL oyster mushroom extract. It was concluded that oyster mushroom extract up to 15 mL/litre of water administered in either steel hutch or wooden hutch did not impair the growth performance and blood profile of rabbit bucks.

Keywords: Rabbit bucks, housing types, blood profile, oyster mushroom, phytochemicals

Introduction

Rabbits are highly social animals and they reproduce rapidly; a feature that is capitalized on by farmers to satisfy the increasing demands of animal protein (Lazzaroni *et al.,* 2009) and their production is crucial to livestock activity in the supply of reasonable amount of meat for the world's teeming population (Oseni, 2012). However, in keeping to the demand and well-being of rabbits, housing in large group is believed to be one of the most important factors. In spite of the restriction on the use of wire net cages for intensive rabbit production from welfare viewpoint (Trocino *et al.,* 2004; Lukefahr, 2007), rabbits are raised intensively on group wire cages because they are easy to clean and meet hygienic requirements. Hence, searching for a better housing condition fitting rabbit's welfare aspects is the aim of several researchers (Szendrő and Luzi, 2006; Verga *et al.*, 2006). Housing types are one of the factors that moderately affect performance noticeably in the blood parameters, carcass yield, meat quality (Pla, 2008; Chodova *et al.,* 2014) and reproductive behaviour (Marai and Rashwan, 2003) of rabbits.

Also, the subsequent introduction of emerging antibiotic resistant strains of microorganisms has led to alternative disease control methods in the use of phytogenics and/or phytobiotics with great intensity (Roe and Pillai, 2003 and Saleha *et al*., 2009). One of such alternatives that is

receiving much interest is the mushroom family and the lectin associated with it (Daneshmand *et al.,* 2012; Willis *et al*., 2012). For instance, Dalloul *et al*. (2006) found that the growth performance of *Eimeria acervulina*-infected chickens was significantly improved by injecting *Fomitella fraxirea* lectin into 18 day old embryos. Also, the polysaccharide extract from oyster mushroom (*Pleurotus ostreatus)* was shown to have immunomodulating effects in chickens (Selegean *et al*., 2009). In the same vein, oyster mushroom (*Pleurotus ostreatus*) has been shown to improve growth, immunity and intestinal health of poultry (Guo *et al*., 2003; Giannenas *et al*., 2010). Besides, oyster mushroom (*Pleurotus ostreatus*) has greatly been used in poultry production unlike in other livestock production like rabbits. This study thereby investigated growth performance and blood profile of rabbit bucks in two housing types on varying levels of oyster mushroom (*Pleurotus ostreatus* Jacq ex fr.) extract

Materials and methods

Experimental site

The experiment was carried out at the Rabbitry Unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located in the rain forest vegetation zone of South-Western Nigeria on altitude of 127 m, latitude 7° 13′ N and longitude 3° 26′ E (Google Earth, 2015).

Experimental rabbits and management

Forty-eight (48), six (6) weeks old weaned male rabbits of cross (Chinchilla and New Zealand White) with live weight range of 600-650g were purchased from a reputable farm and used for the study. The bucks were allotted on weight equalization basis into two different housing types (steel cages and wooden hutches) containing 24 rabbits each and composed of four (4) varying levels (0, 5, 10 and 15 ml per litre of water, respectively) of oral administration of oyster mushroom (*Pleurotus ostreatus*). Six (6) bucks were assigned to each treatment group and each group was replicated three times with two (2) bucks per replicate. The rabbits were acclimatized for a period of two (2) weeks prior to the start of the experiment which lasted ten (10) weeks. The rabbits were maintained on a concentrate diet as shown in 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.

Construction and design of the housing

The bucks were housed individually in well-constructed steel cages and wooden hutches to protect the rabbits from predators and harsh weather conditions with each cell having dimension 60 cm x 60 cm x 50 cm. Feed and water troughs were provided in the cages with unrestricted access to feeds and fresh clean drinking water. The rabbits were raised under natural ambient temperature and light. Daily routine management procedure was adhered to.

Preparation of test material (oyster mushroom extract)

Fresh oyster mushrooms (*Pleurotus ostreatus*) were purchased from a reputable market in Ibadan, Oyo State. The mushrooms were properly rinsed so as to remove any form of dirt on them, all external materials such as stones and leaf debris were also removed. After cleaning, hot water extraction procedure was applied by extending the boiling process of the mushroom so as to fully extract the content which is considered to be medicinal out of the mushroom cell wall. 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 ^{0}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 until needed.

Table 1: Gross composition (%) of concentrate diet for the growing rabbits

Ingredients	Composition
Maize	40.00
Soyabean meal	10.00
Wheat offal	20.00
Rice bran	10.00
Palm kernel cake	14.00
Fish meal (72 %CP)	2.00
Bone meal	2.00
Limestone	1.00
*Premix (Growers)	0.50
Salt (NaCl)	0.50
Total	100.00
Calculated Analysis	
Metabolizable energy (MJ/kg)	10.91
Crude protein (%)	16.73
Crude fibre (%)	9.20

*1 kg of vit/mineral to contain: vit. A, 10 000 000 iu; vit. D_3, 200 000 iu; vit. E, 12 500 iu; vit. K, 1.30 g; vit B_1, 1.30 g; vit. B_2, 4.00 g; Dicalcium-pantothenate, 1.30 g; vit. B_6, 1.30 g; vit. B_{12}, 0.01 g; nicotinic acid, 15. 00 g; folic acid, 0.05 g; biotin, 0.02 g; Co, 0.20 g; Cu, 5.00 g; Fe, 25.00 g; I, 0.06 g; Mn, 48.00 g; Se, 0.10 g; Zn, 45.00 g; choline chloride, 200.00 g; BHT, 50.00 g

Data collection

Determination of phytochemicals in oyster mushroom extract

The aqueous extract of the sample was subjected to Gas Chromatography/Mass Spectrometry (GC/MS) analysis, this group of powerful instruments interface helped to characterize the various bioactive components (Skoog *et al.*, 2007). 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 70v with an ion source temperature at 250 °C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5ms (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was at 80 °C held for 5 minute and increased to 250 degrees while holding for 16 minutes at the rate of 4 degrees/minute 1µ/l was auto injected.

Growth performance characteristics

The following growth performance parameters: feed intake, weight gain and mortality were measured weekly and the feed conversion ratio was calculated as the ratio of the feed intake to the weight gain.

Blood collection and analysis

Peripheral venous blood samples of about 3 mL was collected in the morning between 7:00am and 9:00am at the 10th week by vein puncture from a rabbit from each replicate for white blood cell differential and serum biochemistry analysis. About 1.5 ml was used for white blood cell differentials and was stored in Bijou bottles with ethylene - diamine tetra acetate (EDTA) as anticoagulant while the other 1.5 ml was stored without coagulant for serum biochemistry analysis. Sample bottles containing the collected blood were placed in ice packs to maintain a cool and stable temperature prior to laboratory analysis. The estimate of the total number of white blood cells was carried out immediately

after collection of blood samples from the animals using Neubauer haemocytometer counting chamber (Jain, 1986). About 0.2 ml each of blood samples was pipetted and mixed with 4 ml of white blood cell diluting fluid (white blood cell fluid made up of 3% aqueous solution of acetic acid and 1% gentian violet). The samples were then put into the haemocytometer, cell counted and expressed as 10^9 white blood cell per litre of blood. White blood cell differentials (neutrophils, lymphocytes, monocytes, eosinophils and basophils) were also determined via slide reading under microscope after blood smear and staining.

For the blood serum biochemical indices including the blood lipid profile, the 1.5 ml of blood samples were maintained in collection tubes with no additives for 2 h at 20 to 22 °C and then centrifuged (Minifuge RF, Heraeus, Hannover, Germany) at 1200 × g and 4 °C for 20 min. Serum was separated and stored frozen at –30 °C until assayed. Serum cholesterol, triglyceride, albumin, total protein, creatinine, urea and glucose concentrations were measured by using an auto analyser (Hitachi 747, Boehringer Mannheim, Madrid, Spain).

Experimental design and statistical analysis

The experiment was laid out in a 2 × 4 factorial arrangement of a Completely Randomized Design. The data were subjected to analysis of variance and significantly ($P<0.05$) different means were separated using Tukey′s Test as contained in Minitab 17 (2013) package.

Results

Phytochemicals in oyster mushroom extract

The phytochemicals of oyster mushroom (Pleurotus ostreatus) are presented in Table 2. It shows the active compounds, quality, percentage composition of each compound and their bioactivities. The most abundant of these compounds was 9,12-Octadecadienoic acid (Z,Z)- methyl ester which is linoleic acid; a polyunsaturated fatty acid while least compounds contained in oyster mushroom extract were 2H-Pyran, 3, 4-dihydro and Methyl 20-methyl-heneicosanoate with 1.94 %, respectively.

Effects of housing types and aqueous extract of oyster mushroom extract on growth performance of rabbit bucks

The main effects of housing types and aqueous extract of oyster mushroom on performance of rabbit bucks is presented in Table 3. All parameters measured were not significantly ($p>0.05$) affected by housing types and aqueous extract oyster mushroom extract. In the interaction effects between housing types and aqueous extract of oyster mushroom extract on growth performance of rabbit bucks, only the feed conversion ratio was significantly ($P<0.05$) influenced. It was best (6.08) in bucks reared in wooden cage and administered 15 ml oyster mushroom extract.

Effects of housing types and aqueous extract of oyster mushroom extract on serum biochemistry of rabbit bucks

The main effect of housing types and aqueous extract of oyster mushroom extract on serum biochemistry of rabbit bucks is presented in Table 4. The aqueous extract of oyster mushroom extract significantly ($P<0.05$) affected the serum triglyceride content with the highest value of 72.25 mg/dl) recorded in bucks administered 5 ml oyster mushroom extract and lowest (59.50 mg/dl) in bucks administered 10 ml oyster mushroom extract.

The same trend was obtained in the interaction effects between housing types and aqueous extract of oyster mushroom extract on serum biochemistry of rabbit bucks.

Effects of housing types and aqueous extract of oyster mushroom on white blood cell count and white blood cell

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

Active Compound	Quality	Percentage	Bioactivity
2H-Pyran, 3,4-dihydro-	53	1.94%	Install tetrahydropyranyl (THP) protecting groups on alcohol and amines
Benzene acetaldehyde	90	2.76%	Antimicrobial
2-Pyrrolidinone	80	5.02%	e.g. Doxapram stimulates chemoreceptors in the carotid arteries which in turn, stimulates the respiratory centre in the rats brain.
Methenamine	91	8.14%	Used medically as Mandelic acid salt for th e treatment of urinary tract infection (bactericidal).
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
1,6-Octadiene, 3,7-dimethyl-, (S)- (linalool)	15	2.20%	Anti-stress
9-Octadecenoic acid (Z)-, methyl ester (oleic acid)	99	17.99%	Monounsaturated fatty acid associated with decreased LDL and possibly increased HDL
Methyl octadeca-10,13-dienoate	99	4.25%	Unsaturated fatty acid
Octadecanoic acid, methyl ester	98	10.18%	Saturated fatty acid has antimicrobial activity
9,12-Octadecadienoic acid (Z,Z) - methyl ester (Linoleic acid: Omega 6 fatty acid)	99	30.10%	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor: antiandrogenic, anti-arthritic
Benzamide, 4-ethyl-N-methallyl-	35	2.36%	Cell differentiation inducer: A method of treating a malignant tumour selected from the group consisting of leukaemia, colorectal cancer, ovarian cancer, oral cancer, lung carcinoma, breast carcinoma, prostate carcinoma, and melanoma
Methyl 20-methyl-heneicosanoate	95	1.94%	Silicone elastomers in cosmetic esters: teeth impression moulds
1,2-Benzenedicarboxylic acid, diisooctyl ester	64	2.90%	Plasticizer compound: antimicrobial, antifouling
		100.00%	

differentials of rabbit bucks

Table 5 shows the main effect of housing types and aqueous oyster mushroom extract on white blood cell counts and white blood cell differentials of rabbit bucks. All parameters measured were not significantly (P>0.05) affected by housing types except lymphocyte content. Bucks in steel hutch housing type recorded the highest (66.25%) lymphocyte content than 62.38% recorded in bucks in wooden cage housing type. Also, aqueous extract of oyster mushroom extract significantly (P<0.05) affected the lymphocyte content with the highest value (68.00%) recorded in bucks administered 5 ml oyster mushroom extract and least (61.75 and 63.00%) in bucks administered 0 and 10 ml oyster mushroom extract. In the interaction effects between housing types and aqueous extract of oyster mushroom extract, the lymphocyte content was significantly (P<0.05) influenced.

Table 3: Effects of housing types and varying levels of oyster mushroom on performance of rabbit bucks

Parameters	Housing types			Oyster mushroom						
	Steel hutch	Wooden cage	SEM	0 ml	5 ml	10 ml	15 ml	SEM	HT*OM	SEM
Initial weight (g/rabbit)	760.42	752.08	16.60	775.00	741.67	758.33	750.00	23.50	NS	33.20
Final weight (g/rabbit)	1543.75	1552.08	41.90	1500.00	1554.17	1591.67	1545.83	59.30	NS	83.80
Weight gain (g/rabbit/day)	11.19	11.43	4.29	10.36	11.61	11.91	11.37	0.81	NS	1.00
Feed intake (g/rabbit/day)	77.21	75.84	1.96	77.04	77.68	76.75	74.62	2.78	NS	3.92
Feed conversion ratio	7.15	6.75	0.35	7.70	6.78	6.67	6.66	0.52	S	0.71

Values are means of three replications SEM = Standard error of means HT = Housing type OM = Oyster mushroom
HT*OM = Interaction between Housing types and Oyster mushroom NS = Not significant (P>0.05) S = Significant (P<0.05)

Table 4: Effect of housing types and varying levels of oyster mushroom on serum biochemistry of rabbit bucks

Parameter	Housing types			Oyster mushroom						
	Steel hutch	Wooden cage	SEM	0 ml	5 ml	10 ml	15 ml	SEM	HT*OM	SEM
Total protein (g/l)	69.10	70.90	3.10	68.80	70.50	68.80	72.00	4.60	NS	2.10
Albumin (g/l)	36.80	38.40	1.60	37.80	38.50	37.00	37.00	2.10	NS	1.10
Globulin (g/l)	32.40	32.50	2.10	31.00	32.00	31.70	35.00	3.10	NS	1.40
Total cholesterol (mg/dl)	59.13	58.63	1.87	61.00	56.50	58.50	59.50	2.82	NS	1.38
Triglycerides (mg/dl)	68.50	63.37	2.81	61.25^{ab}	72.25^{a}	59.50^{b}	70.75^{ab}	3.47	S	2.18
HDL (mg/dl)	31.90	30.32	1.67	32.23	29.35	30.88	32.00	2.46	NS	1.19
LDL (mg/dl)	13.52	15.62	1.96	16.53	12.70	15.73	13.35	1.57	NS	0.82
Creatinine (mg/dl)	1.76	1.03	0.57	1.07	0.80	0.95	0.75	0.57	NS	0.49
Urea (mmol/l)	10.50	10.65	0.45	10.20	10.78	10.40	10.93	0.63	NS	0.31
Glucose (mg/dl)	106.63	110.00	4.83	107.50	108.25	107.00	110.50	7.41	NS	3.40

^{a,b} Means on the same row having different superscript are significantly (P<0.05) different HDL = High density lipoprotein LDL= Low density lipoprotein SEM = Standard error of means
HT = Housing type OM = Oyster mushroom HT*OM = Inte raction between Housing types and Oyster mushroom NS = N ot significant (P>0.05) S = Significant (P<0.05)

Table 5: Effects of housing types and varying levels of oyster mushroom on White Blood Cell differentials of rabbit bucks

Parameter	Housing types			Oyster mushroom						
	Steel hutch	Wooden cage	SEM	0 ml	5 ml	10 ml	15 ml	SEM	HT*OM	SEM
White blood cell ($x10^9$/l)	7.40	7.20	0.49	7.40	7.83	6.48	7.50	0.70	NS	0.34
Heterophils (%)	32.13	35.88	1.42	36.00	30.50	36.25	33.25	1.88	NS	1.08
Lymphocyte (%)	66.25^{a}	62.38^{b}	1.27	63.00^{b}	68.00^{a}	61.75^{b}	64.50^{ab}	1.69	S	1.01
Eosinophil (%)	0.75	0.25	0.16	0.25	0.50	0.50	0.75	0.27	NS	0.13
Basophil (%)	0.00	0.50	0.13	0.00	0.25	0.25	0.50	0.25	NS	0.14
Monocyte (%)	0.88	1.13	0.29	0.75	0.75	1.50	1.00	0.40	NS	0.20

^{a,b} Means on the same row having different superscript are significantly (P<0.05) different SEM = Standard error of means HT = Housing type OM = Oyster mushroom
HT*OM = Interaction between Housing types and Oyster mushroom NS = Not significant (P>0.05) S = Significant (P<0.

Discussion

Edible *P. ostreatus* are known to be medically active in several therapies, such as anti-tumour, antibacterial, antiviral and immunomodulating treatments with the therapeutic effects being linked to the presence of bioactive compounds in the mushrooms. Some of these bioactive compounds include glycolipids, aromatic phenols, saturated, unsaturated and poly

unsaturated fatty acid derivatives, polyacetylamine, polyketides (lovastatin, pleuromutilin), sesquiterpenoids, polysaccharides (Patel *et al.,* 2012; Silva *et al.*, 2012). The phytochemical analysis of oyster mushroom extract in this study disclosed the presence of phyto-constituents. Wang *et al.* (2001) stated that the essential oils from *Pleurotus* ostreatus mushroom to be attributable to the presence of benzaldehyde which smells more like almonds. Also, Mohamed and Farghaly (2014) observed the presence of benzene,1-chloro-4-methoxy- and benzene acetic acids fractions in oyster mushroom with Benzene acetic acid being an active plant hormone, possessing a honey-like odour in low concentrations. This study confirmed the reports that oyster mushroom extract has antibacterial, antioxidant, immunomodulating, anti-inflammatory, hypocholesterolemic, hepatoprotective, 5-alpha reductase inhibitor (Wang *et al.*, 2001; Silva *et al.*, 2012; Zang *et al*., 2012).

This study showed no significant effect of housing types on growth performance parameters measured. This was in line with the reports that showed no significant difference in growth performance among the groups of rabbits raised in houses with floors made of wire-mesh, plastic-mesh, steel slats and plastic (Trocino *et al*., 2008; Princz *et al.,* 2009). However, reports from studies by Lambertini *et al.* (2001), McNitt *et al.* (2003) and Pinheiro *et al. (*2011) refuted the results from this study. The authors observed significant difference in weight gain of rabbits as affected by housing systems. This variation could be greatly attributed to differences in housing systems of the various studies. In the same vein, no significant effect of oyster mushroom extract administration was observed in the growth performance of the rabbit bucks. However, the feed conversion ratio of the rabbit bucks was significantly influenced by the interaction of the housing types and varying levels of oyster mushroom extract given the best in bucks housed in a wooden hutch and on 15 ml oyster mushroom extract in a litre of water.

Although, there existed a paucity of information on the attendant effects of housing on serum profile of rabbits, this study however revealed no significance of housing types adopted on all serum indices except in the triglycerides. Though, the highest triglyceride was obtained in rabbit bucks on the administration of 5 ml oyster mushroom extract and also in rabbit bucks housed in a steel hutch and on 5 ml oyster mushroom extract, the values recorded were within the reference values for rabbits (Mitruka and Rawnsley, 1977). On the other hand, the non-significance of oyster mushroom extract inclusion on most of the serum indices of rabbit bucks was in line with the findings of Etim and Oguike (2011) that showed no significant differences among treatments for serum globulin and urea in rabbits with the inclusion of plant-based antibiotics.

Though, it was revealed from this study that housing influenced the lymphocyte contents, the white blood cell counts reported fell within the normal physiological range reported by Mitruka and Rawnsley (1977) hence implying both housing types pose no stress on the rabbits. Similarly, the administration of *Pleurotus ostreatus* extract to rabbit bucks significantly influenced lymphocyte portions but resultant eosinophil, basophil and monocyte fall within the normal ranges reported by Burke (1994). This indicates that body defence system of the rabbits in all the treatment groups were not negatively affected by oyster mushroom extract administration. Hence, the comparable means in white blood cell counts of the rabbit bucks in all the treatment groups in this study ruled out the possibility of

microbial infection.

Conclusion and Recommendation

Oyster mushroom extract up to 15 ml/litre of water should be administered to rabbit bucks in either steel hutch or wooden hutch.

Ethical approval

All applicable international, national and/or institutional guideline for the care and use of animals were followed.

Informed Consent

Consent of every individual included in this study was obtained.

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