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Nutritional, Anti-nutritional and Biochemical Studies on the Oyster Mushroom, *Pleurotus ostreatus*

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Abstract

Nutritional, anti-nutritional and biochemical studies on the Oyster mushroom, *Pleurotus ostreatus* using Wistar albino rats were evaluated. Nutritional compositions such as protein, crude fats, fatty acids, vitamins A, B complex, C, and K; as well as potassium, sodium, calcium, zinc, copper and manganese minerals with high calcium-phosphorus ratio and amino acids were observed in Oyster mushroom, *P. ostreatus.* Saponins, alkaloids, oxalate, tannins and phytate were among the anti-nutrients observed in the studied sample. Amino acid scores of the studied sample relative to FAO/WHO/UNU provisional amino acid scoring pattern revealed that *P. ostreatus* surpassed the total aromatic amino acids, threonine, and tryptophan requirements by 71%, 5% and 30% respectively. The estimated nutrient-anti-nutrient ratios predicted nutrients bioavailability in the body following its consumption. The biochemical studies revealed that haematological indices, liver enzymes such as ALT and AST, and electrolyte ions of rats placed on *P. ostreatus* were significantly influenced ($p < 0.05$) when compared to the control and reference 2, but compared favorably with the rats placed on reference 1 (Nutrend, a standard baby food from Nestle, Nigeria). Lipid profile and lipid profile ratios of rats placed on *P. ostreatus* reduced significantly ($p < 0.05$) against those of the control but compared favorably with rats placed on reference 1. The pattern of effects of *P. ostreatus* on test rats against the control, reference 1 and reference 2 could be indication that the sample has no much deleterious effects on the blood, liver and kidney following consumption. However, ALP enzymes increased significantly ($p < 0.05$) while reproductive hormones reduced significantly ($p < 0.05$) in test rats when compared to the control, reference 1 and reference 2. There is need to extend the effect of *P. ostreatus* to associate organs like the prostate and others in rats. This study has evaluated the nutritional, anti-nutritional and some biochemical studies on *P. ostreatus* using Wistar albino rats.

Keywords: *Nutritional; Anti-Nutritional; Biochemical Studies; Liver Enzymes; Pleurotus ostreatus*

Introduction

The gift of plants by nature could be agreed to be the next in line after human life. The positions occupied by plants before humans and other lower animals cannot be overstated. Plants ultimately serve as food substances to humans and lower animals of the planet Earth. They also bring about geological cycles, provide shelter and materials for industrial productions; and play key role in traditional medicine [1-6]. According to Uwakwe and Ayalogu [7], Okaka and Okaka [8] and Duru., *et al*. [9] food is any edible substance that provides the necessary nutrients required for the proper functioning of the body. Olusanya [10] noted that plant nutrients include water, proteins, fats/lipids, fibre, carbohydrates, vitamins and inorganic matters. Apart from nutrients, different authors [8,11,12] have reported the presence of non-nutritive compounds in plants. It has also been noted that these non-nutritive compounds possess the ability to influence nutrients in plants. Due to their bioactive and physiological nature, some are regarded as plant protectors and hence combine with plant nutrients to foster protection in the body [13-19]. Also, some can complex with nutrients found in plant foods, and fosters their unavailability to the body as anti-nutrients [18,20-22]. However, their ability to bring about any of these effects depends on their concentrations in food substances. The useful plants of planet Earth are too numerous to count [23] and can occur in macro or micro forms. Apart from plant and animal kingdoms, there are also other existing kingdoms of classification that house important organisms. Mushroom is among such important organisms found in one of such existing kingdoms.

Mushrooms are fungi and contain no chlorophyll. They are mostly saprophytic in nature. That is, they obtain their nutrition from metabolizing non-living organic matter. According to Bhattacharjya., *et al.* [24] mushrooms are increasingly being recognized as important food products due to the role they play in human health, nutrition and disease control. The usefulness of certain species of mushrooms as antimicrobial [25-31]; against disease conditions [32-35]; in neutraceutical industry [36,37]; and in environmental bioremediation exercise [38-40] has been reported. In nutrition, different authors have noted the ideal position occupied by mushrooms due to their nutritional constituents [41-44]. Mushrooms could be of edible or non-edible types [45]. Oyster mushrooms which comprise the *Pleurotus* species are predominately edible. According to Tesfaw., *et al.* [46] *Pleurotus* species are edible fungi cultivated worldwide especially in South East Asia, India, Europe, and Africa. Obodai., et al. [47] reported that oyster mushrooms are the third largest commercially produced mushroom in the world.

Pleurotus ostreatus is among the edible *Pleurotus* mushrooms that are widely cultivated globally [46]. Sánchez [48] reported that *P. ostreatus* is the second largest next to *Agaricus bisporus* in the world market*. P. ostreatus* is a fungus and saprophytic eukaryote, which composed of hyphae filament that thrives well in damp or moist condition. The oyster mushroom can thrive on rotten materials [46]. *P. ostreatus* claims few environmental controls, and its fruiting body is not often attacked by diseases and pest. *P. ostreatus* can be cultivated in a simple and cheap way [48]. The cultivation of *P. ostreatu*s provided alternative employment and contributes as a food source to rural disadvantaged groups especially women and elderly people in African villages [46]. Research studies on oyster mushrooms or *Pleurotus* species are mostly centered on *P. tuberregium* [31,39,40,49-57]. Though similar to many mushrooms, few attempts have been made to ascertain nutritional [58] and as well as other possible benefits that are derivable from other lesser known *Pleurotus* species such as *P. florida* [44,59], *P. squarrosulus* [37] and *P. eryngii* [60], but there is scarcity of literature on *P. ostreatus*. This study evaluated the nutritional, anti-nutritional and biochemical studies on the oyster mushroom, *Pleurotus ostreatus.*

Materials and Methods

Collection of mushrooms

Matured *P. ostreatus* samples used in this study were purchased from Orie Amaraku market in Isiala Mbano Local Government Area of Imo State, Nigeria and transported to the laboratory where they were prepared for analysis.

Nutritional compositions

The nutritional compositions evaluated in the present study were proximate analysis, vitamins, mineral elements, amino acids, and fatty acids.

Proximate analysis

Proximate composition analysis was carried out to estimate the percentages of moisture, ash, crude protein, crude fats, and fibre as described by Association of Official Analytical Chemists [61]. The method of difference was used to estimate total carbohydrate while Atwater factors of 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in calories [61] were used for gross energy content of the sample.

Vitamin analysis

Vitamins A, B1, B2, B3, B6, B12, C, D, E and K present in P. ostreatus were analyzed as described by the Association of Official Analytical Chemists [61].

Determination of mineral elements

Preparation of the sample for mineral analysis was completed as described by Amadi., *et al* [62]. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) contents were determined by EDTA versanate complexometric titration method as described by Harbone [63]. Potassium (K^{2+}) and sodium (Na+) ion contents were determined by flame photometry as described by Onwuka [20]. Zinc, manganese, copper, iron, and phosphorus were determined using atomic absorption spectrophotometer. Values of the individual metals were read from spectrophotometer after standardizing with respective elements.

Determination of amino acids and fatty acids

Amino acids in the studied were determined as described by Speckman., *et al* [64]. The amino acid groups and their percentages were calculated as described by Ibegbulem., *et al.* [65] while gas chromatographic method was use for fatty acid analysis.

Antinutritional constituents

Anti-nutritional constituents considered in the presents study such as saponins, flavonoids, alkaloids, tannins, oxalates, and phytates, were screened using the methods as described by Harbone [63] and Ojiakor and Akubugwo (1997). Those found present in the studied sample were quantitatively determined using the standard methods of Association of Official Analytical Chemists [61].

Determination of fatty acid; mineral element; and antinutrient: nutrient mole ratios

The methods described by Duru., *et al*. [67] and Duru., *et al.* [9] were used for fatty acid and mineral element ratios respectively; while the methods of Woldegiorgis., *et al*. [68] were used for antinutrients-mineral ratios.

Experimental animals

Forty-eighty male Wistar albino rats weighing between 70 - 80g obtained from the animal colony of Department of Biochemistry, Abia State University, Nigeria were procured for this study. The rats were housed in clean and dry plastic cages with good ventilation and were given pelletized commercial rat feed (Pfizer Livestock Co., Ltd, Aba, Nigeria), and potable water *ad libitum*. The rats were given the same feed before acclimatization. The acclimatization period lasted for seven days. After acclimatization period, the animals were allocated to six groups of eight rats each. The rats were placed into groups by weight and the groups were equalized as nearly as possible. Three control groups; an experimental control, together with two known reference foods (Nutrend, a known baby food (Reference I) and formulated basal feed (Reference II) were used as controls, while the remaining groups were used as test groups. Treatments given to the rats are expressed as follows.

Control groups

Reference 1 = Nutrend + potable water

Reference 2 = Basal feed (formulated by mixing 640 g/kg of corn flour, 120 g/kg of sucrose, 10 g/kg of mineral mixture, 80 g/kg of red palm oil, and 150 g/kg of cellulose powder) + potable water.

Experimental control group =Normal feed + potable water.

Test groups

Test group I = 5% of *P. ostreatus* + 95% normal feed + potable water.

Test group II = 15% of *P. ostreatus* + 85% normal feed + potable water.

Group III = 25% of *P. ostreatus* + 75% normal feed + potable water.

The treatments of experimental rats were in accordance to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals [68]. The treatment lasted for twenty-eight days (28 days).

Biochemical Studies

Rats from the various groups were weighed and sacrificed while under chloroform anesthesia after the treatment period. Blood was collected by direct cardiac puncture into heparin treated tubes for haematology analysis, while the blood for kidney and liver studies were collected in anticoagulant free tubes. The tubes were properly labeled for analysis.

Haematology indices such as Packed Cell Volume (PCV) was estimated using micro-haematocrit method as described by Alexandar and Griffths [69], haemoglobin level (Hb) was determined using cynomethaemoglobin as described Alexandar and Griffths [70], whereas white blood cells count (WBC) and its differentials, and red blood cell indices were estimated by visual means using the new improved Neubauer counting chamber as described by Dacie and Lewis [71]. Mean cell volume (MCV), Mean corpuscular, haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were estimated using the methods as described by Jain [72].

Urea, creatinine, sodium ion, potassium ion, chloride and bicarbonates; as well as the liver enzymes considered such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were spectrophotometrically determined using the standard ready to use kits from Rondox Laboratory Ltd. Co. Antrim, United Kingdom.

Assay of total cholesterol, HDL-cholesterol, and triglyceride were assayed enzymatically with Randox test kits (Randox Laboratories, England). The method as described by Friedwald., *et al.* [73] [LDL-cholesterol (mg/dl) = Total cholesterol (mg/dl) - (HDL-cholesterol (mg/dl) - TG/5)] was used to estimate the LDL-cholesterol. The mathematical calculation methods as described by Lisa [74] were specifically applied for LDL-cholesterol/HDL-cholesterol; Cardiac Risk Ratio; Atherogenic Coefficient; Triglyceride/HDL; and Atherogenic Index of Plasma

The procedures for the assay as contained in the manufacturer's manual were strictly followed in the determination of testosterone and luteinizing hormones in the present study.

Results and Discussion

Proximate content	Composition
Moisture (%)	83.24 ± ***1.20***
Crude protein (%)	22.61 ± 0.57
Ash content (%)	8.24 ± 0.68
Crude fat (%)	5.01 ± 0.13
Crude fibre (%)	16.28 ± 2.19
Total carbohydrate (%)	47.86 ± 1.04
Gross energy (Kcal/100g)	326.97 ± 4.13

Table 1: *Proximate composition of P. ostreatus.*

Results are mean and standard deviations of triplicate determinations

The importance of proximate composition in nutrition cannot be overstated. The relationship between water and shelf life of plant products have been reported by Onwuka [20] and Chikezie., *et al* [75]. Moisture or rather water in the body of organisms acts as dissolving medium for substrates, transport materials, regulate temperature amongst other functions [7,76]. The moisture content of *P. ostreatus* (83.24%) is higher than that of those of wet and dry *P. tuberregium* sclerotia reported by Ikewuchi and Ikewuchi [55]; dry *L. edodes* by Regula and Siwulski [77], but falls within the range reported by Bhattacharjya., *et al.* [24] on *P. ostreatus* cultivated on different saw dust substrates. The observed moisture content value of the present study is in line with statement of María., *et al.* [60] that mushrooms contain a high moisture percentage that ranged between 80 and 95 g/100 g approximately. Protein is an essential component of diet and assures availability of amino acids (Hassan., *et al.* 2011). Arukwe., *et al.* [77] noted that the relative impact of proteins and amino acids in the body should not be overlooked. According to Olusanya [10], protein and amino acids repair and replace worn out cells, form structural and globular materials that holds the body, form blood proteins, and boost immune system. The crude protein (22.61%) as observed in the present study is lower than those of Ikewuchi and Ikewuchi [55] on wet and dry *P. tuberregium* sclerotia; lower than that of Regula and Siwulski [77] on dried *L. edodes*, and compares to the values reported by Bhattacharjya., *et al* [24]. The protein contents of mushrooms have been reported to vary according to the genetic structure of species, the physical and chemical differences of the growing medium [78]. Effiong., *et al.* [79] and Ali [80] noted that any plant food that provides about 12% of its caloric value from protein is taken as good protein source. Ash content has been related to presence of nutritionally important minerals food substances [10]. Ash content (8.24%) of the present study is lower than the values reported by Ikewuchi and Ikewuchi [55] on wet and dry *P. tuberregium* sclerotia; lower than the value reported by Bhattacharjya., *et al.* [24] on *P. ostreatus* and compares to the values reported by Akhtar., *et al.* [44] on *P. florida.* Fats in diets increase palatability of food by absorbing and retaining flavors [81]. The fatty acid components of fats/oils have been noted to play strategic role in organisms against some disease pathogens and degenerating health conditions [82]. The crude fat (5.01%) content of the studied sample is higher than the values reported on wet and dry *P. tuberregium* sclerotia [55]; dry *L. edodes* [77] and compares to the value reported by Egwim., *et al.* [83] on *P*. *ostreatus*. The importance of fibre in food substances to organisms has been noted by Walker [84], SACN [85], Ikewuchi and Ikewuchi [55], Arukwe., *et al* [76]. The fibre (16.28%) content of *P. ostreatus* in this study is higher than the values reported by Ikewuchi and Ikewuchi [55] and Ijeh., *et al.* [56] on *P. tuberregium,* Adejumo., *et al*. [19] on *T. robusta*; but compared to the values reported by Egwim., *et al.* [83] and Bhattacharjya., *et al.* [24] on *P. ostreatus*. Total carbohydrate (47.86%) as observed in this study is higher than the values of Ikwewuchi and Ikewuchi [55] on wet and dry *P. tuberregium* sclerotia; lower than the value of (2009) on *P. tuberregium*; and compares to the values reported by Patil., *et al.* [58] on the nutritional value of *P. ostreatus* (Jacq:Fr) kumm cultivated on different lignocellulosic agro-waster. *P. ostreatus* becomes a potential source of carbohydrate when compared to the carbohydrate contents of some conventional food sources such as cereals [86] and some leafy vegetables [87-89]. The energy value (326.97 Kcal/100g) of *P. ostreatus* in the present study is lower than the values reported by Ijeh., *et al.* [56], Ikwewuchi and Ikewuchi [55] but higher than the values reported on *A. bispocus, L. edoes, P. eryngii, P. sajor-caju,* and *P. giganteus* by María., *et al* [60].

According to Alais and Linden [89], vitamins are chemicals substances which organisms are not capable of synthesizing. The same authors noted that vitamins are necessary for growth and the functioning of organisms and must therefore be obtained in regular and periodic fashion through the diet. Vitamin content of *P. ostreatus* as presented in figure 1 shows the presence of vitamin A (0.14 mg/100g), B1 (0.10 mg/100g), B2 (0.57 mg/100g), B3 (0.40 mg/100g), B6 (0.91 mg/100g), B12 (0.16 mg/100g), C (0.23 mg/100g), D (0.89 mg/100g), E (0.09 mg/100g), and K (0.28 mg/100g). María., *et al.* (2015) noted that mushrooms are a good source of vitamins with high levels of riboflavin (vitamin B2), niacin, folates, and traces of vitamin C, B1, B12, D and E. Heleno., *et al.* [90] note that edible mushrooms provide a nutritionally significant content of vitamins (B1, B2, B12, C, D and E. All these were observed in the present study. The presence of vitamin D as observed in the present study is in line with the statement of María., *et al.* [60] that mushrooms are the only non-animal food source that contains vitamin D and are the only natural vitamin D ingredients for vegetarians. Vitamins have co-factor role in enzyme and membrane systems [89]. It has been noted that the deficiencies of vitamins are associated with a state of malnutrition, which plays a major role in the clinic picture [89]. Okwu [11] and Amadi., *et al.* [91] have reported the individual functions of the observed vitamins of the present study in the body.

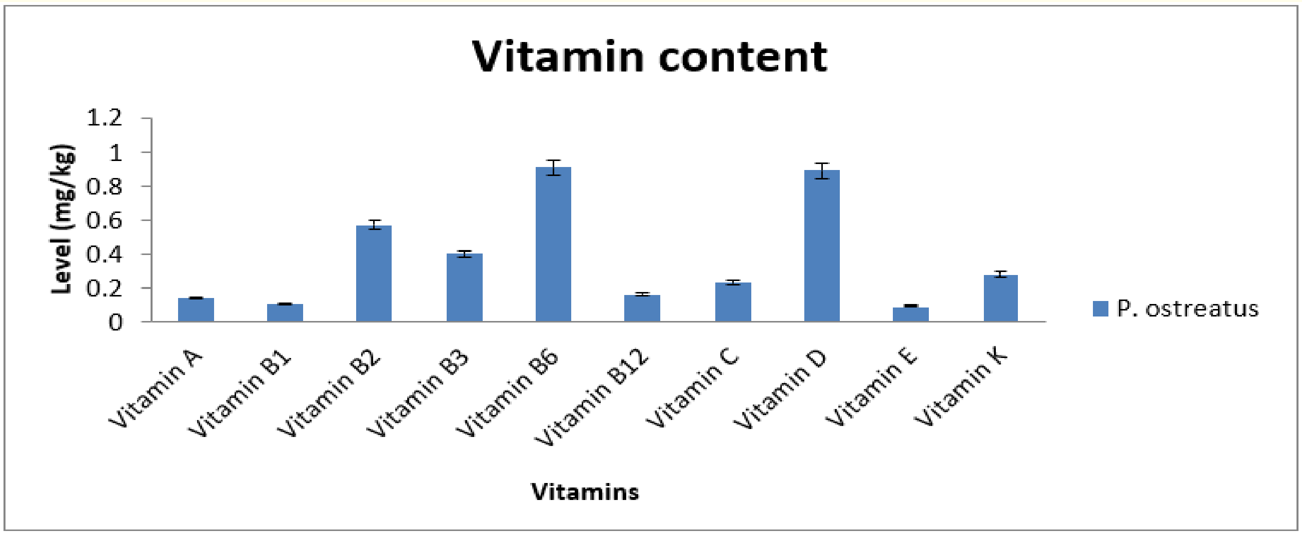

Figure 1: *Vitamin contents of P. ostreatus.*

Olusanya [10] and Amadi., *et al.* [92] reported the relationship between minerals and ash constituent of proximate composition. Minerals found in *P. ostreatus* in this study are presented in figure 2. Observed mineral contents were potassium (18.12 mg/100g), sodium (1.21 mg/100g), phosphorus (3.84 mg/100g), calcium (38.12 mg/100g), magnesium (90.82 mg/100g), zinc (1.68 mg/100g), copper (0.54 mg/100g), iron (14.28 mg/100g), and manganese (0.15 mg/100g). Observed values of potassium, sodium, calcium, magnesium, and copper were lower; whereas those of zinc, iron, and manganese were higher against their respective values as reported by Ijeh., *et al.* [56] on *P. tuberregium* fruit body. The observed high calcium (38.12 mg/100g) content of this study is in line with earlier work of Adejumo., *et al.* [19] on *P. ostreatus*. Function of potassium is typically for acid base balance, sodium for transmission of impulse, phosphorus and magnesium work together for cyclic AMP as well as other second messengers, zinc and copper interwork against diabetes and wound healing, iron known for its role in ferrodoxins and haemoglobin; whereas manganese play strategic role as activators of certain enzymes [92]. The sodium/potassium, calcium/phosphorus, and calcium/magnesium weight ratios of the present study were 0.07, 9.93 and 46.49 respectively, against their respective ratios of 1.0, 1.0 and 2.2 as recommended by NRC [93]. The sodium/potassium ratio of the studied sample is less than one, and it is of great importance to the body due to relationship between sodium ratio and occurrence of high blood pressure [94]. High calcium content of *P. ostreatus* coupled with its low phosphorus and magnesium contents could possibly be behind the high calcium/phosphorus, and calcium/magnesium ratios of the sample. This could be indication of poor intestinal absorption of calcium minerals from the sample when consumed [95].

The protein contents of mushrooms vary according to the genetic structure of species, the physical and chemical differences of the growing medium [19,78]. Invariably, amino acids, the building blocks of protein would also vary the same way. This could be the reason behind the variations observed with the amino acids of the studied sample as presented in figure 3 (leucine (5.10 g/100g), lysine (2.90 g/100g), isoleucine (2.01 g/100g), phenylalanine (3.20 g/100g), tryptophan (0.13 g/100g), valine (3.18 g/100g), methionine (0.72 g/100g), proline (1.89 g/100g), arginine (2.96 g/100g), tyrosine (7.03 g/100g), histidine (1.01 g/100g), cysteine (2.04 g/100g), alanine (4.90 g/100g), glutamic acid (8.12 g/100g), glycine (4.20 g/100g), threonine (4.27 g/100g), serine (0.18) and aspartic acid (6.10 g/100g)) against the values reported by Patil., *et al.* [58] on *P. ostreatus*. Some functions of the individual amino acids observed in this study have been reported by Amadi., *et al.* [92] and Uwakwe and Ayalogu [7].

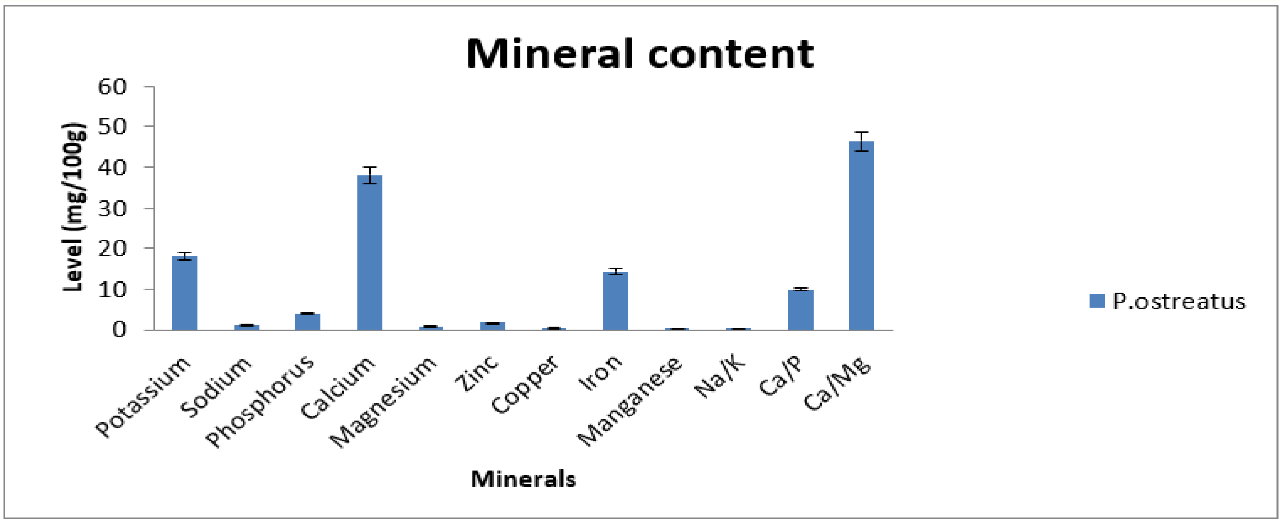

Figure 2: *Minerals found in P. ostreatus.*

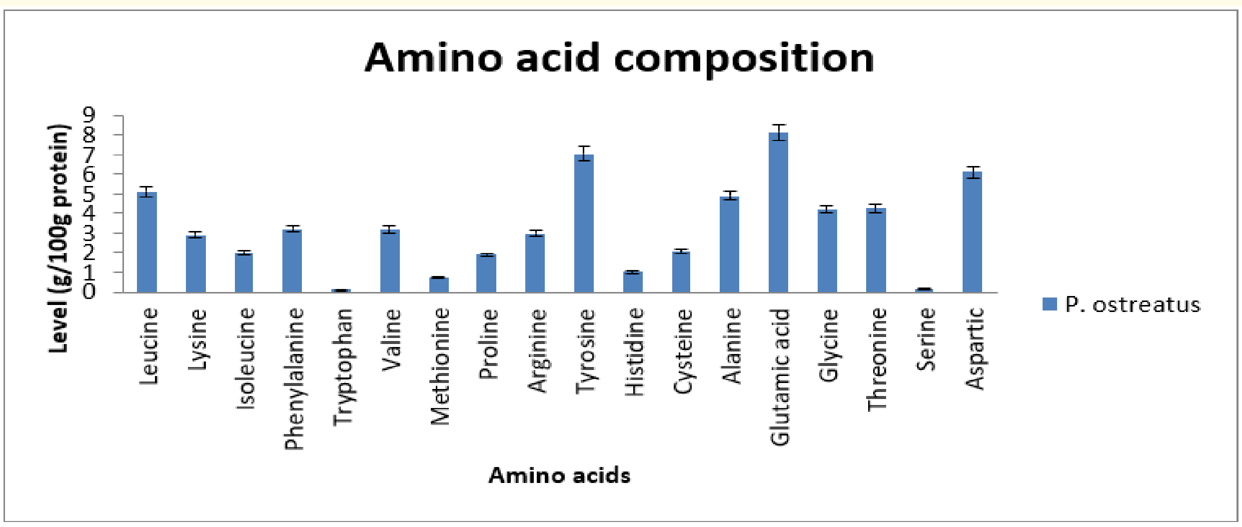

Figure 3: *Amino acid composition of P. ostreatus.*

Table of total amino acid groups of *P. ostreatus* (Table 2) reveals that the sample could be a good source of total non-essential amino acids than total essential amino acids (with histidine or without histidine). *P. ostreatus* sample has more total non-essential amino acids than total essential amino acids with or without histidine (Table 2). However, the total essential amino acids value of the studied sample is higher than 1.7g total essential amino acids of egg white as reported by Connolly [96] and it could also be regarded as a good source of essential amino acids. Total non-essential amino acids value of the studied sample is higher than 2.1g of total non-essential amino acids found in white egg as noted by Connolly [96]. Large neutral amino acids and branched chain amino acids according to Fernstorm [97] modify tryptophan and tyrosine uptake into brain and their conversion to serotonin and catecholamines, respectively. Total neutral amino acid group was the highest against total acidic and basic amino acid groupings (Table 2).

Percentages of amino acid groups of *P. ostreatus* (Table 3) reveal that %Total non-essential amino acids (57.49%) was higher than the %Total essential amino acids with histidine (42.51%) and without histidine (40.82%). %Total non-essential amino acid value of *P. ostreatus* was above 50% and could be indication that proteins of the sample may be charged. T% Total non-essential amino acids of *P. ostreatus* (57.49%) is lower than 59.36%, 59.33%, and 59.98% reported Igwe., *et al.* [98] on raw, boiled and fermented *Prosopis africana* respectively. %Total acidic amino acid (23.72%) and %Total basic amino acid (11.61%) values could be indication that the proteins of the studied sample are negatively charged and could be acidic proteins. This observation is in line with the works of Igwe., *et al*. [98] on *P.*

africana; and Adeyeye., *et al.* [99] on cocoa. % Cys in sulphur-containing amino acids (73.91%) of *P. ostreatus* is high compared to 4.60% of % Total Sulphur-containing amino acids of the sample. High Cys content of the sample could result in methionine sparing action. %Cys in sulphur-containing amino acids of the studied sample is higher than 37.85% reported on *Garcinia kola* and 50.5% on *Anacardium occidentale* by Adeyeye., *et al.* [100] and 60.09% on *P. africana* by Igwe., *et al* [98]. %Cysteine in sulphur-containing amino acids surpassed the expected 50% from a plant source [98]. Consumption of *P. ostreatus* may contribute to cysteine pool, and the contributions of cysteine to formation of glutathione as well as the functions of glutathione in the body have been reported by Nelson and Cox [101], Harris and Crabb [102] and Nelson and Cox [103]. % Total branched chain amino acids (17.17%) surpassed the expected 10% desired energy requirement of proteins as noted by Wardlaw and Kessel [104]. This could be an indication that *P. ostreatus* may provide energy for the body. % Total aromatic amino acid value (17.28%) and %Tyr in total aromatic acids value (67.86%) could be indication that over 20% of phenylalanine in *P. ostreatus* may be spared by tyrosine. Phenylalanine is among the essential amino acids, which takes part in protein synthesis [65].

Parameter	***P. ostreatus***
Total amino acids	59.94
Total non-essential amino acids	34.46
Total essential amino acids	
With histidine	25.48
Without histidine	24.47
Total neutral amino acids	34.04
Total acidic amino acids	14.22
Total basic amino acids	6.96
Total sulphur-containing amino acids	2.76
Total branched chain amino acids	10.29
Total aromatic amino acids	10.36

Table 2: *Total amino acid groups (g/100g protein) of P. ostreatus.*

Note: These results are based on figure 3 above.

Parameter	***P. ostreatus***
%Total non-essential amino acids	57.49
%Total essential amino acids	
With His	42.51
Without His	40.82
%Total neutral amino acids	56.79
%Total acidic amino acids	23.72
%Total basic amino acids	11.61
%Total sulphur-containing amino acids	4.60
%Cys in sulphur-containing amino acids	73.91
%Total branched chain amino acids	17.17
%Total aromatic amino acid	17.28
%Tyr in total aromatic acids	67.86

Table 3: *Percentages of amino acid groups (%) of P. ostreatus.*

Note: These results are based on figure 3 and table 2 above.

Ratios of amino acids and amino acid groupings of *P. ostreatus* (Table 4) reveals that leucine/Isoleucine ratio of the studied sample as being more than one, which invariably means that the studied sample contains more leucine against isoleucine. This may not go down well with the utilization of trp and niacin. According to Igwe., *et al.* [98] and Ibegbulem., *et al.* [65] diets with leucine contents have been reported to impair Trp and niacin metabolism. With the total basic amino acid/total acidic amino acid ratio of less than 1 (< 1), the observation could be indication that the proteins of *P. ostreatus* maybe acidic proteins and may as well serve as acids at physiological pH. The ratio of total amino acids with histidine (0.43) and without histidine (0.41) to total amino acid could be indication that the amino acids of the studied sample may not support protein synthesis.

Parameter	***P. ostreatus***
Leucine/Isoleucine	2.54
Total basic amino acid/Total acidic amino acid ratio	0.49
Total essential amino acid/Total amino acid ratio	
With His	0.43
Without His	0.41

Table 4: *Ratios of amino acids and amino acid groupings of P. ostreatus.*

Note: These results are based on figure 3 and table 2 above.

The amino acid score determines the effectiveness with which absorbed dietary nitrogen can meet the indispensable amino acid requirement at safe level [105-107]. Olusanya [10] that any method employed to measure quality of protein must either determine growth-promoting ability of protein food or its essential amino acid content. In recent years, prediction of protein quality using Protein Digestibility Corrected Amino Acid Score (PDCAAS) has been established. Protein Digestibility-Corrected Amino Acid Score (PDCAAS) is a method of evaluating the protein quality based on both the amino acid requirements of humans and their ability to digest it. It has a relationship with digestibility and amino acid score [107-109]. Amino acid scores of the studied sample relative to FAO/WHO/UNU [110] provisional amino acid scoring pattern (Table 5) reveals that that *P. ostreatus* surpassed the total aromatic amino acids, threonine, and tryptophan surpassed requirements by 71%, 5% and 30% respectively. Those close to their requirements such as leucine and total sulphur-containing amino acids fall short by 27% and 21% respectively. Isoleucine, lysine and valine fall short of their requirements by 50%, 47% and 38% respectively. Isoleucine is the amino acid with the least provisional score, hence the limiting amino acid of the studied sample.

Amino acid	***P. ostreatus***	**FAO/WHO/UNU (1981) (mg/g)**
Isoleucine	0.50	40
Leucine	0.73	70
Lysine	0.53	55
Methionine+Cysteine	0.79	35
Phenylalanine+Tyrosine	1.71	60
Threonine	1.05	40
Tryptophan	1.30	10
Histidine	NC	NA
Valine	0.62	50
Total	7.23	360

Table 5: *Amino acid scores of P. ostreatus based on FAO/WHO/UNU [110] provisional amino acid scoring pattern.*

Results are mean of triplicate determinations.

NC: Not Calculated; NA: Not Available.

The biological significance of fatty acids has been noted [111,112]. Fatty acids observed in the studied sample include lauric acid (9.94%), myristic acid (27.59%), palmitic acid (3.36%), Hexadecadienoic (8.74%), stearic acid (20.985), oleic acid (6.06%), linoleic acid (21.39%), and linolenic acid (2.41%) as present in figure 4. Sums of unsaturated fatty acids, saturated fatty acids and polyunsaturated fatty acids were 38.60%, 61.87%, and 23.80% respectively. Fatty acids from diets are classified into their predominant fatty acids as essential and nonessential fatty acids [10]. Linoleic acid is the fatty acid considered to be essential in the diets of humans [113]. Linoleic acid and alpa-linoleic acid are converted to eicosapentaenoic acid, docosahexaenoic acid, gamma-linolenic acid, arachidonic acid, and cervonic acid, which are important to health [114]. Different authors [115] have noted that linoleic and linolenic fatty acids account for Omega-3-fatty acid and omega-6-fatty acid respectively. The omega fatty acids are known to play specific role in brain and eye development, heart development as well as normal growth development [116-119]. The observed palmitic, stearic, oleic, linoleic and linolenic fatty acids are in line with the work of María., *et al* [60]. The observed polyunsaturated fatty acids agree with the report of María., *et al.* [60] who noted that polyunsaturated fatty acids are contained in mostly edible mushrooms.

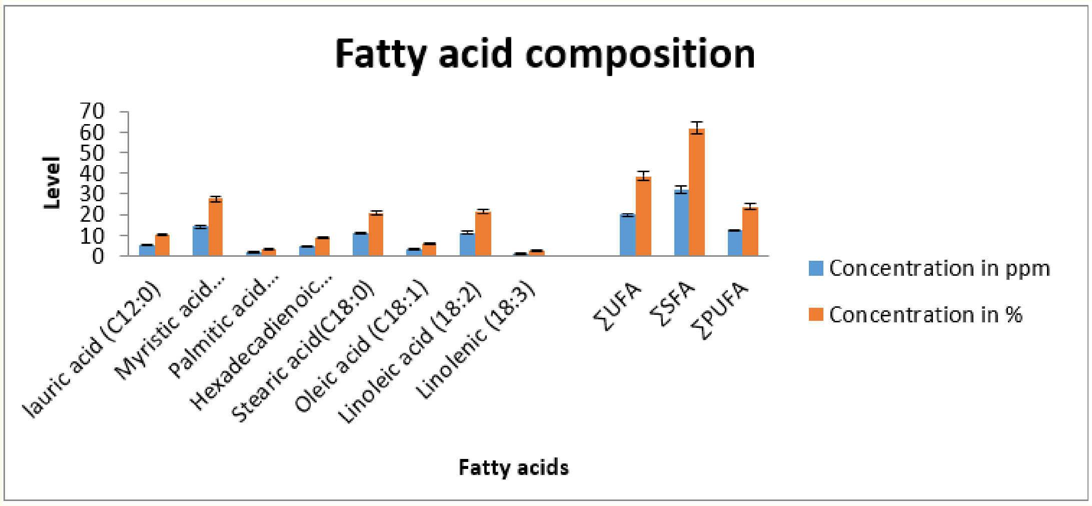

Figure 4: *Fatty acid composition of P. ostreatus.*

∑PUFA: Sum of Polyunsaturated Fatty Acid

∑UFA: Sum of Unsaturated Fatty Acid

∑SFA: Sum of Saturated Fatty Acid

Anti-nutrients are chemical compounds synthesized in natural food by normal metabolism. They exert effects on food nutrients and influence their bioavailability [120,121]. The anti-nutrients found in *P. ostreatus* (Figure 5) showed the presence of saponins (1.64 mg/100g), alkaloids (1.52 mg/100g), oxalate (0.41 mg/100g), tannins (0.11 mg/100g), phytate (0.90 mg/100g) and flavonoids (0.31 mg/100g). Saponin was the highest against flavonoid the lowest as observed in the present study. Saponin has been implicated in the reduction of uptake of certain nutrients including glucose and cholesterol at the gut through intra-lumenal physico-chemical interaction [122]. The importance of alkaloids and flavonoids in foods and in the biological system cannot be overemphasized [123,124]. High oxalate levels in food may reduce the bioavailability of such metals as calcium, magnesium, and zinc thereby interfering with their utilization [125-127]. According to Duru., *et al*. [9] the stringent taste of tannins affects palatability and digestibility of food. Tannins decrease protein quality by decreasing its digestibility and palatability [128-130]. It has been reported that knowledge of phytate level in foods is important because high concentration can bring about adverse effects on digestibility [131]. Ions such as Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+} and Fe^{2+} have been reported to complex with phytate to form stable complexes hence prevent their bioavailability [122].

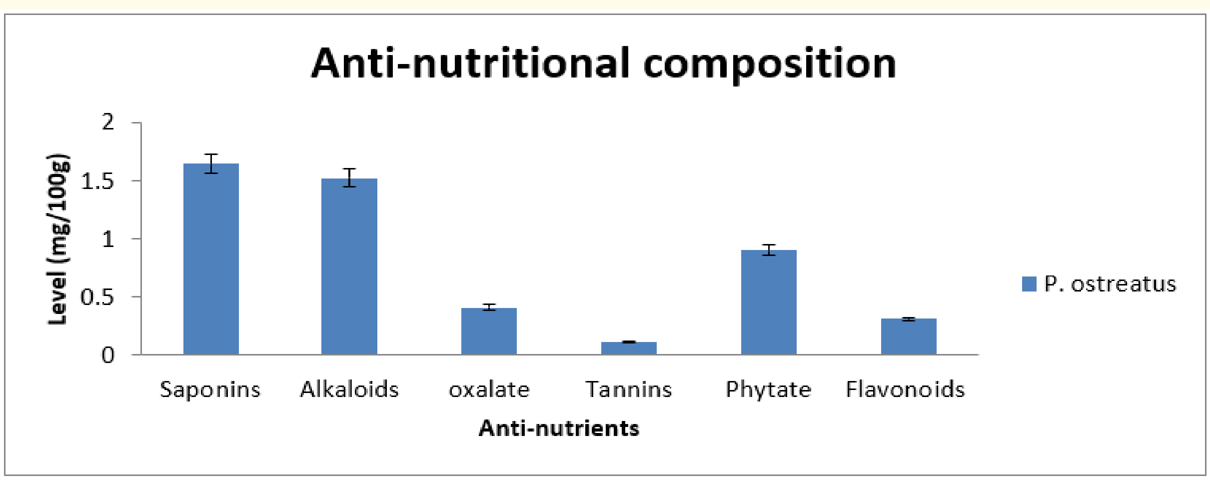

Figure 5: *Anti-nutrient composition of P. ostreatus.*

In distinguishing between nutrients and anti-nutrients in food substances, the knowledge of their relationship is equally important. To predict such relationship, the polyunsaturated fatty acids to saturated fatty acid ratio (PUFA/SFA); and the nutrient-antinutrient mole ratios of *P. ostreatus* were estimated as presented in table 6. The obtained values were compared to the ideal ratio for fatty acid and critical values for each nutrient-anti-nutrient relationships as to predicate their importance and bioavailability as the case maybe in the studied sample. It has been reported that foods with PUFA/SAF ratio less than 0.45 may not be appropriate for human consumption due to linkage of such foods to diseases of the heart [132]. Oxalic acids and its salts are associated with decreasing calcium absorption. According to Frontela., *et al.* [133], oxalate content in plant product in limiting total dietary calcium available is significant only when the ratio of oxalate to calcium is greater than one. Hassan., *et al.* [134] noted that oxalate to calcium mole ratio of not more than 2.5 could be indication of calcium bioavailability. The biological interaction between calcium, magnesium and oxalate in terms of bioavailability is also considered in plants. The mole ratios of [Oxalate]/[Calcium] and [Oxalate]/[Calcium+Magnesium] of the present study were lower than their critical values and could be indication of calcium availability in the studied sample. Phytate is known to inhibit the bioavailability of calcium, iron and zinc [120,121]. The estimated mole ratios for [Phytate]/Iron], [Phytate]/[Calcium], and [Phytate]/[Zinc] were lower than their critical values. Obelease and Harland [135] showed that foods with a molar ratio of zinc less than 10 showed adequate availability of zinc and problems were encountered when the value was greater than 15. Hassan., et al. [136] noted that [Phytate/[Ca] and [Phyate]/[Fe] mole ratios of above 0.2 and 0.4 respectively could be indication of poor bioavailability of calcium and iron. Ellis., *et al.* [137] and Davies and Warrignton [138] indicated that the ratio of Ca x Phy/Zn is a better predictor of Zn availability and that if the values were greater than 0.5 mol/kg, there would be interferences with the availability of Zn. The estimated Ca x Phy/Zn mole ratio of the studied sample is lower than the critical value and could be indication of Zn availability.

Ratio	Value	Critical value/Ideal ratio
PUFA/SFA	0.38	Not lower than 0.45
[Oxalate]/[Calcium]	4.90×10^{-2}	2.5
[Oxalate]/[Calcium+Magnesium]	4.73×10^{-3}	2.5
[Phytate]/Iron]	5.33×10^{-3}	0.4
[Phytate]/[Calcium]	1.43×10^{-3}	0.2
[Phytate]/[Zinc]	5.31×10^{-2}	10
[Calcium][Phyate]/[Zinc]	5.05×10^{-2}	0.5

***Table 6:** Fatty acid ratio and nutrient-antinutrient mole ratios of P. ostreatus.*

Critical values of nutrient-antinutrient molar ratios adopted from Hassan., et al. [134] and Hassan., et al. [121].

The body weight and percentage weight changes of rats as presented in figure 6 showed decreased order in the parameters compared to those of the control. Body weight change of test groups decreased significantly ($p < 0.05$) against those of the control and reference 1 and 2 rats. Agomuo., *et al.* [139] noted increase in sympathetic tone of various resting tissue beds, notably fats as one that stimulate weight loss. This reason could also be behind the weight reduction effect of *P. ostreatus* in test rats.

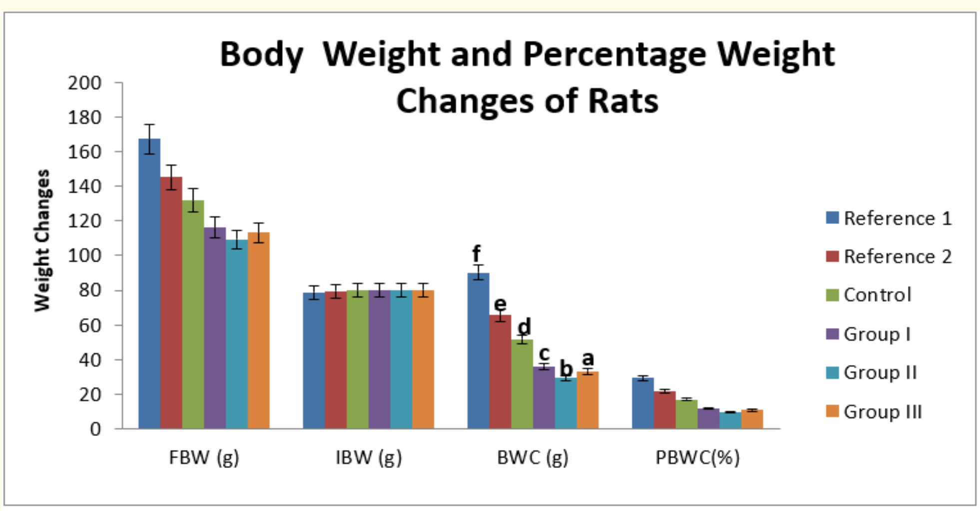

***Figure 6:** Body weight and percentage weight changes of rats.*

***Note:** Bars of an index with different letters of alphabet are statistically significant (p < 0.05).*

***Legend:** FBW: Final Body Weight; IBW: Initial Body Weight; BWC: Body Weight Change and PBWC: Percentage Body Weight Change.*

The various haematological parameters investigated in this study are useful tools for evaluating the status of *P. ostreatus* in the body. The haematological indices of the present study are present in figures 7-9. Hb levels of groups I and II rats significantly ($p < 0.05$) increased against the control and reference 2, but decreased significantly ($p > 0.05$) against reference 1. Hb levels of group III significantly ($p < 0.05$) increased when compared to the control and reference 2; and was insignificantly ($p > 0.05$) affected against reference 1. *P. ostreatus* may have influenced the production of haemoglobin in test rats to a level that is comparable to that of standard known baby food such as Nutrend food. PCV level of group I significantly ($p < 0.05$) increased when compared to those of control and reference 2 but decreased significantly ($p < 0.05$) against reference 1, whereas PCV levels of groups II and III increased significantly ($p < 0.05$) against those of reference 1 and 2; and the control. The known relationship between PCV and Hb levels in blood [140] was also maintained in the present study. RBC level of group I was significantly higher ($p < 0.05$) than those of reference 2 and control, while RBC of groups II and III were significantly higher ($p < 0.05$) against the control and reference 2 but compares favourably with RBC level of reference 1 rats. The relationship between Hb and RBC levels in test rats when compared to control, reference 1 and reference 2 rats in the present study could be a clear indication that *P. ostreatus* may encourage erythrocyte production (erythropoiesis) in the body on consumption. Leukocytosis is stimulation of the immune system, which protects the body against any infection [141]. WBC levels of test groups increased significantly ($p < 0.05$) against the control, but insignificantly ($p > 0.05$) against reference 2, while WBC of test group I reduced insignificantly ($p > 0.05$) against reference 1. WBC levels of test groups II and III reduced significantly ($p < 0.05$) against reference 1. Lymphocyte levels of test groups increased significantly ($p < 0.05$) when compared to the control and reference 1 but reduced significantly ($p < 0.05$) against reference 2. Monocytes and Eosinophil levels increased significantly ($p < 0.05$) in test groups against the control, reference 1 and 2. MCV levels of test groups I and II significantly ($p < 0.05$) reduced when compared to control, reference 1 and 2. MCV of test group III increased significantly ($p < 0.05$)against the control, reference 1 and 2, but insignificantly ($p > 0.05$) reduced against reference 1.MCH levels of test groups I and II reduced significantly ($p < 0.05$) against the control, reference 1 and 2, while MCV level of test group III increased insignificantly ($p > 0.05$) against the control, reference 1 and 2. MCHC levels of test groups I and II reduced significantly ($p < 0.05$)when compared to reference 1 and 2. MCHC level in test group II was insignificantly reduced ($p > 0.05$) against the control, while MCHC level of test group III reduced insignificantly ($p > 0.05$) against reference 1 and 2; and control. MCHC and MCH relate to individual red blood cells while Hb, RBC, and PCV are associated with the total population of red blood cells. MCV, MCH, and MCHC levels of test groups in the present study compared favourably with those of reference I (Nutrend), a known baby food. This could be indication that *P. ostreatus* may not influence these blood parameters negatively. The enhance effect by *P. ostreatus* on blood indices as observed in the present study is in line with earlier report of María., *et al*. [60], who noted that *Pleurotus* mushrooms have haematological properties.

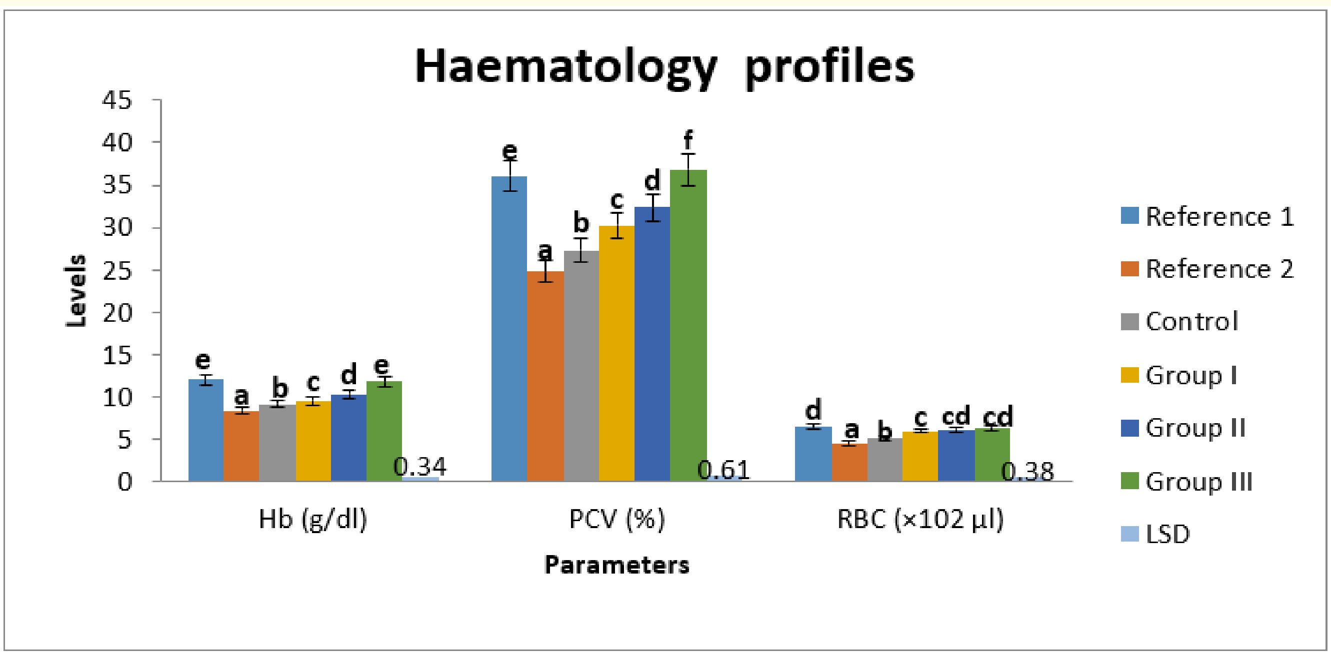

Figure 7: *Hb, PCV, and RBC levels of rats placed on P. ostreatus.*

Note: *Bars of an index with different letters of alphabet are statistically significant (p < 0.05).*

Legend: *Hb: Haemoglobin; PCV: Packed Cell Volume; RBC: Red Blood Cell and LSD: Least Significance Difference.*

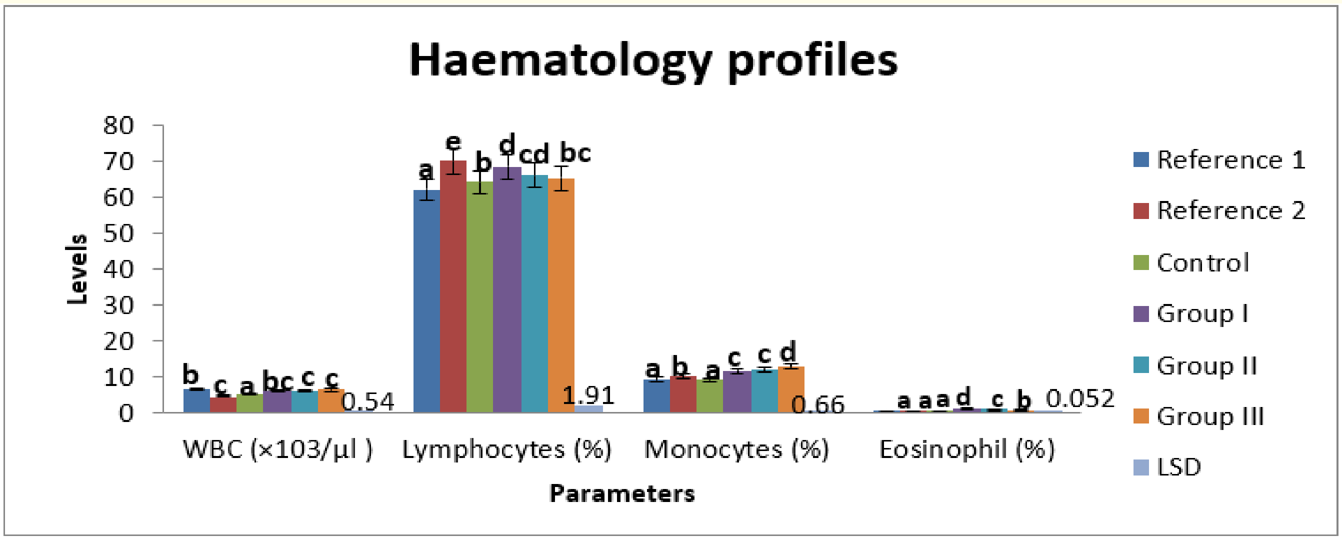

Figure 8: *WBC, lymphocytes, monocytes, and eosinophil levels of rats placed on levels of rats placed on P. ostreatus.*

Legend: WBC: White Blood Cell and LSD: Least Significance Difference.

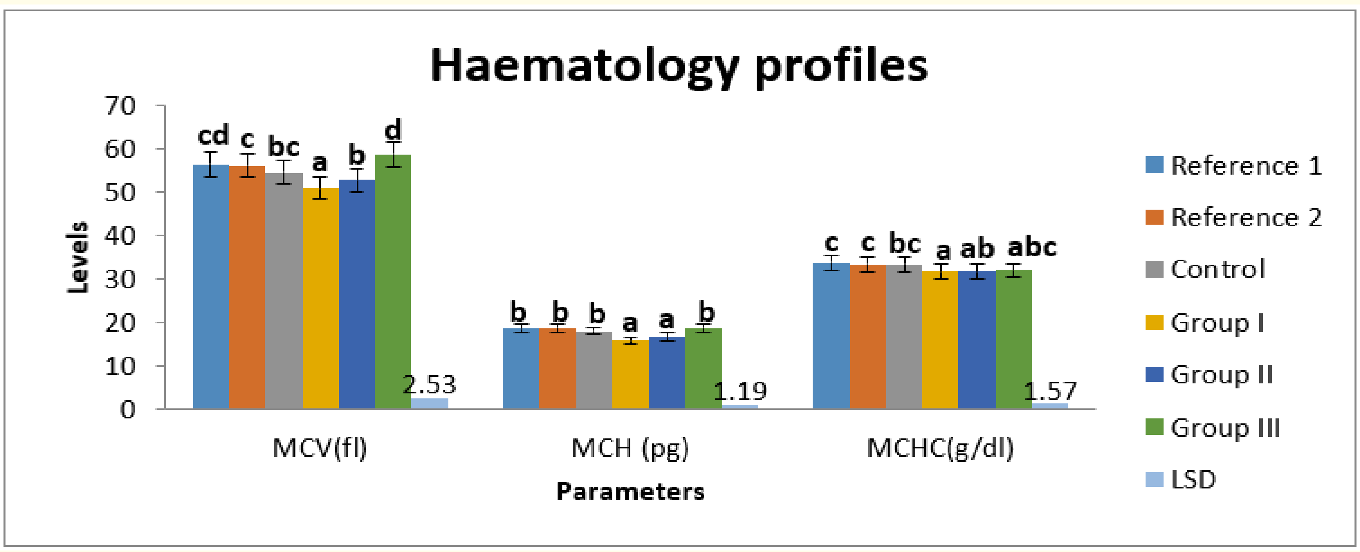

Figure 9: *MCV, MCH and MCHC levels of rats placed on P. ostreatus.*

Note: *Bars of an index with different letters of alphabet are statistically significant ($p < 0.05$).*

Legend: *MCV: Mean Cell Volume; MCH : Mean Corpuscular, Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration and LSD: Least Significance Difference.*

In recent years, it has been reported that nutritional lifestyle can induce dyslipdemia on individuals. Poor nutritional lifestyle could result in hypertension and other cardiac diseases. Total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol as present in figure 10, are indices of lipid profile. Lipid profile is used to determine cardiac risk assessment and helps to determine individual's risk to heart disease condition [9]. The importance of cholesterol in the body cannot be over emphasized [142-144]. However, a rise in the blood cholesterol has been associated with diseases such as arthrosclerosis, which is among the leading cause of death in developing countries of the world [144]. The therapeutic benefits of natural foods have been the main focus of some scientific studies aimed at dietary regulation of cholesterol and reducing of cardiovascular diseases [145]. Total cholesterol levels of test groups reduced significantly ($p < 0.05$) when compared to the control, reference 1 and reference 2. Bainton., *et al.* [146] and Cullen [147] noted that evidences have shown that high triglyceride levels are associated with coronary atherosclerosis. Triglyceride levels of test group I reduced significantly (p < 0.05) against reference 2, and insignificantly (p > 0.05) against the control and reference 1. Test groups II and III had significant reduction (p < 0.05) in triglyceride levels against the control, reference 1, and reference 2. According to Cullen [147], the link between LDL-cholesterol and atherosclerosis are interconvertible. LDL-cholesterol levels of test groups reduced significantly (p < 0.05) when compared to the control, reference 1 and reference 2. Aside the role of LDL-cholesterol in the development of arthrosclerosis, emerging evidences suggest that growing level of high density lipoprotein cholesterol (HDL-cholesterol) is a powerful predictor of cardiovascular disease (CVD). Notwithstanding the HDL-cholesterol level of test group I that increased insignificantly (p > 0.05) against the control, reference 1 and 2; test groups II and III had significant increase (p < 0.05) in HDL-cholesterol levels when compared to the control, reference 1 and reference 2. According to María., *et al.* [60], polyunsaturated fatty acids are found in edible mushrooms and they may contribute to the reduction of serum cholesterol. The same authors implicated linoleic acid, an essential polyunsaturated fatty acid to humans as having a wide range of physiological functions; and reduce cardiovascular diseases, triglyceride levels, blood pressure, and arthritis. The presence of polyunsaturated fatty acids and linoleic acid in *P. ostreatus* in this study could be behind the observation made on the lipid profile indices of test rats against the control rats, reference 1 and reference 2 (Figure 10).

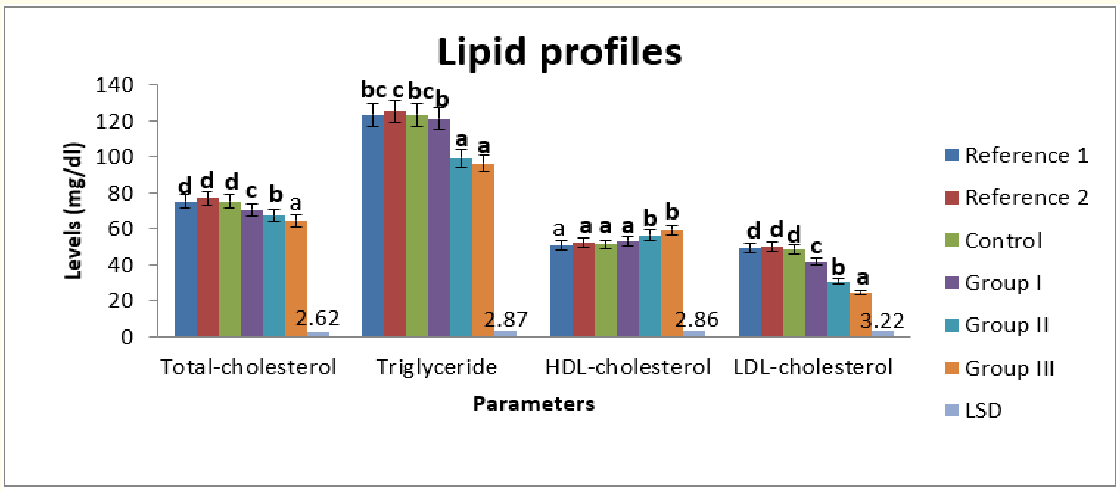

Figure 10: *Total-cholesterol, triglyceride HDL-cholesterol and LDL-cholesterol levels of rats placed on of rats placed on P. ostreatus.*
Bars of an index with different letters of alphabets are statistically significant (p < 0.05).
Legend: LSD: Least Significance Difference.

Lipid profile ratios, which include cardiac risk ratio, atherogenic coefficient, triglyceride/HDL-cholesterol, atherogenic index of plasma and LDL-cholesterol/HDL-cholesterol are powerful indicators of the risk of heart disease [148]. All the lipid profile ratios estimated in the present study as present in figure 11, significantly reduced (p < 0.05) in test groups when compared to those of the control, reference 1 and reference 2. These estimations could point to the fact that consumption of P. ostreatus in the body could be an advantage over lipid profile indices. The observations on lipid profile indices and lipid profile ratios of the present study may have placed *P. ostreatus* away from the risk of heart disease condition as predicted by 0.38 value of PUFA/SFA ratio of the sample, having been lower than the ideal ratio of 0.45 as noted HMSO [132] and Benjamin., *et al* [149].

It has been reported that tissue or organ damage is marked by the release of enzymes specific to such tissue or organ in circulation [150-152]. Figure 12 and 13 showed the ALT, AST and ALP levels; and bilirubin levels of rats placed on *P. ostreatus* respectively. Different authors have noted that AST is less specific than ALT as indicator of liver function [63,153,154]. The observed ALT level of test group I significantly reduced (p < 0.05) against reference 2; and insignificantly reduced (p > 0.05) affected when compared to those of the control. ALT levels of test groups II and III were insignificantly (p>0.05) affected against the control, reference 1 and reference 2. AST levels of test groups I and II reduced significantly (p < 0.05) against the control, reference 1 and reference 2. AST level of test group III reduced significantly (p < 0.05) against the control and reference 2 but reduced insignificantly (p > 0.05) against reference 1. ALP levels in test groups increased significantly (p < 0.05) when compared to the control, reference 1 and reference 2. The same order was followed by total bilirubin. The conjugated bilirubin levels of test group I significantly reduced p < 0.05) when compared to the control, reference 1 and reference 2; increased significantly (p < 0.05) in test group II against the control, reference 1 and reference 2; and was insignificantly affected (p > 0.05) in test group III when compared to those of the control, reference 1 and reference 2. The observations made on ALT and AST levels in the present study could be a clear indication that *P. ostreatus* did not affect the liver function in test rats. However, a lot of attribute could be given to the observed increase in ALP levels of test rats. A look at associate organ/glands other than the liver could be the sources of the ALP in test rats.

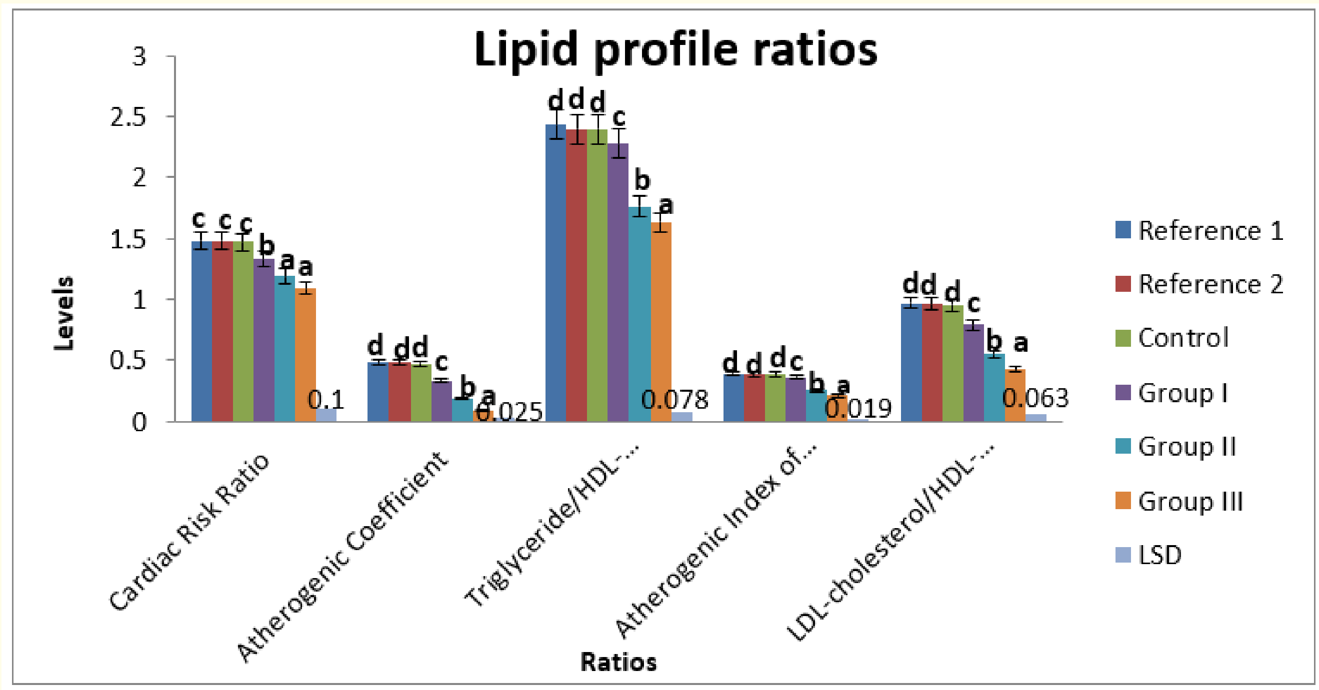

Figure 11: *Cardiac risk ratio, Atherogenic coefficient, Triglyceride/HDL-cholesterol, Atherogenic Index of Plasma and LDL-cholesterol/HDL-cholesterol ratios of rats placed on P. ostreatus.*

Bars of an index with different letters of alphabets are statistically significant (p < 0.05).

Legend: LSD: Least Significance Difference.

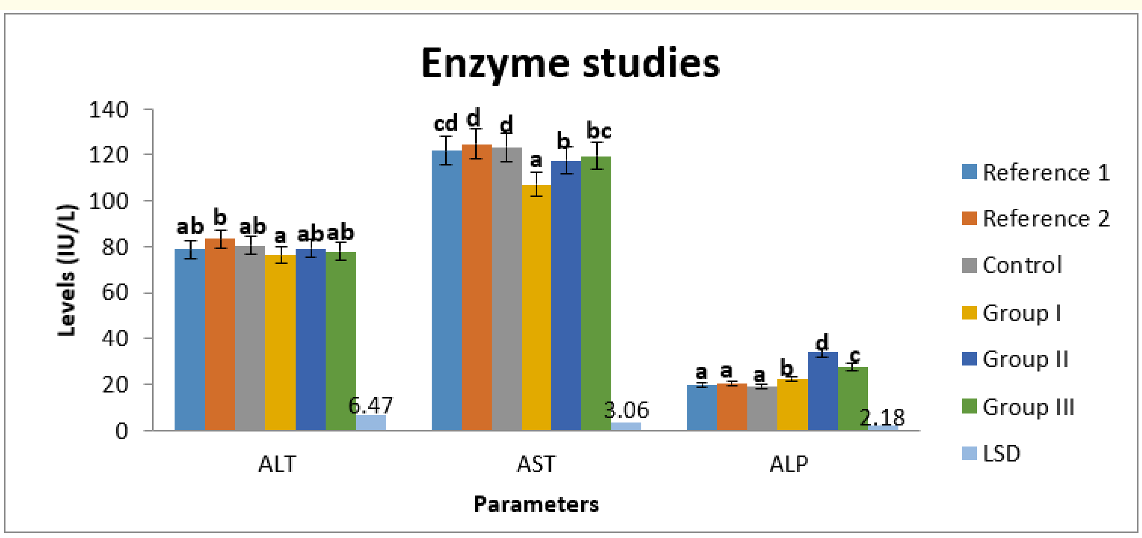

Figure 12: *ALT, AST, and ALP levels of rats placed on P. ostreatus.*

Bars of a parameter with different letters of alphabets are statistically significant (p < 0.05).

Legend: ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase and LSD: Least Significant Difference.

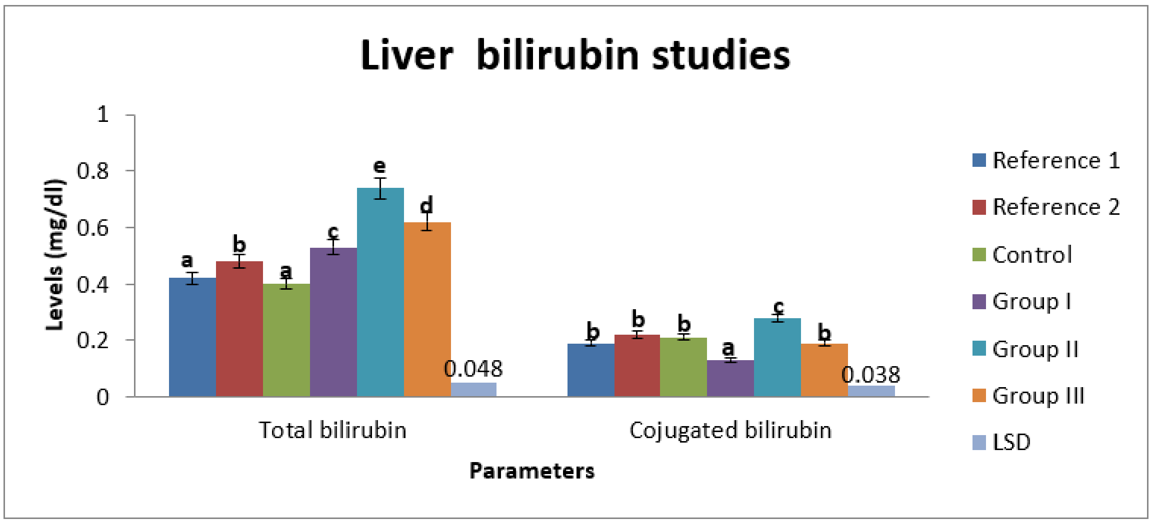

Figure 13: *Total bilirubin and conjugated bilirubin levels of rats placed on P. ostreatus.*

Bars of a parameter with different letters of alphabets are statistically significant (p < 0.05).

Legend: *LSD: Least Significant Difference.*

According to Aliyu., *et al.* [151], increase in level of creatinine is as indication of renal failure. Creatinine and urea levels are presented in figure 14. From, the figure, the creatinine level of test group I reduced significantly ($p < 0.05$) against the control and reference 2. Levels of creatinine in test groups II and III increase insignificantly ($p > 0.05$) when compared to reference 2. According to Nduka [155], high blood urea is associated with increased tissue protein catabolism, excess breakdown of blood protein and diminished excretion of urea. Increased urea in the blood azotaemia, was observed in test group I with significant increase ($p < 0.05$) in level of urea against the control, reference 1 and reference 2. However, at higher concentrations of test groups II and III, the urea levels reduced significantly ($p < 0.05$) against the control, reference 1 and reference 2.

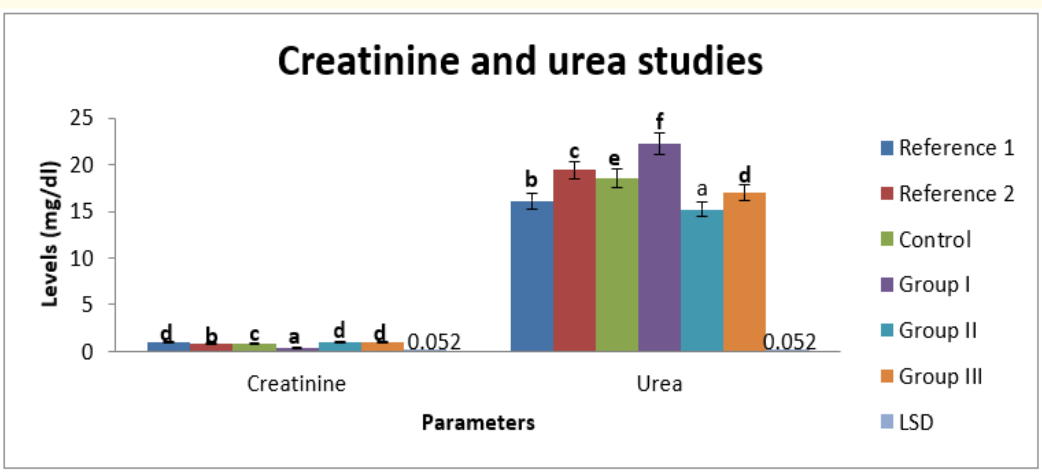

Figure 14: *Creatinine and urea levels of rats placed on P. ostreatus.*

Electrolyte levels of rats placed on *P. ostreatus* (Figure 15) revealed significant decrease ($p < 0.05$) in levels of Na^+ in test group I against the control and reference 2. Na^+ levels in test groups II and III reduced significantly against the control, reference 1 and reference 2. K^+ levels of test groups increased significantly ($p < 0.05$) against the control. K^+ levels of test groups II and II increased significantly ($p < 0.05$) against reference 2; while K^+ level of test group II was insignificantly ($p > 0.05$) affected against reference 1. Cl^- levels of test groups I and II increased significantly ($p < 0.05$) when compared to the control and reference 1; but compared favourably to reference I. Chloride level of test group III increased significantly ($p < 0.05$) when compared to the control, reference I and reference II. HCO_3^- levels of test groups I and II reduced significantly ($p < 0.05$) against the control and reference II. HCO_3^- level in test group III compared favourably to reference II; while HCO_3^- level of test group II increased significantly ($p < 0.05$) when compared to those of the control, reference I and reference II. The observed pattern for electrolyte ions in the present study could be an indication that *P. ostreatus* poses no threat on the electrolyte ion levels of the body and ultimately the renal system either following consumption.

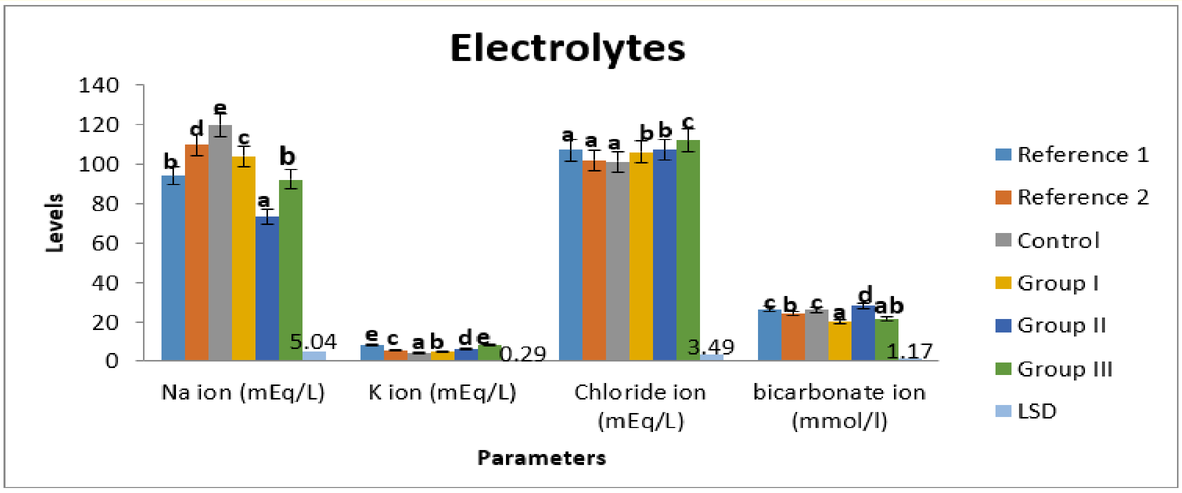

Figure 15: *Electrolyte levels of rats placed on P. ostreatus.*

Results of reproductive hormones as presented in figure 16 revealed significant decrease ($p < 0.05$) in testosterone levels of test groups against the control, reference I and reference II. Luteinizing hormonal levels also decreased significantly ($p < 0.05$) in test rats when compared to the control. The importance of normal reproductive hormonal levels for procreation is paramount. The observed reduction of these hormonal levels of the rest rats could be linked to the reduction in sizes of testis of the rats note in the present study. Both observations could imply that the activity of testis could be influence by *P. ostreatus* in the body [156-159].

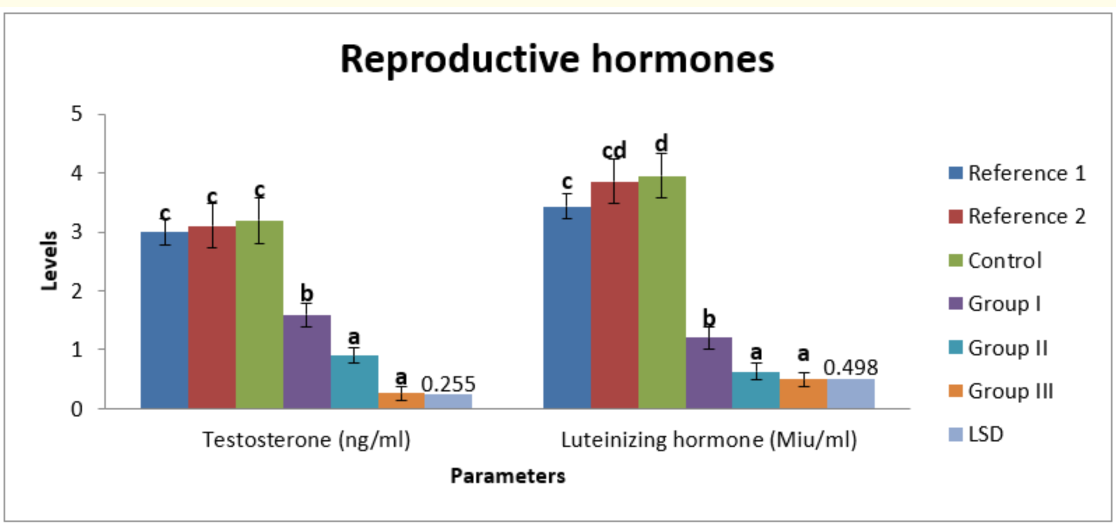

Figure 16: *Reproductive hormone levels of rats placed on P. ostreatus.*

Conclusion

Conclusively, *P. ostreatus* is high in proteins and fibre, with appreciable vitamins, minerals, fatty acids and anti-nutrients. Its nutrient-antinutrient mole ratios could ensure mineral nutrient bioavailability. Biochemically, *P. ostreatus* consumption may not have negative effects in the body since most observations made on biochemical indices compared favourably with those of Nutrend (reference I; a well-known standard baby food). However, ALP enzyme levels increased significantly in rats given *P. ostreatus* when compared to those of the control, reference I and reference II. Also, with the observed reduction in reproductive hormones and testis size of test rat, there may be need to extend the study on *P. ostreatus* to associate organs of the body. This study has evaluated the nutritional, anti-nutritional and biochemical effects of oyster mushroom, *P. ostreatus.*

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Volume 14 Issue 1 January 2019