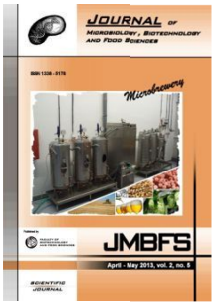

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HEMATOLOGICAL ASSESSMENT OF ALBINO RATS FED WITH *Pleurotus ostreatus* CULTIVATED ON TWO TROPICAL TREES' SAWDUST (*Pycnanthus angolensis* AND *Spondias mombin*)

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ABSTRACT

Pleurotus ostreatus, an edible mushroom in the tropics was artificially cultivated on the mixture of the sawdust of two different tropical trees(African nut (*Pycnanthus angolensis*, Welw, Warb; Family: Myristicaceae) and hog plum (*Spondias mombin,* L; Family: Anacardiaceae)) . The fruitbodies obtained were used to feed experimental animals (Wistar strain *albino* rats) while the subsequent toxicological effects was investigated on the hematological parameters of the experimental animals. There was a significant decrease ($P > 0.05$) in the Packed Cell Volume (34.0%), Red Blood Cell (3.2 x 10^{12}/L), White Blood Cell (3.5 x 10^{3}/L), Mean Corpuscular Volume (8.0 g/fL) and Hemoglobin (11.33 g/dL) of the rats fed with protein free diet compared to the rats fed with the mushroom composed diet (PCV, 40.0%; RBC, 5.0 x 10^{12}/L; WBC, 8.5 x 10^{3}/L; MCV, 90.0 g/fL and Hb, 13.33 g/dL. The values obtained from the rats fed mushroom composed diet however compared favourably with the results obtained in rats fed with the soybean composed diet (positive control) which is given as (PCV, 35.0%; RBCs, 6.6 x 10^{12}/L; WBCs, 6.5 x 10^{3}/L), MCV (93.0 g/fL) and Hb, 11.67 g/dL. It was therefore concluded that the hematological parameters of the experimental animals was not in any way affected as a result of consumption of mushroom (*Pleurotus ostreatus*) cultivated on sawdust of these two tropical trees.

Keywords: Hematological, *Pleurotus ostreatus*, *Pycnanthus angolensis, Spondias mombin*

INTRODUCTION

Edible mushrooms include many fungal species that are either harvested wild or cultivated, they are nutritionally endowed fungi that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials **(Lindequist *et al.*, 2005; Iwalokun *et al.*, 2007)**. They are saprophytes which include some members of the *Basidiomycota* and some members of the Ascomycota. Mushrooms are cultivatable and the common wild mushrooms are often available in markets. Before assuming that any wild mushroom is edible, it should be identified, also some preparations like sun-drying, boiling, fermentation and cooking may render certain poisonous mushrooms fit for consumption. Proper identification of a species is the only safe way to ensure edibility **(**Rubel and Arora, 2008). Some mushrooms that are edible for most people can cause allergic reactions in some individuals, also, old or improperly stored specimens can cause food poisoning **(Holick *et al.*, 2008)**. Deadly, poisonous mushrooms that are frequently confused with edible mushrooms and responsible for many fatal poisonings include several species of the genus *Amanita*, in particular, *Amanita phalloides*, the 'death cap' **(Enjalbert *et al.*, 2002)**.

The exploitation of substrate for mushroom production however varies with the species, strain and cultivation technology employed **(Zadrazil and Dube, 1992)**. *Pleurotus* is one of the most common edible mushrooms available in the tropical region and are prospective source of valuable food protein with the ability to effectively bio-convert various lignocellulosic materials to protein **(Dianxia *et al.*, 2002)**. However, the low availability of lignocellulosic materials from the family of Graminae for the cultivation of mushrooms in recent years has led to the search for an alternative source of raw materials as substrates for the cultivation of *Pleurotus* species. Attention has therefore been turned to the use of sawdust of tropical trees from our saw-mills which of course have been yielding tremendous results. In particular is *Spondias mombin, a* tropical tree that naturally supports the growth of the mushroom. This is to be achieved by maximizing the wastes through engaging them in the cultivation of mushroom instead of careless burning which has environmental implications. Unnecessary accumulation of wastes like sawdust from sawmills and its subsequent environmental pollution as a result of burning is of great environmental concern. Hence, there is a need for effective management and utilization of these waste products. The specific objective of the study is therefore to examine the toxicological effects of the *P. ostreatus* when cultivated on sawdust of two tropical trees.

MATERIAL AND METHODS

Source of sample

Fresh wild *P. ostreatus* was obtained from a forest reserve located near Ilara Mokin, Ondo State, Nigeria and was identified at the Department of Microbiology, Federal University of Technology, Akure and authenticated at the Department of Botany and Microbiology, University of Ibadan, Ibadan. Artificial cultivation of the mycelium was done on Potato dextrose agar (PDA) and subsequently inoculated into sterile sorghum seeds to produce the grain mother spawn, which was maintained at 4ºC as stock culture.

Substrates preparation and sample cultivation

Trunks of two tropical trees: African nut (*Pycnanthus angolensis*, Welw, Warb; Family: Myristicaceae) and hog plum (*Spondias mombin,* L; Family: Anacardiaceae) were obtained and authenticated in the Department of Forestry and Wood Technology, Federal University of Technology, Akure and later processed into sawdust in a sawmill in Akure. They were filled into transparent plastic bucket of 100 g each and sterilized by autoclaving at 121ºC for 15 minutes. Sterilized substrates were cooled and inoculated with the grain mother spawn. The inoculated substrates were incubated in wooden, ventilated boxes (1.0 m x 0.8 m x 0.5 m) for 24 days at 25 ± 1ºC.

Mushroom Sample Preparation

The fruitbodies were harvested at the matured stage (5 days) and oven dried at 55ºC. It was subsequently pulverized using a premium quality MGM blender and kept in a polythene bags inside a desiccator until needed.

Hematological assay

Wistar strain *albino* rats weighing between 25–38 g were purchased from the Department of Biochemistry, University of Ilorin, Nigeria, and acclimatized for 2 weeks, during which period they were maintained *ad libitum* on commercial diet. The rats were subsequently divided into three treatment groups. Animals in group A were fed the protein free diet (PFD), while animals in group B were fed the

soybean composed diet (SCD) and animals in groups C were fed the mushroom composed diet (MCD) as shown in table 1. The feed and water were given *ad libitum* throughout the duration of feeding trial experiment. The experiment lasted for 30 days of feeding trials after which the *albino* rats were anaesthetized with chloroform and whole blood was collected into sample tubes containing EDTA as anticoagulant and plain sample tubes for serum by decapitation. The serum was subsequently subjected to hematological analysis. The hematological tests for packed cell volume (PCV), red blood cell (RBC) counts, white blood cell (WBC) counts i.e basophil, lymphocyte, monocyte, eosinophil, neutrophil), hemoglobin (Hb), mean corpuscular volume (MCV), were conducted according to the conventional methods reported by **Aning *et al*., 1998**.

Statistical analysis

The result of the three replicates were pooled and expressed as mean ± SE. Data was analyzed using analysis of variance (ANOVA) and the Least Significance Difference test were carried out using Duncan's Multiple Range test at 5% level of significance i.e. $P \leq 0.05$ **(Zar, 1984)**.

Table 1 Feed formulation for the evaluation of protein quality (g/1000g)

Components	Diets		
	PFD (g)	SCD	MCD
Cellulose	40.0	40.0	40.0
Sucrose	100.0	100.0	100.0
Corn oil	40.0	40.0	40.0
Min.mix/Vit mix	50.0	50.0	50.0
Methionine	4.0	4.0	4.0
Soya bean meal	-	250.0	-
Mushroom	-	-	125.0
Corn Starch	766.0	516.0	641.0
Total	1000	1000	1000

Keys:
PFD - Protein free diet
SCD - Soya bean Composed diet
MCD - Mushroom Composed Diet

RESULTS AND DISCUSSION

Hematological changes in rats fed diet with composed diets.

The hematological parameters of rats fed with protein free diet, soybean composed diet and mushroom composed diets are shown in Table 2. There was a significant decrease in the PCV (34.0%), RBC (3.2 x 10^{12}/L), WBC (3.5 x 10^{3}/L), MCV (8.0 g/fL) and Hb (11.33 g/dL) of the rats fed with protein free diet compared to the rats fed with the mushroom composed diet (PCV, 40.0%; RBC, 5.0 x 10^{12}/L; WBC, 8.5 x 10^{3}/L; MCV, 90.0 g/fL and Hb, 13.33 g/dL. The values obtained from the rats fed mushroom composed diet however compared favourably with the results obtained in rats fed with the soybean composed diet (positive control): (PCV, 35.0%; RBCs, 6.6 x 10^{12}/L; WBCs, 6.5 x 10^{3}/L), MCV (93.0 g/fL) and Hb, 11.67 g/dL.

Table 2 Mean Hematology Volumes of experimental animals fed with various composed diets

Feed Samples	Hematological Parameters (WBC)				
	PCV (%)	RBC (X10^{12}/l)	WBC (X10^{3}/l)	Hb (g/dl)	MCV (fl)
PFD	34.0±2.0^{c,d}	3.2±0.12^{d}	3.5±0.1^{d}	11.33±0.2^{b,d}	8.0±0.4^{d}
SCD	35.0±1.2^{a,b}	6.6±0.22^{b}	6.5±0.4^{c}	11.67±0.1^{a}	93.0±0.2^{b,c}
MCD	40.0±2.2^{b}	5.0±0.10^{c}	8.5±0.2^{b,c}	13.33±0.1^{b,c}	90.0±0.3^{b}

Values are means of triplicates ± SD, Samples carrying the same superscripts in the same row are not significantly different at ($p<0.05$)

Keys:
PCV : Packed Cell Volume
RBC : Red Blood Cell
WBC : White Blood Cell
MCV : Mean Corpuscular Value
Hb : Hemoglobin
PFD: Protein free diet
SCD: Soyabean composed diet
MCD: Mushroom composed diets

Mean Hematological Parameters For The WBC Components

The mean hematological parameter for the WBC components is shown in Table 3. The results of mushroom composed diet compared favourably with the positive control (neutrophil, 6.0% and 8.0%; lymphocyte, 92.0 and 90.0%) respectively.

Table 3 Mean Hematology Volumes (WBC) of experimental animals fed with various composed diets

Feed Samples	Hematological Parameters (WBC)				
	NTP (%)	LPC (%)	MNC (%)	ESP (%)	BSP (%)
PFD	14.0±0.06^{b,c}	85.0±2.0^{d,e}	1.0±0.02^{d}	0.0±0.00^{a,d}	0.0±0.00^{a,c}
SCD	8.0±0.11^{b}	90.0±1.0^{a,b}	1.0±0.01^{b}	1.0±0.01^{a,b}	0.0±0.00^{a}
MCD	6.0±0.10^{b}	92.0±2.0^{c}	2.0±0.01^{c}	0.0±0.00^{a}	0.0±0.00^{a}

Values are means of triplicates ± SD, Values are means of triplicates ± SD, Samples carrying the same superscripts in the same row are not significantly different at ($p<0.05$)

Keys:
BSP: Basophil
ESP: Eosinophil
MNC: Monocyte
NTP: Neutrophil
LPC: Lymphocyte
PFD: Protein free diet
SCD: Soyabean composed diet
MCD: Mushroom composed diets

Nutrient utilization and growth performance of rats fed with mushroom composed diet (MCD), Soybean composed diet (SCD) and Protein free diet (PFD).

Figure 1 shows the growth performance and nutrient utilization of rats fed with composed diets using mushroom from these tropical trees as the sole basal medium and also with other composed diets. Sample meal which is mushroom composed diet and positive control (soybean composed diet) gave an average daily feed intake of 6.0g/rat/day and 5.8g/rat/day, daily weight gain of 1.42g/rat/day and 1.68g/rat/day and feed gain ratio of 4.22 and 3.45 respectively. Also, the protein free diet which is the negative control had an average daily feed intake of 5.8g/rat/day, daily weight gain of 0.95g/rat/day) and feed gain ratio of 6.11.

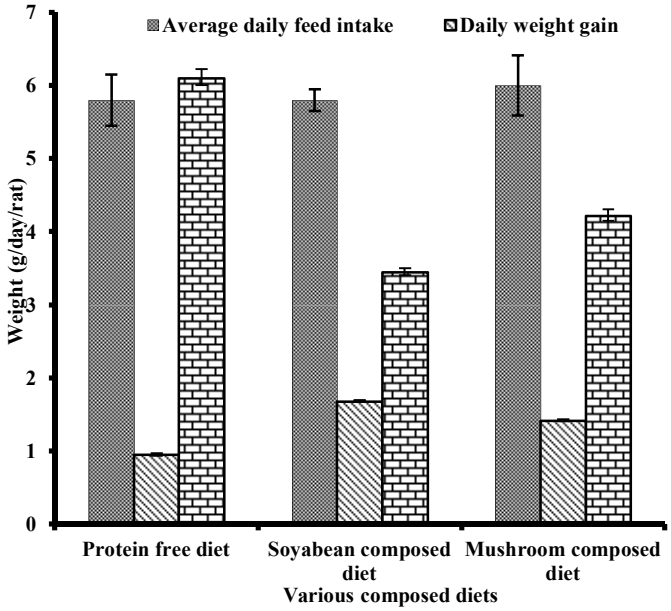

Figure 1 Growth performance and nutrient utilization and of albino rats fed with different composed diets

Hematological indices are an index and a reflection of the effects of dietary treatments on the animal in terms of the type, quality and amounts of the feed ingested and were available for the animal to meet its physiological, biochemical and metabolic necessities **(Ewuola *et al*., 2004)**. In this study, in which mushroom composed diet, soyabean composed and protein free diets were used to feed experimental rat. The results obtained for the PCV, Hb and RBC were consequently implicated and significantly influenced by the dietary treatments. This agrees with the reports of **Aletor and Egberongbe (1992)** that the blood variables most consistently affected by dietary influences include RBC counts, PCV, plasma protein e.t.c. The hematological values obtained from the rats fed with the protein free diet decreased significantly, while that of mushroom composed diets and soyabean composed diets compared favourably with normal hematological parameter standards which implies that the experimental rats were not by no means affected. Also the increased Hb concentration in albino rats fed with Soyabean and Mushroom composed diets may be supported by previous reports of **Alada *et al.* (2004)** that protein rich diets increased both haematocrit levels and haemoglobin concentrations in human and animal studies.

However, the results of the protein free diets could therefore probably be attributed to its effects on the blood profile that depressed the parameters in animals placed on such diets as compared to Sample meal and positive control. The changes in PCV values suggest that dietary protein levels and probably treatment duration may modulate the effects PCV values which agree with the results that PCV levels can reflect the extent and efficiency of oxygen uptake **(Ots, 1998).** There was a significant decrease in the mean corpuscular value (MCV) of albino rats fed with protein free diet compared to the rats fed with mushroom composed and soyabean composed diets which is an indication of the presence of hemolytic anemia. There was also a significant decrease in the Packed cell volume, Hemoglobin, red blood cell and white blood cell of the rats fed diet with protein free diet compared to the rats fed diet with mushroom composed diet. This reduction in hematological parameters could be attributed to the ability of the protein free diet to induce damage on the erythrocyte membrane **(Flora *et al*, 2008)**. Also, the above assertion and the decrease in the values of RBC, MCV, WBC and Hb in the rats that was dosed with protein free diet in this experiment suggests that protein free diet (absence of mushroom as a basal protein source) may have a hepatotoxic potential, the severity of which may be dependent on the level of exclusion of mushroom in the feed. Also the moderation in the hematological parameters of the animals fed with mushroom and soyabean composed diets fed rats also indicates that both have no toxicological and implicative effects. This result suggests that adequate nutrition is essential in stabilizing the immune system of the body **(Blackburn *et al*., 1992).** The result also shows that the higher the feed gain ratio, the lower the average weight gain of the albino rats. This also agrees with the findings of **Edet *et al*. (2010)** that growth is indicated by weight gain and increase in size, weight lose or reduction in size (as obtained in the protein free diet) shows that the taste diet is either not supporting growth or that it has some interferences.

CONCLUSION

In conclusion, the mushroom harvested from the tropical trees sawdust' used in this studies for the mushroom cultivation has no toxicological or noxious consequence(s) when consumed as observed in the experimental animals, hence, consumption of the mushroom by man is therefore declared safe and poisonous free.

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