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Full Length Research Paper

Evaluation of nutritional, anti-nutritional and some biochemical studies on *Pleurotus squarrosulus* (Mont.) singer using rats

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Evaluation of nutritional, anti-nutritional and some biochemical studies on *Pleurotus squarrosulus* (Mont.) Singer using rats was undertaken with standard methods. Results of nutritional contents such as proximate composition revealed moisture (11.67%), crude protein (21.31%) and total carbohydrate (51.58%). Vitamin composition showed the presence of vitamins A (0.17 mg/kg), B2 (0.62 mg/kg), B3 (0.45 mg/kg), B6 (0.84 mg/kg), and D (0.82 mg/kg). Important minerals such as potassium (20.75 mg/100 g), calcium (41.14 mg/100 g), magnesium (0.70 mg/100 g), and iron (17.80 mg/100g) were found in the studied sample with nine essential amino acids. *P. squarrosulus* sample revealed sums of polyunsaturated fatty acid of 13.14% and saturated fatty acid of 86.85%. Result of anti-nutritional composition of *P. squarrosulus* showed the presence of saponins (5.11 mg/100 g), oxalate (0.28 mg/100 g), phytate (0.75 mg/100 g) among others. The anti-nutrient to mineral molar and mineral weight ratios of the studied sample were within the recommended critical values/ideal ratios for bioavailability. Amino acid groups positioned the studied sample as having more non-essential amino acids than essential ones; with total neutral amino acids being the highest while total sulphur-containing amino acid was the lowest. Results of biochemical studies implicated *P. squarrosulus* as a weight reducing recipe, which can lower cholesterol levels, influence haematological indices positively, and lower aspartate aminotransferase (AST) and alanine transaminase (ALT) liver enzymes. However, *P. squarrosulus* increased alkaline phosphatase (ALP) and lowered reproductive hormonal levels in test rats when compared to experimental control and those placed on reference foods. Since ALP increase is associated with the prostate, there is need to extend the study on *P.squarrosulus* to accommodate its effect on prostate and reproduction. This study has evaluated the nutritional, anti-nutritional and some biochemical studies on *P.squarrosulus* (Mont.) Singer using rats.

Key words: Nutritional, anti-nutritional, biochemical studies, mushroom, *Pleurotus squarrosulus.*

INTRODUCTION

Mushrooms are among the saprophytic fungus of which majority are used as food materials (Aletor and Aladetimi 1995; Egwim et al., 2011; Omar et al., 2011; Jonathan et al., 2012; Hussein et al., 2014). They are known to have a distinctive fruiting body that could grow above the ground or underground (Boa, 2004; Oei and Nieuwenhuijzen, 2005; Stevenson and Lentz, 2007). Generally, mushrooms are macro in nature and large enough to be seen by an unaided eye (Fasidi, 2008). Such mushrooms are found in many parts of tropical Africa and other parts of the world (Chan, 1981; Kalac, 2009; Heleno et al., 2010; Khurdishul et al., 2010; Heleno et al., 2012).

According to Adejumo and Awosanya (2005), most mushrooms belong to the phylum Basidiomycota, while some members also belong to phylum Ascomycota. They have stem in the form of stipe; cap in the form of pileus and gills in the form of lamellae on the other side of the cap (Kirk et al., 2008). Different authors have noted that edible mushrooms contain proteins, carbohydrates, crude fibre, ash, minerals like iron, copper, manganese, potassium, calcium and sodium; and moisture (Kadiri and Fasidi, 1992; Hayes, 1997; Adejumo and Awosanya, 2005; Chandravadana et al., 2005; Akindahunsi and Oyetayo, 2006; Aremu et al., 2009; Ezeibekwe et al., 2009; Ikewuchi and Ikewuchi, 2009; Adedeyo et al., 2010; Adedayo, 2011; Kayode et al., 2013; Okwulehie and Ogoke, 2013). Jiskani (2001); Okwulehie and Odunze (2004); Adejumo and Awosanya (2005) noted that mushrooms are considered as sources of proteins, vitamins, fats, carbohydrates, amino acids and minerals.

According to Buigut (2002), all essential amino acids, water soluble vitamins, and minerals are present in mushrooms. Chang and Buswell (1996) noted the presence of vitamins such as riboflavin, biotin and thiamine in mushroom. These reported constituents could be behind the reason why mushrooms, especially edible ones have occupied a central role in diets as diet supplements in foods (Agomuo, 2011). Apart from mushrooms` role as food supplements, they have also been reported to play important medicinal role against some disease conditions due to their antimicrobial properties (Breene, 1990; Cheung and Cheung, 2005; Andrew et al., 2007; Chang and Miles, 2008; Kuforiji, 2008; Akinyele et al., 2011; Motey et al., 2015). Common species of mushrooms have been implicated against diseases such as headache, colds and fever, asthma, smallpox, high blood pressure, and dysentery (Fukushima, 2000; Jonathan, 2002; Boa, 2004; Guillamón et al., 2010). They are also used in the treatment of hemorrhoids, various stomach ailments and in cleansing blood. Some mushrooms have anti-diabetic, anti-cholesterol, anti-allergic, anti-tumor and anti-cancer properties (Chang and Buswell, 1996; Fillipie and Umek, 2002; Ferreira et al., 2010; Omar et al., 2011). Attempts have also been made by different authors (Atlas and Bartha, 1992; Isikhuehmen et al., 2003; Adenipekun, 2008) on the use of some mushroom species in bioremediation exercise.

Pleurotus squarrosulus (Mont.) Singer is among the edible mushrooms found in tropical Africa as well as other parts of the world (Chan, 1981). It belongs to phylum Basidiomycota, and it is used as food. It is a lesser known *Pleurotus specie* mushroom than *Pleurotus tuber-regium*. In Nigeria, the consumption of *P. squarrosulus* is seasonal just like any other edible mushroom. It is mostly hunted and harvested from wild, just before the on-set of rainy season. *P. squarrosulus* is good looking with smooth body, and has attractive flavor, which many postulate could be behind its consumption among the locals who do not have the knowledge of its nutritional constituents.

Studies on *Pleurotus species* of mushroom on cultivation (Okhuoya and Okogbo, 1991; Okhuoya and Etugo, 1993; Kuforiji and Fasidi, 1998; Kuforiji et al., 2003; Isikhuemhen et al., 2003; Isikhuemhen and LeBauer, 2004;Onuoha, 2007), induction (Okhuoya and Okogbo, 1990; Chiejina and Olufokunbi, 2010; Akande and Kuforjji, 2013), nutritional (Kalac, 2009; Adebayo et al., 2010; Khurdishul et al., 2010; Agomuo, 2011;Beluhan and Ranogajec, 2011; Motey et al., 2015), non-nutritional (Ikewuchi and Ikewuchi, 2009), remediation (Adenipekun, 2008), and enzymes (Kadiri and Fasidi,1990) have centered much on *P. tuber-regium,* leaving other members of the specie with little or no literature.

There is need to enlarge the scope of the study on *Pleurotus species*. The present study aimed at evaluating the nutritional, anti-nutritional composition of *P. squarrosulus* and its effect on some selected biochemical parameters in rats following consumption. Inferences made from this study will acquaint those that consume the mushroom with its constituents, and its possible biochemical status in the body. This can directly stimulate its cultivation by farmers.

MATERIALS AND METHODS

Collection of mushrooms

Matured *P. squarrosulu* samples used in this study were purchased

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from a village market in Ngor-Okpuala L.G.A of Imo State, Nigeria. The purchased samples were transported to the laboratory where they were prepared for analysis.

NUTRITIONAL COMPOSITIONS

Proximate analysis, vitamins, mineral elements, amino acids, and fatty acids were among the nutritional compositions that were considered in the present study**.**

Proximate analysis

The methods as described by the Association of Official Analytical Chemists (AOAC, 2006) were used for the analysis of moisture content, ash, crude protein, crude fat, crude fiber, and carbohydrate. The method of difference was used for total carbohydrates while the Atwater factor of 4:9:4 was used to obtain the energy value of the sample.

Vitamin analysis

Vitamins such as A, B-complex, C, D, E and K present in *P. squarrosulus* were analyzed as described by the Association of Official Analytical Chemists (AOAC, 2006).

Determination of mineral elements

Preparation of the sample for mineral analysis was done as described by Amadi et al. (2013). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) contents were determined by EDTA versanate complexometric titration method as described by Harbone (1973).

Potassium (K^{2+}) and sodium (Na^{+}) ion contents were determined by flame photometry as described by Onwuka (2005). 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

The amino acids in the studied sample were determined using the method as described by Speckman et al. (1958) while gas chromatographic method was use for fatty acid analysis.

Anti-nutritional constituents

The anti-nutritional constituents considered in the presents study such as saponins, alkaloids, tannins, oxalates, and phytates were screened using the methods as described by Harbone (1973); Ojiakor and Akubugwo (1997). Those found present in the studied sample were quantitatively determined using the standard methods of AOAC (2006).

Determination of fatty acid ratio; mineral element ratios; and anti-nutrient: nutrient molar ratios

The methods described by Duru et al. (2013a); Duru et al. (2015) were used for fatty acid and mineral element ratios, respectively, while the methods as described by Hassan et al. (2011); Hassan et al. 2014; and Woldegiorgis et al. (2015) were used for antinutrients-mineral ratios.

Experimental animals

Forty- eighty male wistar albino rats weighing between 70 to 80 g, obtained from the animal colony of Department of Biochemistry, Abia State University, Nigeria were procured for this study. The animals 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 7 days. After acclimatization period, the animals were allocated to six groups of eight rats each. Their weights were equalized as nearly as possible. Three control groups; experimental control, alongside with two known reference foods (Nutrend, a known baby food (Reference 1) and formulated basal feed (Reference 2)) were used as controls, while the remaining groups were used as test groups. Treatments given to the animals 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. squarrosulus* + 95% Normal feed+ potable water.
Test group II= 15% of *P. squarrosulus* + 85% Normal feed + potable water.
Group III= 25% of *P.squarrosulus* + 75% Normal feed + potable water.

The treatments of experimental animals were in accordance to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals (NIH, 1985).

Biochemical Studies

At the end of the administration period (28 days), rats from the various groups were weighed, and sacrificed under chloroform anesthesia. 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 (1993a), haemoglobin level (Hb) was determined using cynomethaemoglobin as described Alexandar and Griffths (1993b), whereas white blood cells count (WBC), 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 (1991). MCV, MCH, and MCHC were estimated using the methods as described by Jain (1986).

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 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,

Table 1. Proximate composition of *P. squarrosulus*.

Proximate content	Composition
Moisture content (%)	11.67±0.46
Ash content (%)	7.84±0.23
Crude protein (%)	21.31±1.03
Crude fats (%)	4.90±0.21
Crude fibre (%)	6.12±0.16
Total carbohydrate (%)	51.58±2.40
Energy value (Kcal/100g)	335.66±4.83

Results are mean and standard deviations of triplicate determinations.

England). The method as described by Friedwald et al. (1972) [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 (2008) 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

According to Okaka and Okaka (2001); Okaka and Okaka (2005); Amadi et al. (2014), percentage moisture content of food materials and the state it exists in foods are important in determining their storage stability. Foods noted with high moisture contents that exist in free form are known to have a shorter life span (Okaka and Okaka, 2005; Amadi et al., 2014). The percentage moisture content of the *P. squarrosulus* (11.67%) as presented in Table 1 is lower than that of *P. tuber-regium* as reported by Ijeh et al. (2009); higher than that of *Pleurotus ostreatus* as reported by Adejumo et al. (2015), but in line with the earlier work of Ezeibekwe et al. (2009) on *P. squarrosulus*.

According to Amadi et al. (2017), ash content could be an insight of nutritional important minerals. The ash content of *P. squarrosulus* (7.84%) in this study is higher than that *P. tuber-regium* (Ijeh et al., 2009; Ikewuchi and Ikewuchi, 2009); *P.ostreatus, Pleurotus eryngii*, and *Pleurotus sajor-caju* (Maria et al., 2015). Adejumo et al. (2015) noted that mushrooms are rich in protein. The observed crude protein content (21.31%) of *P. squarrosulus* (Table 1) could be indication that it can contribute significantly to daily human protein requirements, usually about 23 to 56g (FAO/WHO/UNU, 1991; Chaney, 2006). The crude protein content of the present study is lower than those of *Pleurotus pulmonarius* (Adejumo et al., 2015), *P. sajor-caju (*Adejumo et al., 2015; Maria et al., 2015), *Pleurotus florida* (Akhtar et al., 2012) and *P. tuber-regium* (Ijeh et al., 2009; Ikewuchi and Ikewuchi, 2009*)* but higher than those of *P.erynii* (Maria et al., 2015), and *P. giganteus* (Maria et al., 2015).

The importance of having fats/lipids in edible foods cannot be overemphasized (Ugbogu and Amadi, 2014). Dietary fats increase palatability of food by absorbing and retaining flavors (Antia et al., 2006). The crude fats content (4.90%) of the studied sample is higher than those of *P. tuber-regium* (Ikewuchi and Ikewuchi, 2009), *P. giganteus (*Maria et al., 2015), *P. florida* (Akhtar et al., 2012), and *P. erynii* (Maria et al., 2015), but lower than those of *P. ostreatus* and *P. pulmonarius* (Adejumo et al., 2015).

In recent times, emphasis is being made on the need for low fiber intake in the nutrition of infants and weaning children to avoid irritation of the gut of mucosa (Bello et al., 2008; Amadi et al., 2017). The crude fiber content (6.12%) of *P. squarrosulus* in the present study is lower than those of *P.tuber-regium* (Ijeh et al., 2009), *P. pulmonarius* (Adejumo et al., 2015), and *P. florida* (Akhtar et al., 2012), but higher than those of *P. ostreatus* (Adejumo et al., 2015) as well as other species such as those of *Termitomyces robusta, Volvariella volvacea* and *Termitomyces microcarpus* (Adejumo et al., 2015). Also, evidence from epidemiological studies suggest that increased fiber consumption may contribute to a reduction in the incidence of certain diseases such as diabetes, coronary heart disease, colon cancer, high blood pressure, obesity, and various digestive disorders (SACN Report, 2006).

Total carbohydrate content (51.58%) of the studied sample (Table 1), is lower than those of *P. tuber-regium* (Ijeh et al., 2009), *Lentinus edodes, P. eryngii* and *P. sajor-caju* (Maria et al., 2015), but higher than those of *P. pulmonarius and P. ostreatus* (Adejumo et al., 2015) as well as those of other fungi species such as *Tetanocera robusta, Volvariella volvacea, Termitomyces microcarpus* (Adejumo et al., 2015). Maria et al. (2015) noted that many wild-grown mushrooms have low energy levels.

The energy value (335.66 Kcal/100 g) of the present study (Table 1) is higher than those of *P. tuber-regium* (Ijeh et al., 2009; Ikweuchi and Ikewuchi, 2009; Onyeike and Ehirim, 2001), but lower than those of *P. ostreatus, P. eryngii, P. sajor-caju* and *P. giganteus (*Maria et al., 2015).

Vitamins though required in minute quantity but are as important as other nutrients found in edible food substances (Okaka and Okaka, 2001; Okaka and Okaka, 2005; Okwu, 2005). Figure 1 reveals the vitamins observed *P. squarrosulu*s in the present study. Vitamins A, B1, B2, B3, B6, B12, C, D, E and K were among the vitamins observed in the studied sample. Amadi et al. (2011) noted that vitamins are important in the body as their deficiencies adversely affect the metabolism of the body. These vitamins become very important when their functions (Paul and Pearson, 2005; Nkafamiya et al.,

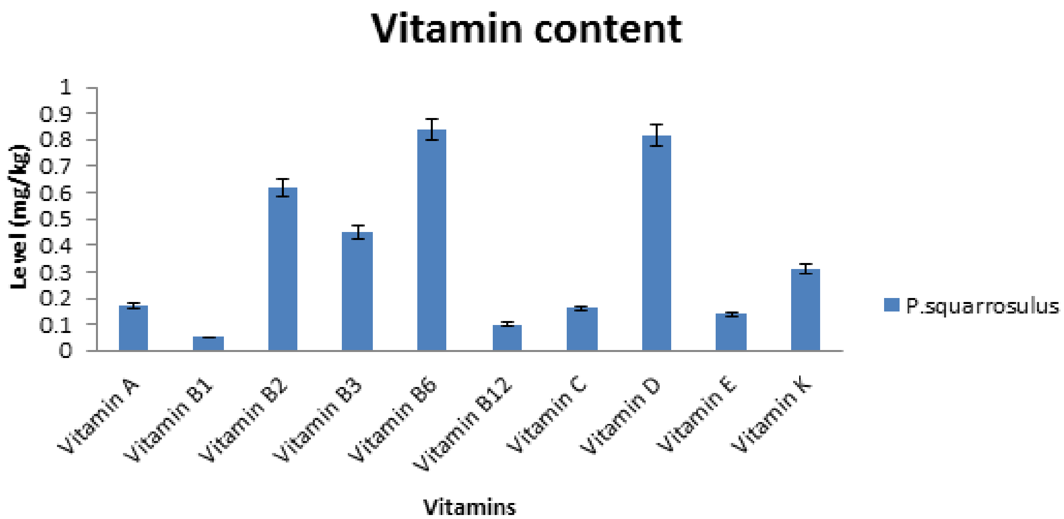

Figure 1. Vitamin contents of *P. squarrosulus.*

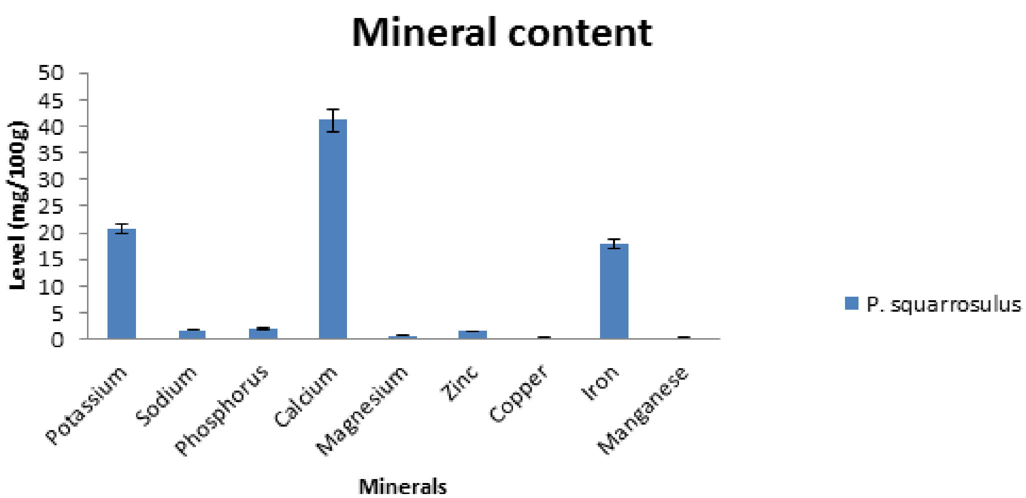

Figure 2. Minerals found in *P.squarrosulus.*

2010) are considered in the body. According to Maria et al. (2015) and Heleno et al. (2010), edible mushrooms provide a natural significant content of vitamins B1, B2, B12, C, D and E. Maria et al. (2015) again noted that mushrooms are a good source of vitamins with high levels vitamins B2, B3, and traces of vitamins B1, B12, C, D and E. The presence of these vitamins in the studied sample is in line with above statements of Maria et al. (2015) and that of Heleno et al. (2010). The presence of vitamin D as observed in P. squarrosulus in the present study is in line with mushrooms being the only non-animal food source that contain vitamin D (Maria et al., 2015), and this qualifies the studied sample as being among the natural vitamin D mushrooms for vegetarians (Maria et al., 2015). The presence of vitamins A and K as observed in the studied sample may be indication of its linkage to bio-vision and blood clotting, respectively.

Figure 2 depicts the minerals found in *P. squarrosulus.* Important mineral elements (Ibanga and Okon, 2009; Soetan et al., 2010; David, 2010; Valvi and Rathod, 2011; Ullah et al., 2012; Mlitan et al., 2014) such as potassium, sodium, phosphorus, calcium, magnesium, zinc, copper, iron and manganese were observed in the studied sample. The minerals followed the order calcium>potassium> iron>phosphorus>sodium>zinc> magnesium> manganese >copper. The presence of these minerals is in line with the statement of Maria et al., (2015), who noted that edible mushrooms contain high

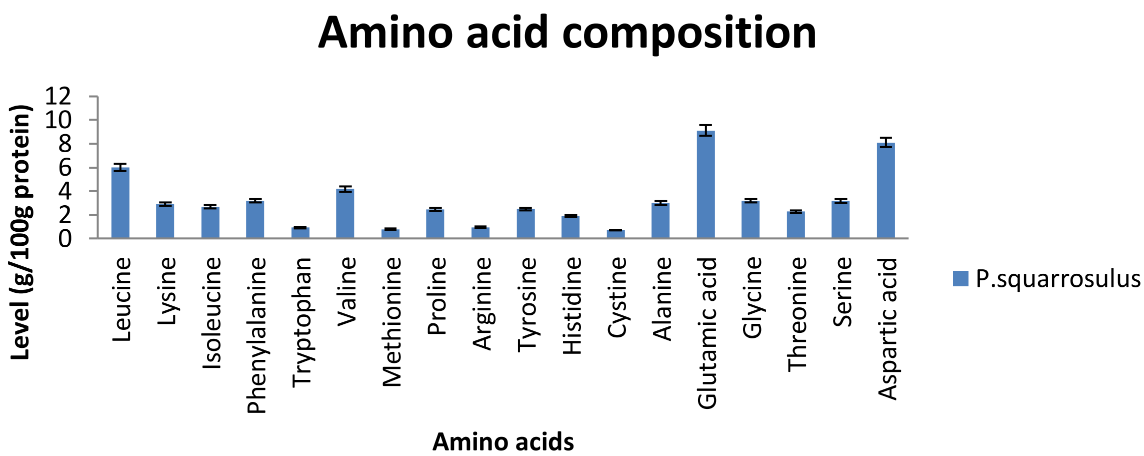

Figure 3. Amino acid composition of *P. squarrosulus.*

amounts of ash, which are mainly potassium, phosphorus, magnesium, calcium, copper and zinc. The low level of sodium in *P. squarrosulus* could be an added advantage due to the relationship of sodium intake and hypertension in the body (Dahl, 1972; Duru et al., 2012b). Potassium (20.75 mg/100 g), sodium (1.83 mg/100 g), calcium (41.14 mg/100 g), magnesium (0.70 mg/100 g), zinc (1.52 mg/100 g), copper (0.27 mg/100 g) and manganese (0.32 mg/100 g) elements found in *P. squarrosulus* are lower than those of *P. tuber-regium* (Ijeh et al., 2009). *P.squarrosulus* iron content (17.80 mg/100 g) is higher than those of other *Pleurotus species* as reported by (Ijeh et al., 2009; Adejumo et al., 2015). The presence of potassium, sodium, calcium, magnesium, and phosphorus minerals observed in the present study is in line with the earlier study of Ezeibekwe et al., (2009) on *P. squarrosulus*.

Amino acids are the building blocks of proteins and facilitate the action of vitamins and minerals (Young and pellet, 1990; Uwakwe and Ayalogu, 1998; Olusanya, 2008). Figure 3 reveals the amino acid composition of *P. squarrosulu*. From the Figure, essential amino acids present in the studied sample include leucine, lysine, isoleucine, arginine, phenylalanine, tryptophan, valine, methionine, histidine and threonine. The non-essential amino acids are proline, arginine, tryrosine, cystine, alanine, glutamic acid, glycine, serine, and aspartic acid. The functions of these amino acids have been duly reported by Lillian (2004); Okaka and Okaka (2005); Mary and Shawn (2006); Olusanya (2008). The observation of these amino acids in *P. squarrosulus* is in line with Maria et al. (2015), who noted that bioactive proteins are important part of functional components in mushrooms and have great value for their pharmaceutical potential.

Fatty acids found in diets are classified into their predominant fatty acids (Okaka and Okaka, 2005; Olusanya, 2008; Duru et al., 2013a). Figure 4 shows the fatty acid composition of *P. squarrosulus*. Lauric acid (C12:0), myristic acid (C14:0), palmitic acid and (C16:0), and stearic acid (C18:0) observed in the studied sample are among the essential fatty acids (Lisa and Paula, 2010; Duru et al., 2013b). The presence of lauric, myristic, palmitic, stearic, and linoleic fatty acids observed in *P. squarrosulus* in the present study is in line with the work of Maria et al. (2015), who reported the fatty acids in *Pleurotus edodes, P. eryngii,* and other mushroom species.

Functionally, Lauric acid is a medium chain fatty acid that is responsible for some health benefits. It is the precursor of manolaurin in humans. Manolaurin could act as antifugal, antibacterial, antiprotozoan and antiviral monoglyceride (Fife, 2000). Enig (1998) noted that monolaurin dissolves and destroys the lipid coated viruses such as herpes, cytomegalovirus, influenza, HIV as well as bacteria like *Helicobacter pylori* implicated in digestive ulcers, *Listeria monocystogens* and protozoan *Giardian lamblia*. Linoleic acid is among the polyunsaturated fatty acids and accounts for omega-3 fatty acids (Rodriguez-Cruz et al., 2005; Sampath and Ntambi, 2005; Lisa and Paula, 2010; Duru et al., 2013a; Duru et al., 2013b), which is essentially needed for health (Duru et al., 2013b). Omega-3 fatty acid has been reported to facilitate brain and eye development especially in growing foetus during pregnancy and maintenance of health throughout life (Rodriguez-Cruz et al*.,* 2005; Duru et al., 2013a). Palmitic, stearic, oleic, and linoleic fatty acids of the present study are lower than those of *P. edodes, P. eryngii*, and those of other mushroom species as reported by Maria et al. (2015). The studied sample also has more saturated fatty acids than unsaturated and polyunsaturated fatty acids but no monosaturated fatty acids (Figure 4).

Anti-nutrients are naturally synthesized chemical

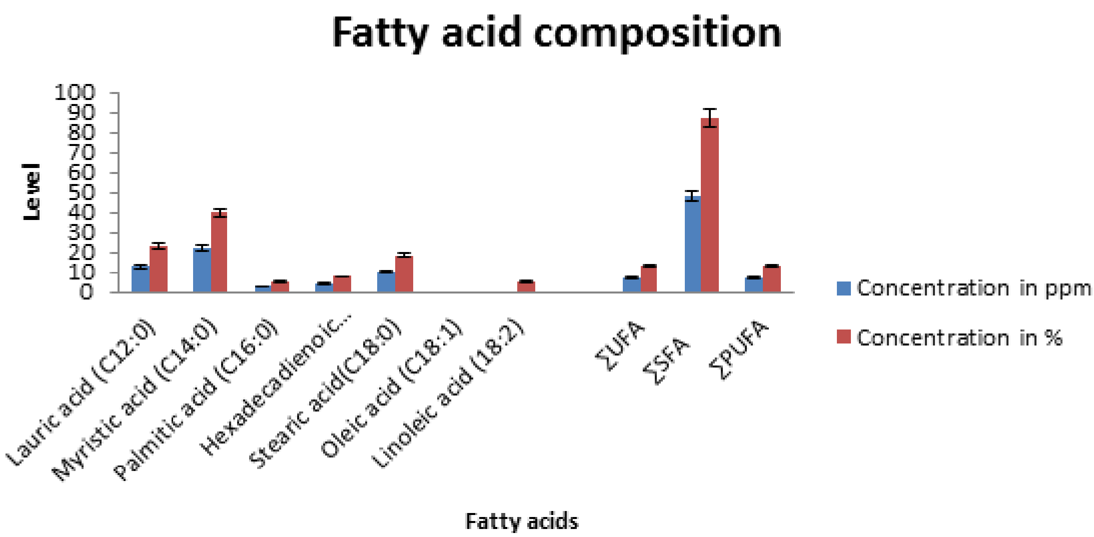

Figure 4. Fatty acid composition of *P.squarrosulus.* (∑PUFA: Sum of polyunsaturated fatty acid, ∑UFA: Sum of unsaturated fatty acid, ∑SFA: Sum of saturated fatty acid).

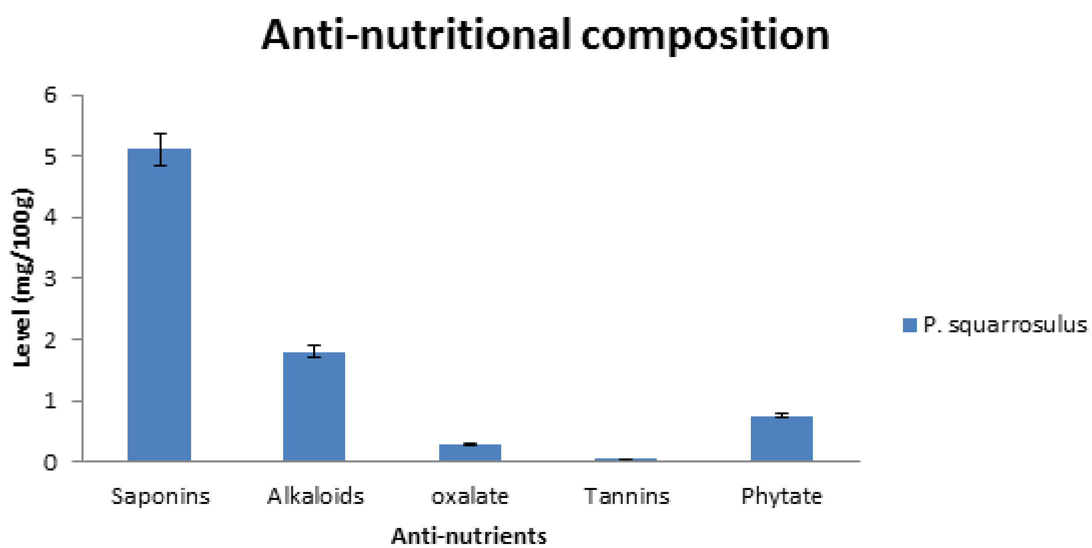

Figure 5. Anti-nutritional composition of *P. squarrosulus*

compounds in food substances (Kadiri and Fasidi, 1992; Okaka and Okaka, 2005; Gemede and Ratta, 2014). Anti-nutritional composition of *P. squarrosulus* as presented in Figure 5 reveals the presence of saponins, tannins, alkaloids, oxalates, and phytate. Anti-nutrients are known to reduce the maximum utilization of nutrients especially proteins, vitamins and minerals present in food substances, decreasing their nutritive value (Fekadu et al., 2013). The observed anti-nutrients in the present study are in line with earlier studies of Ikewuchi and Ikewuchi (2009); Egwim et al. (2011) on *P. tuber-regium* and some selected Nigerian edible mushroom species, respectively. As anti-nutrients, saponins are known to have foamy properties and impact bitter taste at high concentration in foods (Duru et al., 2012b); tannins complex with proteins in foods and make them unavailable for consumption (Amadi et al., 2012; Duru et al., 2012b); tannic acid is also known to impact astringency in foods (Duru et al., 2012b); alkaloids are known to impact bitter taste in foods and as well affect the nervous system with disruption or inappropriately augmenting electrochemical transmission in animals (Duru et al., 2012a; Fereidon, 2012; Fereidoon, 2014); oxalates form insoluble complexes with calcium,

Table 2. Fatty acid ratio, mineral element ratio, and anti-nutrient: mineral molar ratio of *P. squarrosulus*

Ratio	Value	Critical value/Ideal ratio
PUFA/SFA	0.15	Not lower than 0.45
[Calcium]/[Phosphorus]	20.17	1.0
[Calcium]/[Magnesium]	58.77	2.2
[Sodium]/[Potassium]	0.088	Less than 1.0
[Oxalate]/[Calcium]	3.10×10^{-3}	2.5
[Oxalate]/[Calcium+Magnesium]	3.01×10^{-3}	2.5
[Phytate]/Iron]	3.56×10^{-3}	0.4
[Phytate]/[Calcium]	1.10×10^{-3}	0.2
[Phytate]/[Zinc]	4.89×10^{-2}	10
[Calcium][Phyate]/[Zinc]	5.02×10^{-2}	0.5

Ideal values of mineral element ratios were adopted from NRC (1989); while critical values of anti-nutrients: mineral molar ratios were adopted from Hassan et al., (2011, 2014).

magnesium, iron, and zinc thereby interfering with utilization of these mineral elements (Duru et al., 2012a) and phytate works in a broad pH-region as a highly negatively charged ion, and therefore its presence in the diet has a negative impact on the bioavailability of divalent, and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, $Ca^{2+,}$ Mg^{2+}, $Mn^{2+,}$ and Cu^{2+}.

However, anti-nutrients are also useful under certain circumstance as "phytochemicals (plant protectors)" especially, when they do not exceed the toxic limit consumable by animals (Ikewuchi and Ikewuchi, 2009).

This maybe the case with *P. squarrosulus* with very low levels of anti-nutrients as observed in the present study. For instance, saponins through their Na^+-Ca^{2+} antiporter relationship strengthen the muscles (Schneider and Wolfling, 2004; Egwim et al., 2011); aside the stringent taste of tannins, they have been noted to hasten the healing of wounds and inflamed mucous membrane (Amadi et al., 2012); and alkaloids act as stimulants, topical anaesthetic in Ophthalmology, powerful pain reliever, bactericidal agent, antipuretic action among other uses (Edeoga and Enata, 2001; Egwim et al., 2011).

Determining nutritional and anti-nutritional relationships in food substances are much more important than knowing their levels alone. Table 2 shows the fatty acid ratio, mineral element ratios, and anti-nutrient: mineral molar ratios of *P. squarrosulus*. The knowledge of polyunsaturated fatty acids (PUFA)/ saturated fatty acids (SFA) ratio is important and should be known for correlation of food material to heart diseases (Duru et al., 2013a; Duru et al., 2013b). According to Benjamin et al. (2012) and HMSO (1994), foods with PUFA/SFA ratio lower than 0.45 are considered inappropriate for human health due to their linkage to heart disease. The [Calcium]/ [Phosphorus] and [Calcium]/[Magnesium] weight ratios as calculated in the present study are higher than their recommended values 1.0 and 2.20, respectively by NRC (1989). The observed high values could be as a result of high content of calcium in *P. squarrosulus* in this study. According to Benjamine et al. (2012), [Sodium]/ [Potassium] ratio of less than one is recommendable. The low ratio of [Sodium]/[Potassium] in the present study could be an indication that consumption of *P. squarrosulus* may not be connected with high blood pressure.

It has been noted that, high content of anti-nutrients exert negative effects on the bioavailability of some mineral nutrients (Agbaire and Emoyan, 2012). With the calculated values of [Oxalate]/[Calcium] and [Oxalate]/[Calcium] + [Magnesium] in this study, it can be predicted that oxalate has no effect on the bioavailability of calcium and magnesium minerals since their calculated values are lower than their respective critical values (Table 2). For adequate iron bioavailability, It has been argued that [Phytate]/ [Iron] ratio should not exceed 0.4 (Mitchikpe et al., 2008). The [Phytate]/ [Iron] ratio for *P. squarrosulus* in the present study is less than 0.4. The [Phytate]/ [Calcium] mole ratio is also lower than the critical value of 0.2. Different authors have reported that the effectiveness of phytate can reduce zinc availability in diets. The phytic acid and zinc expressed in molar ratio could be satisfactory means of predicting if a phytate containing diet may result in poor availability of zinc (Urga and Narasimba, 1998).

A molar ratio values of less than or equal to 10 has been recommended as the borderline dietary conditions providing adequate zinc homeostasis and those resulting in inadequate zinc nutriture (Urga and Narasimba, 1998). [Phytate]/[Zinc] mole ratio of the present study is lower than 10. However, according to Ellis et al. (1987); Davies and Warrignton (1986), [Calcium] [Phyate]/ [Zinc] ratio is a better predictor of zinc availability. The value for the ratio should not be greater than 0.5 mol/kg, to avoid

Table 3. Total amino acids groups (g/100g protein) of *P. squarrosulus.*

Parameter	***P. squarrosulus***
Total amino acids	58.15
Total non-essential amino acids	33.27
Total essential amino acid acids	
With His	24.88
Without His	22.99
Total neutral amino acids	34.44
Total acidic amino acids	17.22
Total basic amino acids	5.75
Total sulphur-containing amino acids	1.54
Total branched-chain amino acids	12.89
Total aromatic amino acids	6.63

Note: These results are based on Figure 3 above.

Table 4. Percentages of amino acids groups of *P. squarrosulus.*

Parameter	***P. squarrosulus***
%Total non-essential amino acid	57.21
%Total essential amino acid	
With His	42.79
Without His	39.54
%Total neutral amino acids	59.22
%Total acidic amino acids	29.61
%Total basic amino acids	9.89
%Total sulphur-containing amino acids	2.64
%Cys in total sulphur-containing amino acid	48.05
%Total branched-chain amino acids	22.17
%Total aromatic amino acids	11.40
%Try in total aromatic amino acids	37.71

Note: These results are based on Table 2 above.

interference with the availability of zinc in humans and rats (Urga and Narasimba, 1998). Molar ratio above 3.5 mol/kg dry diet would mean poor zinc balance in humans (Urga and Narasimba, 1998). That of *P. squarrosulus* in this study is lower than 0.5, and could depict zinc availability.

Amino acid groups as presented in Table 3 shows a total amino acids of 58.15 (g/100 g protein) with total non-essential amino acids of 33.27 (g/100 g protein) being higher than total essential amino acid either with histidine (24.88 g/100 g protein), or without histidine (22.99 g/100 g protein) for the studied sample. Other constituent amino acid groups followed the order total neutral amino acids>total amino acids> total branched amino acids>total aromatic amino acids>total basic amino acids>total sulphur-containing amino acids>total basic amino acids in the present study.

The percentages of amino acid groups of *P. squarrosulus* as presented in Table 4 shows %total non-essential amino acid of 57.21% against %total essential amino acid with histidine (42.79%) and without histidine (39.54%). %total neutral amino acids > %total acidic amino acids > %total branched-chain amino acids > %total aromatic amino acids > %total amino acids > %total sulphur-containing amino acids. Cysteine in animal proteins does not contribute more that 50% of

Table 5. Ratios of amino acids and amino acid groupings of *P. squarrosulus.*

Parameter	*P. squarrosulus*
Leucine/Isoleucine	2.21
Total basic amino acid/Total acidic amino acid ratio	0.33
Total essential amino acid/Total amino acid ratio	
With His	0.43
Without His	0.40

Results are based on Figure 3, and Table 3 above.

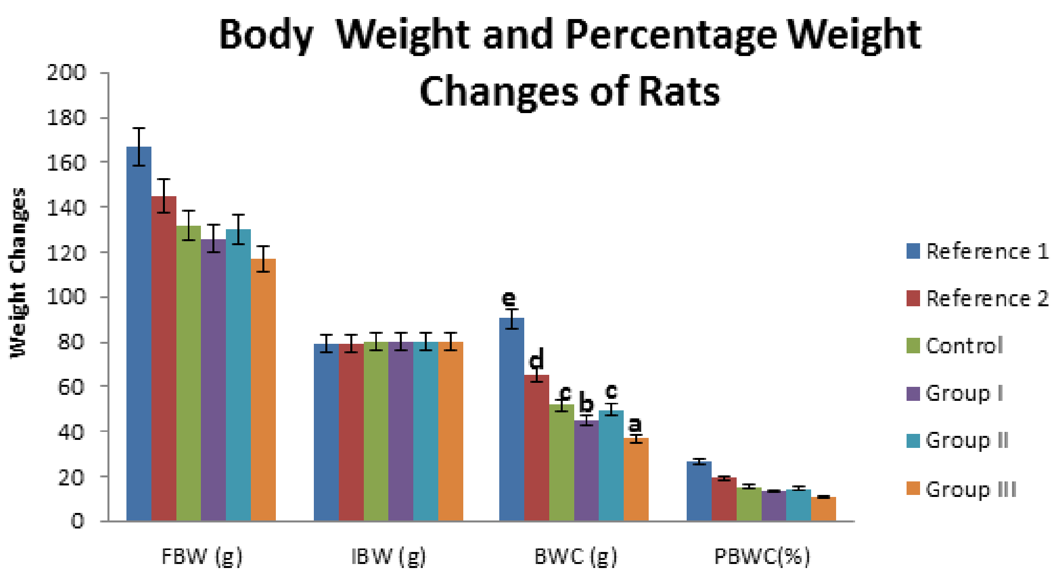

Figure 6. Body weight and percentage weight changes of rats.

total sulphur-containing amino acids. Though, vegetables like legumes may contribute more cysteine substantially than methionine (Adeyeye et al., 2010). The %cysteine of the present study is lower than 50% as expected from plant source. According to Harris and Crabb (2006), the synthesis of glutathione is limited by availability of cysteine. Hence, consumption of *P. squarrosulus* can contribute to the pool of cysteine in the tissues and organs. The %total branched-chain amino acids of the present study could be indication that over 22% of the amino acids of the studied sample can contribute significantly to energy production in the body. This compares favorably with 10% desired energy requirement from proteins as noted by Wardlaw and Kessel (2002).

The Leucine/Isoleucine ratio (Table 5) of amino acid constituent of *P. squarrosulus* could be indication of metabolic antagonism especially with tryptophan and niacin metabolism due to low leucine content (Igwe et al., 2012). Total basic amino acids/Total acidic amino acids ratio of the studied sample is 0.33, indicating more acidic amino acids than the basic amino acids. This could be an indication that the studied sample could support protein synthesis. The ratio of total essential amino acids/total amino acids with histidine (0.43) or without histidine (0.40) collaborates with the results in Tables 3 and 4. The body weight and percentage weight changes of rats as presented in Figure 6 shows insignificant ($p>0.05$) weight reduction in test group II, when compared toexperimental control, and significant ($p<0.05$) weight reduction against those of reference 1 and 2. Test groups I and III reduced significant ($p<0.05$) in weight against those of experimental control, reference 1 and 2. The reduction in weight as observed in the present study could be indication that *P. squarrosulus* may induce body weight reduction on consumption.

The study of haematology is important for assessment of blood relating functions of materials that go into the body (Yakubu et al., 2007 Duru et al., 2012c). It has been noted that analysis of blood parameters is relevant in risk evaluation as changes in the haematological system

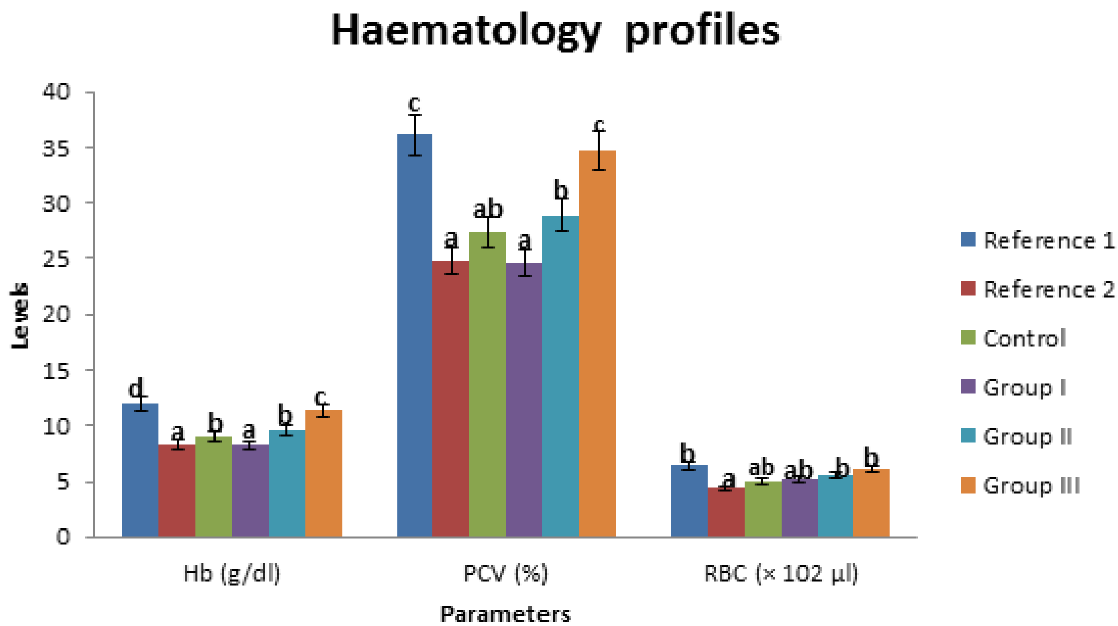

Figure 7. Hb, PCV, and RBC levels of rats placed on *P. squarrosulus.*

have higher predictive value for human toxicity when the data are translated from animal studies (Olson et al., 2000). Figure 7 reveals the Hb, PCV, and RBC levels of rats placed on *P. squarrosulus.* Hb in group I rats reduced significantly (p<0.05) when compared to those of experimental control and reference 1, but was insignificantly (p>0.05) affected when compared to those of reference 2. Hb in group II increased insignificantly (p>0.05) against the experimental control, increase significantly (p<0.05) against those of reference 2; but decreased significantly (p<0.05) against those of reference 1. Hb in group III increased significantly (p<0.05) against those of experimental control and reference 2; but decreased significantly (p<0.05) against those of reference 1.

The observed increase in Hb levels of groups II and III when compared to those of experimental control and reference 2 in the present study could be as a result of stimulating effect of *P. squarrosulus* on erythropoietin. Erythropoietin is the glycoprotein that stimulates red cell production (Duru et al., 2012c; Krishan and Veena, 1980). This observation is in line with earlier report of Maria et al. (2015) on haematological properties of *Pleurotus* specie mushrooms. The high iron content of the studied sample as noted earlier in the present study could be behind this property. The relationship between Hb and PCV levels (Duru et al., 2012c) were maintained in all the considered groups in the present study. PCV in group I rats reduced insignificantly (p>0.05) against those of experimental control and reference 2, but reduced significantly (p<0.05) against reference 1 rats. PCV in group II was insignificantly (p>0.05) affected against those of the experimental control and reference 2, and reduced significantly (p<0.05) against reference 1 rats. PCV in group III increased significantly (p<0.05) against those of the experimental control and reference 1, but was insignificantly affected (p>0.05) when compared to reference 1 rats.

RBC levels in test groups increased significantly (p<0.05) against reference 2, but were insignificantly (p>0.05) affected against those of reference 1 and experimental control (Figure 7). The insignificant effect against the experimental control as observed in the present study could be indication that the balance between erythropoiesis and destruction of the blood corpuscles was not altered. WBC, lymphocytes and platelets levels of rats placed on *P. squarrosulus* are presented in Figure 8. WBC in group I increased significantly (p<0.05) against the experimental control and reduced significantly (p<0.05) against reference 2. WBC in group II increased significantly (p<0.05) when compared to those of the experimental control and reference 2, but reduced insignificantly (p>0.05) against reference 1. WBC in group III increased significantly (p<0.05) against the experimental control, reference 1 and 2. Observed increase in WBC levels of test rats against the experimental control and reference 1 and 2 in the present study, could be due to normal reaction of test rats to foreign substances as a result of combined physiological and chemical effect in metabolic system of the rats (Celik and Suzek, 2008; Duru et al., 2012c).

Lymphocytes levels in test groups I and III increased significantly (p<0.05) when compared to those of experimental control and reference 1, but were

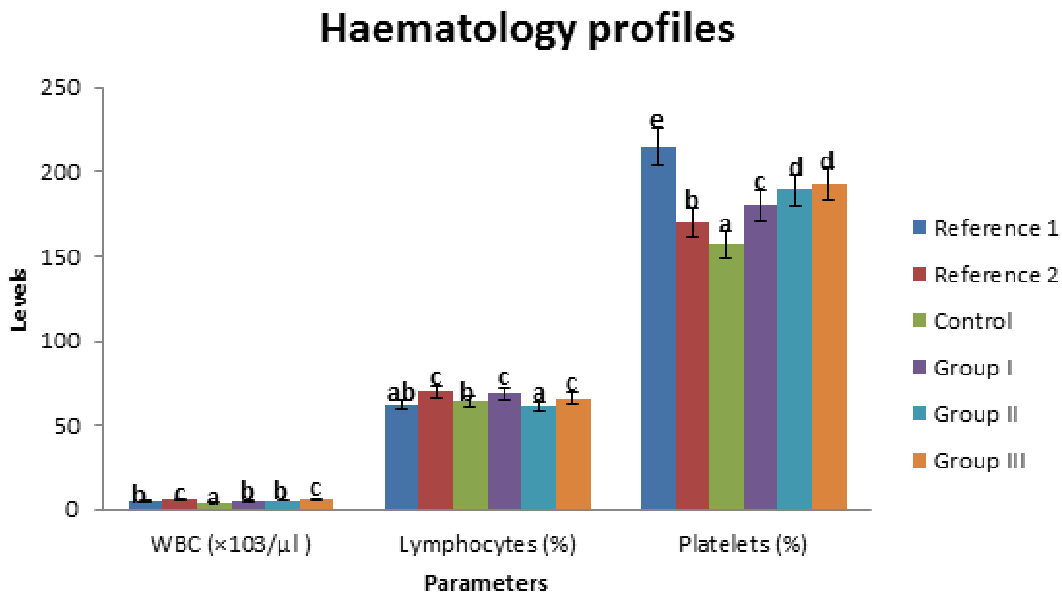

Figure 8. WBC, lymphocytes and platelets levels of rats placed on *P.squarrosulus.*

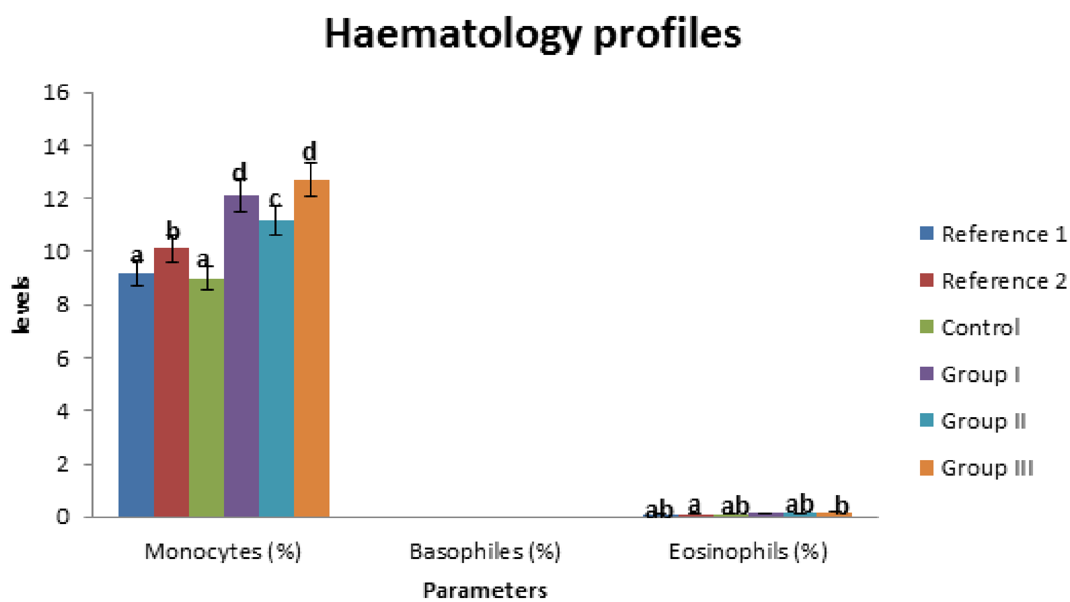

Figure 9. Monocyte, basophile and eosinophil levels of rats placed on *P. squarrosulus.*

insignificantly ($p>0.05$) affected against that of reference 2 (Figure 9). Lymphocytes in group I reduced significantly ($p<0.05$) against experimental control and reference 2; but was insignificantly ($p>0.05$) affected when compared to reference 1. Monocyte levels in test groups increased significantly ($p<0.05$) against those of the experimental control, reference 1 and 2. Eosinophil levels in test groups I, II and III were insignificantly ($p>0.05$) affected against those of the experimental control and reference 1, but test groups I and II increased significantly ($p<0.05$) when compared to reference 2 (Figure 9). The observed increase in WBC levels and its differentials as the case maybe against experimental control rats in the present study could be indication that *P. squarrosulus* induced leucocytosis in test rats. Platelets aggregation is known to play important role on physiopathology of thrombotic diseases. Platelets in test rats increased significantly ($p<0.05$) against those of the experimental control and

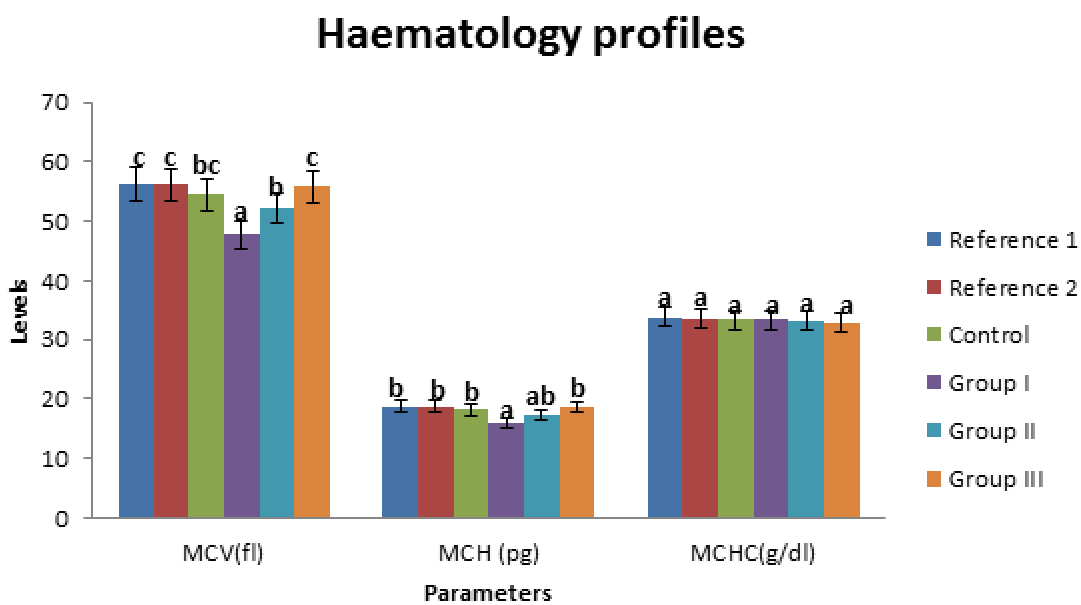

Figure 10. MCV, MCH and MCHC levels of rats placed on *P. squarrosulus.*

reference 2, but decreased significantly (p<0.05) when compared to reference 1 rats (Figure 8).

Adebayo et al. (2005); Duru et al. (2012c) noted that MCHC, MCV, and MCH relate to individual red blood cells while Hb, RBC and PCV are associated with total population of red blood cells. Figure 10 depicts the MCV, MCH and MCHC levels of rats placed on *P. squarrosulus.* MCV in test group I was significantly (p<0.05) reduced against the experimental contol; and reference 1 and 2. At higher concentration in test groups II, MCV was insignificantly (p>0.05) affected when compared to that of experimental control (Figure 10). However, at the highest concentration in test group III, MCV was insignificantly (p>0.05) affected against those of the experimental control, reference 1 and 2 (Figure 10). Apart from MCH of test group I, those of test groups II and III were insignificantly (p>0.05) affected against those of experimental control, reference 1 and 2 (Figure 10). MCHC levels in the test groups were insignificantly (p>0.05) affected against those of the experimental control, reference 1 and 2. The observations made on MCHC, MCV and MCH with test rats in the present study are clear indications that *P. squarrosulus* may not be connected with either normocytic or hypochromic anaemia in the body.

It has been reported that cardiovascular disease is one of the world`s leading causes of death, and has dyslipidemia as one of its major risk factors (Range et al., 2005; Martirosyan et al., 2007; Brunzell et al., 2008). Dyslipidemia may be primary or secondary (Adebayo et al., 2005). It is normally followed by a re-arrangement of triglyceride, total-cholesterol, VLDL-cholesterol and HDL-cholesterol from their normal ranges (Martirosyan et al., 2007). In recent times, it has been reported that nutritional life style can induce dyslipdemia on individuals and can cumulate to hypertension or other cardiac disease. Total-cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol levels of rats placed on *P. squarrosulus* are presented in Figure 11. From the Figure, total-cholesterol reduced significantly (p<0.05) in test groups against those of the experimental control, reference 1 and 2. The reduction in cholesterol levels in test rats is in line with the earlier studies of Guillamón et al. (2010) who reported that *P. ostreatus* exhibits hypocholesterolemic effect on rats with normal cholesterolemia or hypercholesterolemia on one with hereditary cholesterol disorders; and Bobek et al. (1998) on other species of *Pleurotus* with this hypocholesterolemic effect. At higher concentrations of test groups II and III, triglyceride levels reduced significantly (p<0.05) against those of the experimental control, reference 1 and 2.

At low concentration of group I, the triglyceride level insignificantly (p>0.05) reduced against those of experimental control and reference 1. Ijeh et al. (2009) reported similar reduction of triglyceride levels on mice given diet incorporated with *P. tuber-regium.* HDL-cholesterol, the good cholesterol increased significantly (p<0.05) in test groups II and III when compared to those of experimental control, reference 1 and 2. HDL-cholesterol level in test group 1 increased significantly (p<0.05) against those of reference 1 and experimental control but compares to reference 2. LDL-cholesterol levels reduced significantly (p<0.05) in test rats against

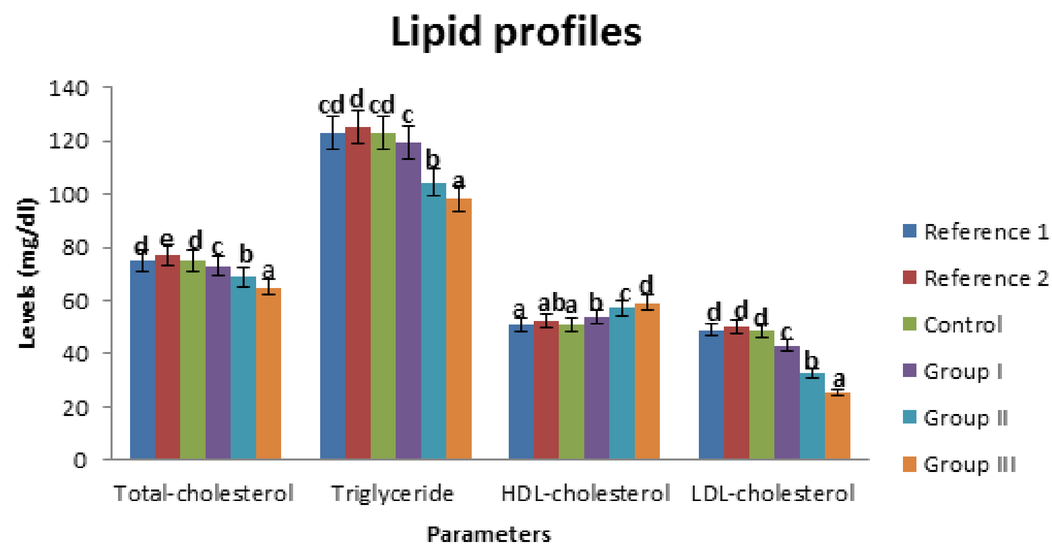

Figure 11. Total-cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol levels of rats placed on *P.squarrosulus.*

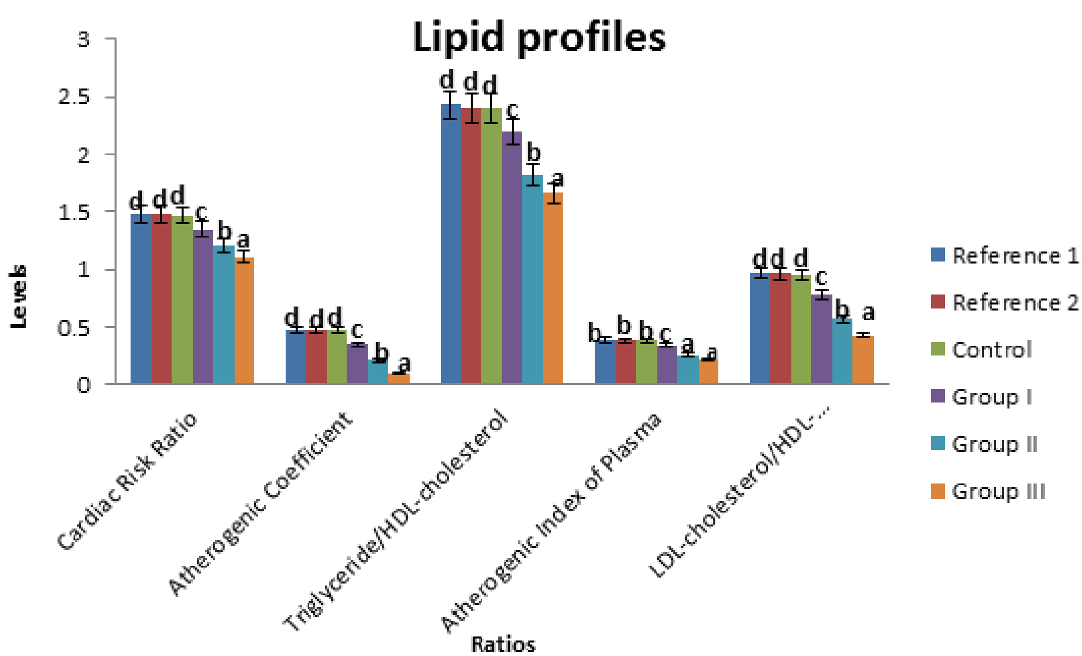

Figure 12. Cardiac ratio, Atherogenic Coefficient, Triglyceride/HDL-cholesterol, Atherogenic Index of Plasma and LDL-cholesterol/ HDL-cholesterol ratios of rats placed on *P. squarrosulus.*

those of experimental control, reference 1 and 2. Lipid profile ratios such as total cholesterol/HDL-cholesterol also known as Cardiac Risk Ratio and LDL/HDL cholesterol ratio are of great interest as major predictors of cardiovascular disease than isolated parameters used independently (Millán et al., 2009). Atherogenic indices are powerful indicators of the risk of heart disease; the higher the value, the higher the risk of developing cardiovascular disease and vice versa (Usoro et al., 2006; Martirosayan et al., 2007).

Results of lipid profile ratios of this study are present in Figure 12. From the Figure, LDL/HDL cholesterol ratios of test rats significantly reduced ($p<0.05$) against those of the experimental control, reference 1 and 2. Levels of

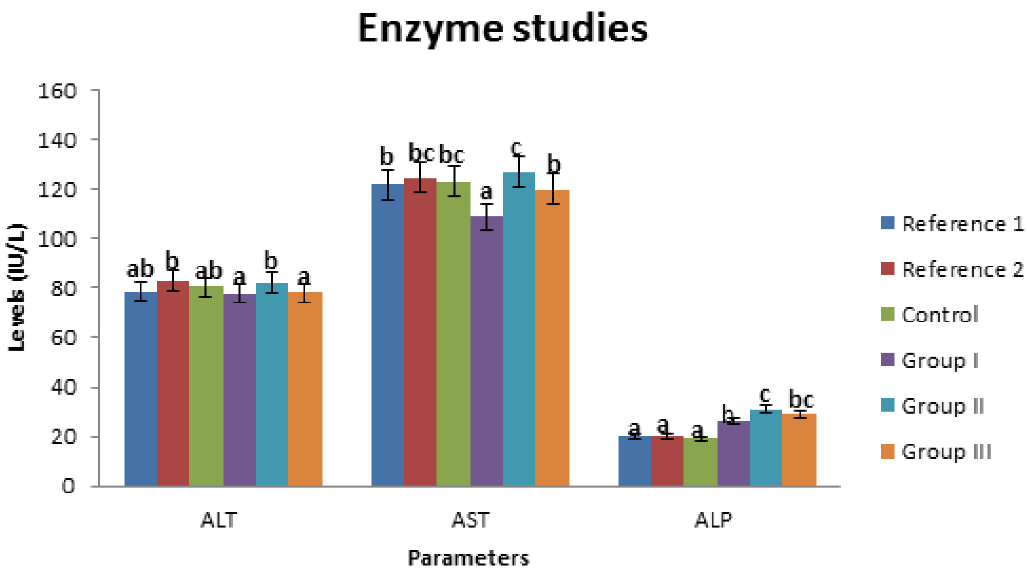

Figure 13. ALT, AST, and ALP levels of rats placed on *P.squarrosulus.*

cardiac risk ratio in test rats significantly ($p<0.05$) reduced against those of the experimental control, reference 1 and 2. Levels of triglyceride/HDL-cholesterol ratio and atherogenic coefficient also followed the order as in cardiac risk ratio in the present study. Levels of atherogenic Index of Plasma reduced significantly ($p<0.05$) in test groups II and III against those of experimental control rats, reference 1 and 2, while test group I was insignificantly ($p>0.05$) affected against those of experimental control, reference 1 and reference 2.

The observations made on lipid profile of rats placed on *P. squarrosulus* are in line with earlier study of Marial et al. (2015) who reported the cardiovascular protective and anti-cholesterolemic properties of *Pleurotus* Mushrooms. Conflicting results obtained with PUFA/SFA ratio; and Sodium/Potassium weight ratio earlier in this study has been put to rest (Table 2). According to HMSO (1994); Benjamin et al. (2012), PUFA/SFA ratio of 0.15 for *P. squarrosulus* is considered inappropriate for human health which linked the consumption of the mushroom to heart disease. This is also against the Sodium/Potassium ratio of 0.088 that distanced the studied sample from having any hypertensive effect. However, results of lipid profile studies of the present study showed that *P. squarrosulus* cannot be connected to any form of dyslipidemia, hypertension or cardiovascular disease.

Increased plasma activity of an enzyme is the consequence of tissue or organ damage (Aliyu et al., 2006; Duru et al., 2012c). ALT, AST, and ALP levels of rats placed on *P. squarrosulus* as presented in Figure 13, reveals insignificant reduction ($p>0.05$) in activity of ALT in test groups I and III against those of reference 1 and experimental control, while that of group II was insignificantly affected ($p>0.05$) against those of experimental control, reference 1 and 2. Activity of AST, which is a less specific enzymes than ALT as indicator of liver damage (Duru et al., 2012c) reduced significantly ($p<0.05$) in test group I against those of experimental control, reference 1 and 2. AST activity in test group II was insignificantly ($p>0.05$) affected against those of experimental control and reference 2, whereas AST activity in test group III was insignificantly ($p>0.05$) affected when compared to those of experimental control rats, reference 1 and 2. ALP, an enzyme associate with the prostate increased significantly ($p<0.05$) in all the test groups against those of the experimental control, reference 1 and 2. Total bilirubin levels of test groups I and II increased significantly ($p<0.05$) against those of experimental control, reference 1 and 2. Total bilirubin of group II rats increased significantly ($p<0.05$) against those of experimental control and reference 1. Conjugate bilirubin level in test group I reduced significantly ($p<0.05$) against experimental control, reference 1 and 2; that of test group II increased significantly ($p<0.05$) against experimental control, reference 1 and 2; whereas test group III had conjugated bilirubin level that was insignificantly ($p>0.05$) affected against those of the experimental control rats, reference 1 and 2 (Figure 14).

Creatinine is major catabolic product of the muscle and it is excreted in the kidney (Adebayo et al., 2010; Duru et al., 2012c). According to Aliyu et al. (2006), creatinine is used as indicator of renal failure. Levels of creatinine in test group II and III increased insignificantly ($p>0.05$) against those of the experimental control, reference 1 and 2 (Figure 15). That of test group I was insignificantly ($p>0.05$) affected against that of reference 2 (Figure 15).

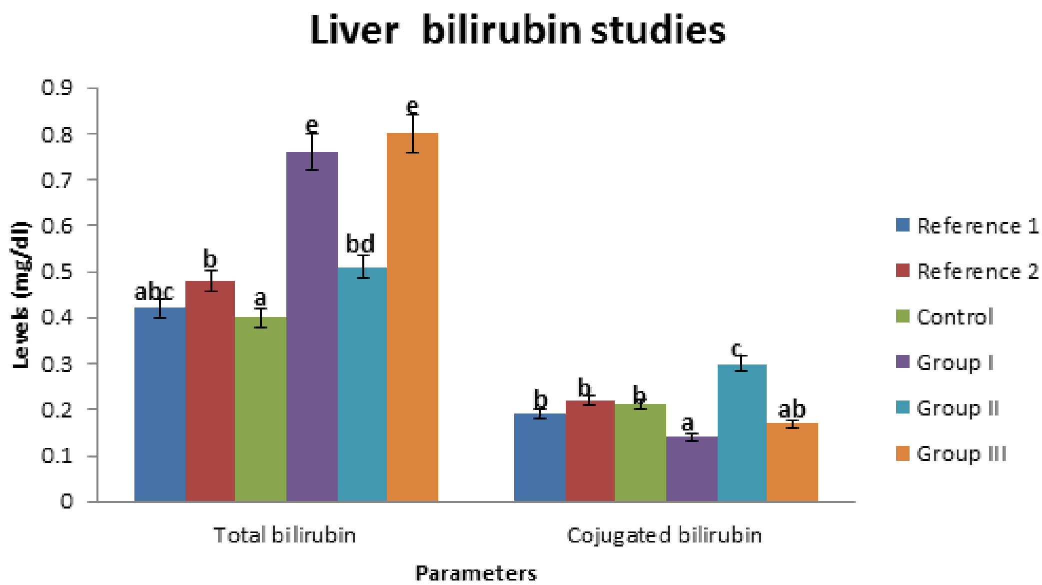

Figure 14. Total bilirubin and conjugated bilirubin levels of rats placed on *P. squarrosulus.*

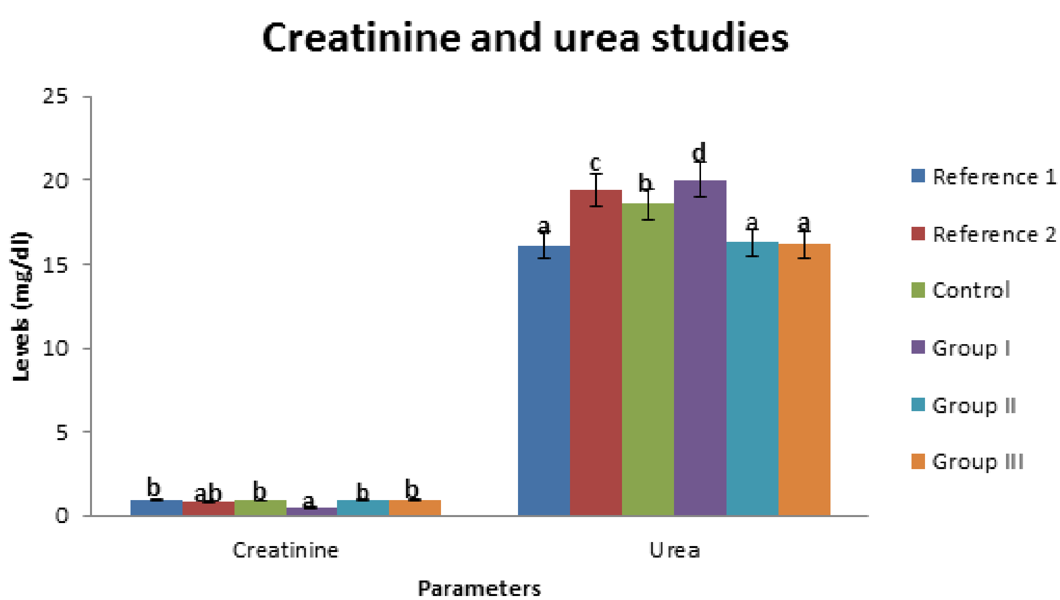

Figure 15. Creatinine and urea levels of rats placed on *P. squarrosulus.*

Retention of creatinine in the blood is an evidence of kidney impairment. Urea is the main end product of protein catabolism. It is excreted through urine. Urea levels of test group I increased significantly ($p<0.05$) against those of experimental control, reference 1 and 2; those of test groups II and III rats increased significantly ($p<0.05$), when compared to those of experimental control and reference 2 and were insignificantly ($p>0.05$) affected against that of reference 1. The observed increase at the lowest concentration could be azotaemia.

However, at higher concentrations, the urea levels of rats placed on *P. squarrosulus* compared favourably with those of reference 1 and 2 (Reference foods), which could be an indication of normal functioning kidney. Na^+, K^+, Cl^- and HCO_3^- are electrolyte ions that are excreted by a functional kidney (Duru et al., 2012c). Levels of these ions as presented in Figure 16 revealed that Na^+ in test groups I and II were significantly ($p<0.05$) affected

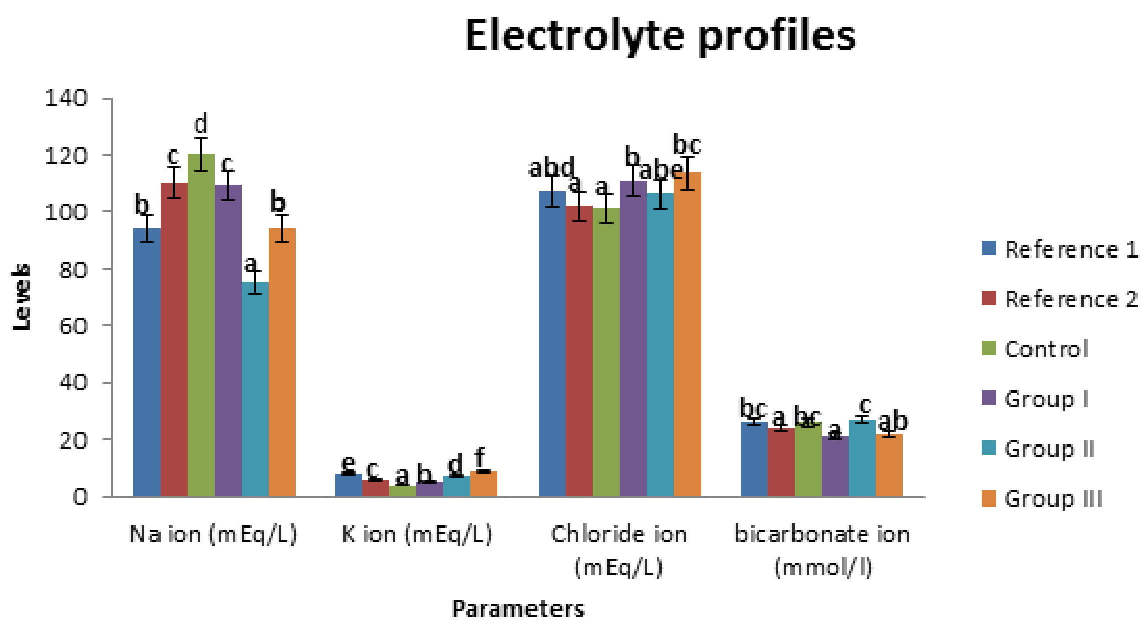

Figure 16. Electrolyte levels of rats placed on *P. squarrosulus.*

against those of the experimental control, reference 1 and 2. However, at the highest concentration in test group III, Na^+ level was insignificantly (p>0.05) affected against that of reference 1. K^+ ion in test rats were significantly increase or decreased as the case maybe against those of experimental control, reference 1 and 2. Cl^- levels in all the test groups were insignificantly affected (p>0.05) against that of reference 1. Cl^- level of test group II was also insignificant (p>0.05) against experimental control and reference 2. HCO_3^- level in test group I reduced insignificantly (p>0.05) against that of reference food 2.

That of test group II increased insignificantly (p>0.05) against those of experimental control and reference 1, whereas that of test group III reduced insignificantly (p>0.05) against those of experimental control and reference 1. The pattern of excretion of these ions in test rats, when compared to both experimental control and the reference foods in the present study could be a clear indication that *P. squarrosulus* on consumption may have no effect on renal function.

The importance of reproductive hormones in procreation can never be over emphasized. Results of testosterone and luteinizing hormones as presented in Figure 17, revealed significant (p<0.05) reduction in the levels of testosterone in test rats against those of experimental control, reference 1 and 2. The same order was followed by luteinizing hormone in the present study. The reduction in cholesterol levels as observed earlier in this study may have been the cause of the reduced testosterone levels since it is known to have a cholesterol nucleus. The reduction could also be correlated with the reduction in size of test rats' testicles as observed in this study. The reduction in both levels of the studied hormones and the testis size may be an indication that *P. squarrosulus* could influence reproductive exercises negatively when consumed.

Conclusion

Observations made on nutritional and anti-nutritional composition of *P. squarrosulus* in this study are in line with earlier reports of Jiskani (2001); Okwulehie and Odunze (2004), who noted that mushrooms are considered as sources of proteins, vitamins, fats, carbohydrates, amino acids and minerals, and in line with other reports on *Pleurotus species* by different authors.

Most of the inferences made on some biochemical studies with the studied sample revealed that *P. squarrosulus* on consumption even at high concentrations may not hamper important biochemical processes or organs in the body. However, the rise in ALP, and reduction in reproductive hormonal levels as observed in this study, should be looked into as a recommendation for further studies on *P. squarrosulus* mushroom. This study has evaluated the nutritional, anti-nutritional and some biochemical studies on *P. squarrosulus* (Mont.) Singer using rats.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

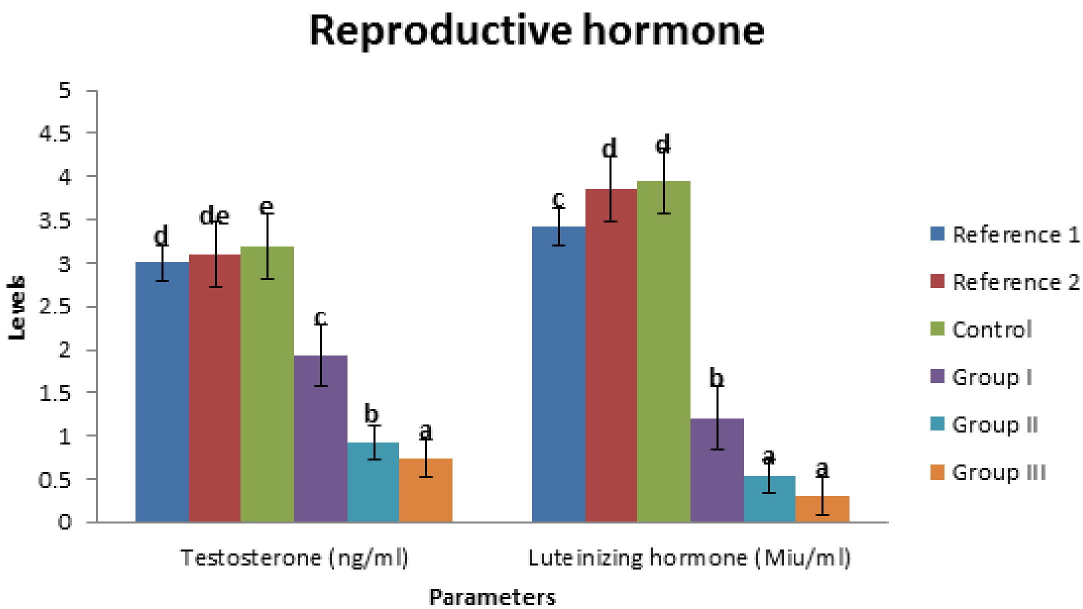

Figure 17. Reproductive hormone levels of rats placed on *P. squarrosulus.*

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