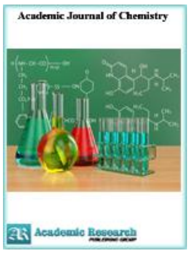

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Bioactive Constituents of *Pleurotus Squarrosulus* (Mont.) Singer and Effect of Its Dietary Incorporation on Body/Organ Weights and Lipid Profile Levels of Rats Placed on High Cholesterol Diet

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Abstract: Bioactive constituents of *Pleurotus squarrosulus* and effect of its dietary incorporation on body and organ weights; and lipid profile levels of rats placed on high cholesterol diet was investigated. Saponins, flavonoids and the variety such as chalcones, aurones, flavones, flavonol, and leukoanthocyanins; tannins alkaloids , proteins, amino acids, as well as individual amino acids such as tyrosine, phenylalanine, arginine, and cysteine were among the bioactive constituents detected in *Pleurotus squarrosulus.* The effect of *Pleurotus squarrosulus* dietary incorporation was effective against weight reduction and dyslipidemia. Rats placed on different proportions of *Pleurotus squarrosulus* incorporated diets had significantly ($p<0.05$) reduced body weight, total cholesterol, triglyceride, VLDL and LDL cholesterol levels against those of the experimental control. The incorporated diets also influenced the atherogenic indices positively in the test rats when compared to those of experimental control rats. The ability of the diet incorporated with *Pleurotus squarrosulus* to bring about the observed effects may be attributed to the presence and properties of these bioactive constituents detected in *Pleurotus squarrosulus.* This study has shown the bioactive constituents of *Pleurotus squarrosulus* (Mont.) Singer and effect of its dietary incorporation on body/organ weights and lipid profile levels of rats placed on high cholesterol diet.

Keywords: Atherogenic indices; Bodyweight; Diet; Dyslipidemia; *Pleurotus squarrosulus.*

1. Introduction

Dyslipidemia, a condition of high or low lipid bloodstream level is among the major risk factor of cardiovascular diseases [1]. It can be primary or secondary, and can accompany diseases such as hypertension, diabetes mellitus, as well as obesity [2]. Dyslipidemia usually involves a deviation from the normal levels of plasma triglyceride, total-LDL-cholesterol, VLDL-cholesterol and HDL-cholesterol [1, 3]. According to Gordon and William [4], the most common types of dyslipidemia are high levels of low-density lipoprotein (LDL or "bad") cholesterol; low levels of high-density lipoprotein (HDL or "good") cholesterol and high levels of triglycerides. Secondary causes have been reported to contribute to many cases of dyslipidemia in adults. Robert [5] noted that the secondary causes of dyslipidemia are type 2 diabetes mellitus, excessive alcohol consumption, cholestatic liver diseases, and nephritic syndrome. Sedentary lifestyle with excessive dietary intake of saturated fats, cholesterol and trans-fats has also been implicated as one of the causes of dyslipidemia in adult [6]. According to Anne [6], trans-fats are polyunsaturated or monounsaturated fatty acids to which hydrogen atoms have been added; they are used in many processed foods and are as atherogenic as saturated fat.

Apart from pharmacologic intervention, research studies have revealed the property of herbs in normalizing dyslipidemia. Fresh leaves of *Cymbopogon citratus* [7]; garlic [8-10]; *Stachytarpheta jamaicensis [11]*; *Tridax procumbens* [12]; *Acalypha wilkesiana* [13]; *Chromolaena odorata* [14] among others have been identified to remedy dyslipidemia levels. Other studies on *Persea americana* [15]; fresh leaves of *Cymbopogon citratus* [16, 17], and *Telfairia occidentalis*[18] have reported the ability of some plants to normalize dyslipidemia associated with a disease condition. In recent times, there is increasing awareness on nutritional intervention on normalizing dyslipidemia.

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Mushrooms are among those plants, which have occupied a strategic position in this nutritional dyslipidemia intervention. According to Adejumo, *et al.* [19], mushrooms are fruit bodies of macroscopic, filamentous and epigeal fungi. Majority of the known mushrooms belong to the phylum Basidiomycota. Adejumo, *et al.* [19] noted that mushrooms are made up of hypha which form interwoven web of tissue known as mycelium in the substrate upon which the fungi feed. Adejumo, *et al.* [19]; and Rai, *et al.* [20] reported that 2,000 out of the known 14,000 species of mushrooms are safe for human consumption while 650 of the known species possess medicinal values. Different authors have noted that edible mushrooms contain proteins, carbohydrates, crude fibre, ash, minerals like iron, copper, manganese, potassium, calcium and sodium; and moisture [11, 21-30]. Jiskani [31], Okwulehie and Odunze [32]; and Adejumo and Awosanya [24]; noted that mushrooms are considered as sources of proteins, vitamins, fats, carbohydrates, amino acids and minerals. According to Buigut [33], all essential amino acids, water soluble vitamins, and minerals are present in mushrooms. Chang and Buswell [34] noted the presence of vitamins such as riboflavin, biotin and thiamine in mushroom. There is no doubt that these reported constituents could be behind the reason why mushrooms, especially edible ones have occupied a central role as diet supplements in foods [35]. The medicinal potentials of some mushrooms have also received attention. For instance, Maria, *et al.* [36] reviewed edible mushrooms and related them to improving human health and promoting quality life. Yu, *et al.* [37]; Zhang, *et al.* [38]; Chang and Wasser [39]; and Maria, *et al.* [36]; noted that more than 100 medicinal functions are produced by mushrooms and fungi and the key medicinal uses are antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protector, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, and hepatoprotective effects. Maria, *et al.* [36] noted that mushrooms protect against tumor development and inflammatory processes.

Studies on the medicinal potentials of genus *Pleurotus* have also been reported. *Pleurotus* mushrooms are known as oyster mushrooms and are mostly edible in nature [36, 40]. Apart from the nutritional constituents of members of *Pleurotus species*, the medicinal properties in relation to the beneficial and health-promoting effects of some of the species have been identified [36]. Members of the genus *Pleurotus* are known to contain several important pharmacological and neutraceutical bioactive substances [36]. According to Maria, *et al.* [36], species such as *P. pulmonarius, P. ostreatus*, *P. florida, P. ostreatus, P. sajor-caju, P. giganteus and P. tuberregium* have been implicated to posses medicinal potentials. However, apart from the nutritional constituents of *Pleurotus squarrosulus* (Mont.) Singer, nothing much has been reported on its medicinal potential. The specie though less popular than *P. tuberregium,* is used as food [26]. Attempt made to study the specie looked into the nutritional [26], some anti-nutritional and biochemical effect of its consumption. There is need to extend the study on its possible medicinal potential.

This study investigated the bioactive constituents of *Pleurotus squarrosulus* (Mont.) Singer and effect of its dietary incorporation on body/organ weights and lipid profile levels of rats placed on high cholesterol diet.

2. Materials and Methods

2.1. Collection of Mushrooms

Matured *Pleurotus squarrosulus* samples used in this study were purchased from a village market of Ngor in Okpuala L.G.A of Imo State, Nigeria. The purchased samples were properly identified and transported to the laboratory where they were prepared for usage.

2.2. Bioactive Constituents

Bioactive constituents such as flavonoids and the variety such as chalcones, aurones, flavones, flavonols, and leukoanthocyanins were screened using the methods as described by AOAC [41]. Saponins, tannins, alkaloids, proteins, amino acids, aromatic amino acids and the individual amino acids such as tyrosine, phenylalanine, tryptophan, arginine, cysteine, cystine, and proline were qualitatively analyzed following the methods described Ojiako and Akubugwo [42].

2.3. Feed Preparation for Experimental Animals

Milled *Pleurotus squarrosulus* was mixed with standard growers mash to obtain a 5%, 15%, and 25% dietary incorporation of the mushroom. The method as described by Ijeh, *et al.* [43] was used to transform the mixtures obtained into pelletized form.

2.4. Experimental Animals

Forty-five male wistar albino rats weighing between 50g-70g 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* for four weeks to enable them to acclimatize to their new environment. After acclimatization, the rats were divided into five groups of nine rats each with almost equalized weights. Three groups served as the test groups and received the prepared standard growers mesh incorporated with the mushroom *Pleurotus squarrosulus*. The remaining two groups served as experimental control and reference. Apart from the reference, experimental control and all the test groups were placed on 10.00g of cholesterol diet. Treatments given to the animals are below

2.5. Control Groups

Reference =Nutrients +potable water

Experimental control=Standard growers mesh + 10.00g of cholesterol diet +potable water

2.6. Test Groups

Test group I= 5% of *Pleurotus squarrosulus* incorporated diet+10.00g of cholesterol diet + potable water.

Test group II= 15% of *Pleurotus squarrosulus* incorporated diet +10.00g of cholesterol diet + potable water.

Group III= 25% of *Pleurotus squarrosulus* incorporated diet +10.00g of cholesterol diet + potable water.

The cholesterol diet was introduced on more than 2.5% per kilogram body weight of animal as reported by other studies. The treatments of experimental animals were in accordance to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals [44], and the treatment lasted 28days.

2.7. Blood Sample and Tissue Collection

The experimental animals were sacrificed under light chloroform anesthesia after 4 weeks of feeding and overnight fasting of 10-14 hours. Blood samples were collected by cardiac puncture into EDTA-bottle for lipid and lipoproteins profiles. The plasma samples were separated from cells by centrifugation. The plasma samples were prepared under refrigeration until analyzed. The heart, kidney, liver and spleen organs of each animal were also excised, blotted on filter paper to remove blood and the wet weight were measured. The final weights of the experimental animals were also taken before sacrifice.

2.8. Plasma Lipid Profiles Determination

Assay of plasma total cholesterol, HDL-cholesterol, HDL-cholesterol, and triglyceride were assayed enzymatically with Randox test kits (Randox Laboratories, England). The methods as described by Friedwald, *et al.* [45] [LDL-cholesterol (mg/dl) = Total cholesterol(mg/dl) - (HDL-cholesterol (mg/dl) - TG/5)]; and VLDL-cholesterol = [Triglyceride/5] were used to estimate the LDL-cholesterol and VLDL-cholesterol respectively. Plasma non-HDL cholesterol concentration was determined as reported by Bruzell, *et al.* [46].

2.9. Statistical Analysis

Results were presented as mean and standard errors in figures. + (presence) and – (negative) signs were used for the bioactive constituents. Significant difference was taken at 5% levels using LSD.

3. Results and Discussion

Table 1 shows the screening of phytochemical constituents in *Pleurotus squarrosulus.* Saponins, flavonoids and the variety such as chalcones, aurones, flavones, flavonol, and leukoanthocyanins; tannins and alkaloids were among the bioactive phytochemical found present. Saponin is glycoside phytochemical, which has attracted human interest from ancient times with its foamy characteristics and diuretic effect [47]. Studies have illustrated the beneficial effects of saponins on cancer, bone health and stimulation of the immune system. Saponins have also been implicated against reactive oxygen species as antioxidants [48-50]. Das, *et al.* [51] noted that a number of studies have shown that saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects. Flavonoids have well documented anti-inflammatory, anti-oxidant, anti-viral, and anticarcinogenic properties [52-55]. The widespread distribution of flavonoids, their variety and their relatively low toxicity when compared to other active plant compounds show that many animals, including humans, take in significant quantities in their diet [56]. Tannins, commonly referred to as tannic acid are water-soluble polyphenols that are present in many plant foods [55, 57]. The anticarcinogenic and antimutagenic potentials of tannins have been reported [58]. Their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation has also been reported [50, 55]. Tannins also have well documented evidence of antimicrobial activities. For instance, tannic acid has been shown to inhibit the growth of organisms such yeast, fungi, bacteria, and viruses. Other physiological effects exerted by tannins are acceleration of blood clotting, reduction of blood pressure, decrease in serum lipid level, production of liver necrosis, and modulation immunoresponses. Alkaloids are among the secondary metabolites of plants. They act as lifesaving drugs in some serious disorders like heart-failure, cancer, and blood pressure and are of relative importance in medical field.

Table-1. Screening of phytochemical constituents in *Pleurotus squarrosulus.*

Constituents	Status
Saponins	++
Flavonoids	+
*Chalcones	+
*Aurones	+
*Flavones	+
*Flavonol	+
*Leukoanthocyanins	+
Tannins	+
Alkaloids	+

Key: +=present; ++= highly present.

The qualitative test for proteins and amino acids in *Pleurotus squarrosulus* as represented in Table 2 shows the presence of proteins, amino acids, peptides and individual amino acids such as tyrosine, phenylalanine, tryptophan, arginine, cysteine, cystine and proline.

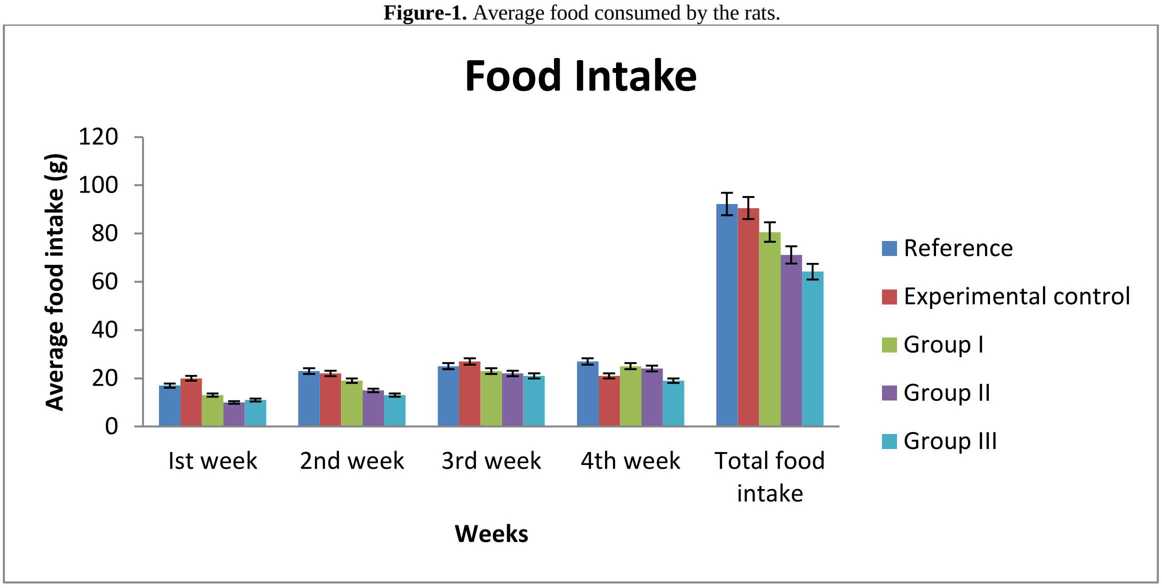
Table-2. Qualitative tests for proteins and amino acids in *Pleurotus squarrosulus.*

Constituents	Status
Protein	++
Amino acid	++
peptide	+
Aromatic amino acid	++
Tyrosine	++
Phenylalanine	++
Tryptophan	+
Arginine	+
Cysteine	+
Cystine	+
Proline	+

Key: +=present; ++= highly present.

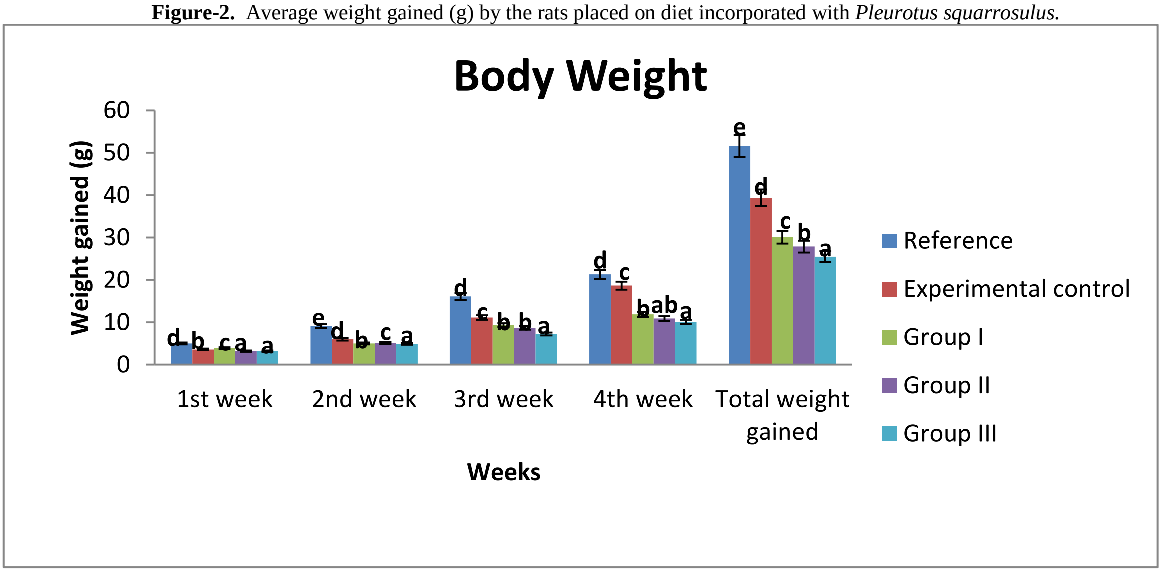
Different authors [55, 59] have noted that amino acids are the building blocks of proteins. By definition, amino acid is known as any of a class of organic compounds containing the amino (NH_2) and the carboxyl (COOH) groups, occurring naturally in plant and animal tissues and forming the chief constituents of protein. Peptide, a compound consisting of two or more amino acids linked in a chain, with the carboxyl group of each acid being joined to the amino group of the next by a bond of the type -OC-NH- is normally found in proteins [59]. Peptides are smaller than proteins. Depending on the number of amino acids, peptides are called dipeptides, tripeptides, tetrapeptides, and so on [59]. Dietary aromatic amino acids are needed to meet the requirements for phenylalanine and tyrosine for protein synthesis. The observed individual amino acids become important when their functions are considered in the body. For instance, tyrosine is the precursor of the catecholamines and thyroid hormone [60]. Phenylalanine is an essential amino acid and the precursor for the amino acid tyrosine in the liver and kidney [61]. Like tyrosine, it is the precursor of catecholamines in the body (tyramine, dopamine, epinephrine and norepinephrine). Tryptophan is another essential amino acid observed in *Pleurotus squarrosulus* in this study, which is the precursor of serotonin. Serotonin is a neurotransmitter of the brain, platelet clotting factor and neurohormone found in some organs throughout the body. Arginine decreases the growth of cancer and tumours by enhancing immune function [59]. It increases the size and activity of the thymus gland, which manufactures T lymphocytes (T cells), crucial components of the immune system. It is also good for liver disorders such as cirrhosis of the liver and fatty liver [62]. Cysteine is among the sulphur-containing amino acids, which is present in alpha-keratin, main protein constituent of the fingernails, toenails, skin, and hair. It promotes elasticity of the skin texture and helps in the production of collagen. Cysteine is present in several digestive enzymes and variety of other body proteins [59]. Cysteine is precursor to glutathione. Glutathione is known for its role in detoxifying the liver by binding with potentially harmful substances in it. Cystine the dimer of cysteine was also detected in this study. Generally, cystine is more stable than cysteine; though both can be converted into the other form as required by the body. Proline aids in the healing of cartilage and the strengthening of joints, tendons, and heart muscle. It also plays important role in the production of collagen and improves the skin, by reducing the loss of collagen through aging process [62].

Figure-1. Average food consumed by the rats.



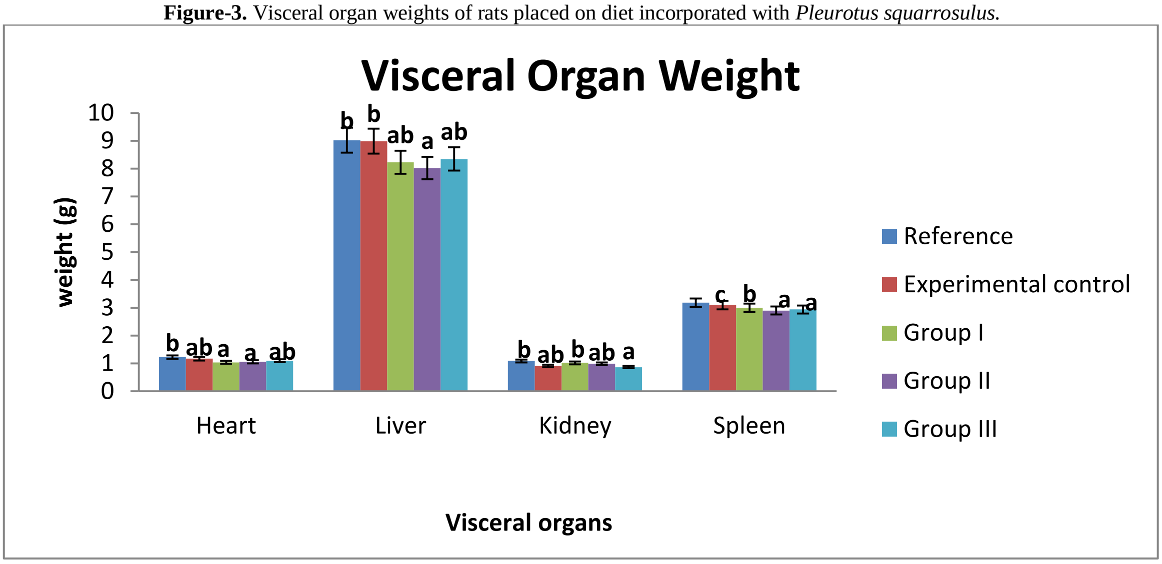
The diet as prepared and incorporated was based on induction of hypercholesterolemia. The average food intake of the rats as presented in Figure 1, shows reduced food intake by the test groups against those of experimental control and reference group for weeks 1, 2 and 3. The palatability of the prepared food could be behind the reduced intake. There was a rise in the average food intake of groups I and II against that of the experimental control in week 4 (Figure 1).The average total food intake of the groups for the weeks followed the order reference >experimental control>group I>group II> group III. Palatability and adaptation to the prepared food by the rats could be behind the observed order.

Figure-2. Average weight gained (g) by the rats placed on diet incorporated with *Pleurotus squarrosulus.*



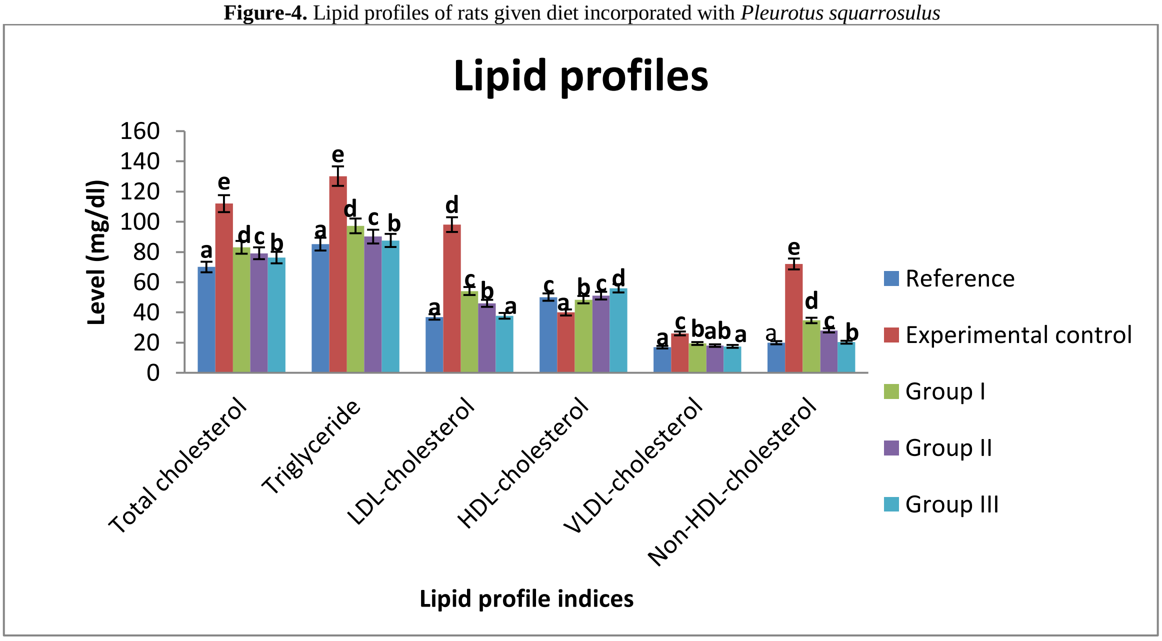
The result of average weight gained by the rats as presented in Figure 2 shows weekly weight gained by the animals and the total average weight gained by the rats at the end of the 4^{th} week. Apart from group I, the weight of groups II and III rats were significantly reduced ($p<0.05$) against the experimental control and reference in 1^{st} week. However, from the preceding weeks (2^{nd}, 3^{rd} and 4^{th}) all the average weight of test groups were significantly ($p<0.05$) reduced against those of the experimental control and reference. The cumulated total average weight of the rats also revealed a significant ($p<0.05$) weight reduction of test rats against those of the experimental control rats and reference. The observed reduction in weight could be attributed to the diuretic activity of the fungi (*Pleurotus squarrosulus*) incorporated diet. According to Freis, *et al.* [63], diuresis leads to weight loss due to volume loss, which correlates with reduction in blood pressure. Different authors have implicated fungi as a weight reducing recipe in the body system [36].

Figure-3. Visceral organ weights of rats placed on diet incorporated with *Pleurotus squarrosulus.*



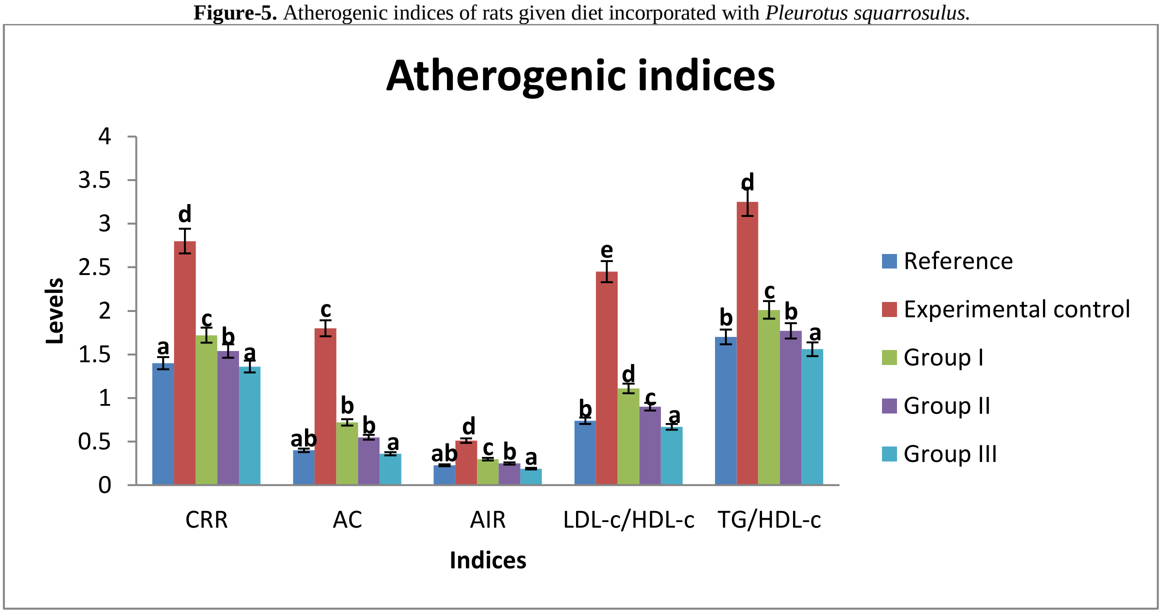
According to Duru, *et al.* [64], expansion of organs adds to the body weight. The same authors noted that expansion and injury of the organ could be as a result of disease condition. No death was observed among the test rats in this study. Weight of hearts and kidneys of test rats were insignificantly ($p>0.05$) reduced against the experiment control (Figure 3). Liver in test groups I and II reduced insignificantly ($p>0.05$) when compared to those of the experimental control and reference (Figure 3). Spleen in test rats significantly ($p<0.05$) reduced against those of experimental control and reference. *Pleurotus squarrosulus* incorporated in the diet could be behind the observed reduction.

Figure-4. Lipid profiles of rats given diet incorporated with *Pleurotus squarrosulus*



Weight loss helps improve and control coronary risk incidence, diabetes mellitus, dyslipidemia, hypertension, obesity, and physical functioning [46, 65-67]. It is also one of the strategies for increasing low HDL cholesterol levels [68]. According to Duru, *et al.* [69], lipid profile indices are used to determine or predict the status of the body in relation to heart diseases such as atherosclerosis, heart attack; stroke, etc. Cholesterol is produced in the liver and is insoluble in blood [70]. Raised plasma total cholesterol level is a recognized and established risk factor for developing atherosclerosis [71]. Lipid profiles of rats given feed incorporated with *Pleurotus squarrosulus* as presented in Figure 4 revealed that total cholesterol levels of the test rats increased significantly ($p<0.05$) against reference and decreased significantly ($p<0.05$) against experimental control rats. The reduced cholesterol levels in the test rats could be due to the saponin and tannin contents of *Pleurotus squarrosulus* in the incorporated diets. Elevated plasma triglyceride level is a synergistic risk factor for cardiovascular diseases [46, 72, 73]. The triglyceride levels of test rats reduced significantly ($p<0.05$) against experimental control and increased significantly ($p<0.05$) against the reference. Decrease in plasma LDL, the bad cholesterol has been linked to reduced risk of coronary heart diseases [3]. LDL levels decreased significantly ($p<0.05$) in test rats against experimental control and increased significantly ($p<0.05$) against the reference. Decrease in plasma HDL cholesterol is a risk factor for coronary heart disease [73, 74], and is often associated with hypertension [3, 46]. The *Pleurotus squarrosulus* in the incorporated diets increased HDL cholesterol levels significantly (p<0.05) in test rats against the experimental control. Increase in plasma HDL cholesterol has been considered to reduce the risk of coronary heart disease [3, 75, 76]. Total cholesterol, triglycerides and LDL cholesterol levels of test groups decreased significantly (p<0.05) among the groups as the proportion of *Pleurotus squarrosulus* increase in the incorporated diets; whereas the reverse was the case with HDL cholesterol in the incorporated diets. Very-low-density lipoprotein (VLDL) cholesterol is produced in the liver and released into the bloodstream to supply body tissues with a type of fat (triglycerides) [74, 76]. This produced cholesterol has several types, each made up of lipoproteins and fats. Each lipoprotein contains a mixture of cholesterol, protein and triglycerides in varying amounts. It has been noted that about half of a VLDL particle is made up of triglycerides. High levels of VLDL cholesterol have been associated with the development of plaque deposits on artery walls, which narrow the passage and restrict blood flow [3, 74, 77]. VLDL cholesterol levels of test rats in this study reduced significantly (p<0.05) against the experimental control. VLDL levels of groups II and III rats were insignificantly (p>0.05) affected when compared to reference. Measuring non-HDL cholesterol levels gives a better assessment of the risk for heart diseases than measuring only LDL [3]. Non-HDL cholesterol is a better predictor of cardiac death than LDL cholesterol, and should be used as a treatment target for cholesterol [76]. Non-HDL cholesterol levels of test rats reduced significantly (p<0.05) against experimental control and increased significantly (p<0.05) against the reference. It also reduced significantly (p<0.05) among the test groups as the proportion of *Pleurotus squarrosulus* increase in the prepared diets. The reduced Non-HDL cholesterol levels of test rats against the experimental control in the present study could mean that *Pleurotus squarrosulus* has the potential of to reduce cardiovascular risk.

Figure-5. Atherogenic indices of rats given diet incorporated with *Pleurotus squarrosulus.*



Legend: CRR=Cardiac Risk Ratio; AC= Atherogenic Coefficient; AIP= Atherogenic Index of Plasma; TG=Triglyceride; LDL-c= LDL cholesterol and HDL-c =HDL cholesterol.

Atherogenic indices are powerful indicators of the risk of heart disease [75, 78]. It has been reported that the higher their values, the higher the risk of developing cardiovascular disease and vice versa [73, 77, 78]. In this study, test rats had significantly reduced (p<0.05) Cardiac Risk Ratio (CRR), Atherogenic Coefficient (AC), and Atherogenic Index of Plasma (AIP), when compared to those of experimental control (Figure 5). LDL-cholesterol and TG/HDL-cholesterol also followed the same order as the above mentioned antherogenic indices (Figure 5). These are further indications that diet incorporated with the mushroom *Pleurotus squarrosulus* could remedy dyslipidemia condition.

4. Conclusion

The bioactive constituents of *Pleurotus squarrosulus* showed the presence of saponins, flavonoids and the variety, tannins and alkaloids. Important essential and non-essential amino acids were qualitatively detected. Rats placed on diet incorporated with *Pleurotus squarrosulus* had reduced weigh and improved dyslipidemia condition against the experimental control. The ability of diet incorporated with *Pleurotus squarrosulus* to bring about the observed effects may be attributed to the presence and properties of these bioactive constituents detected in *Pleurotus squarrosulus.* This study has shown the bioactive constituents of *Pleurotus squarrosulus* (Mont.) Singer and effect of its dietary incorporation on body/organ weights and lipid profile levels of rats placed on high cholesterol diet.

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