AJFAND ONLINE
Volume 10 No. 3
March 2010
ISSN 1684 5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

NUTRITIONAL AND HYPOCHOLESTEROLEMIC PROPERTIES OF *TERMITOMYCES MICROCARPUS* MUSHROOMS

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Volume 10 No. 3
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ABSTRACT

Wild edible mushrooms, *Termitomyces microcarpus* are widely consumed in Uganda, partly because of their taste, flavour and because they are believed to have medicinal benefits. This study investigated the nutrient composition of the *Termitomyces microcarpus* mushrooms and the effect of the mushroom on feed intake, weight gain, serum cholesterol and triglycerides of male albino rats. Semi-dried mushrooms collected from Kyenjojo District in western Uganda were analyzed for nutrient composition using standard procedures. To determine the effect of dietary intake of mushrooms, a completely randomized study design was used with experimental treatments having diets containing 25, 45 and 60% air -dried mushroom flour mixed with the basal feed and 0.5% cholesterol. These were compared to a control diet consisting of only commercial (basal) feed and to a diet containing basal feed and 0.5% cholesterol. The rats were fed on the five diets for ten weeks and were monitored for changes in feed intake and weight at weekly intervals for six weeks and in serum total cholesterol, High Density Lipoprotein (HDL)-cholesterol, Low Density Lipoprotein (LDL)-cholesterol and triglycerides at two weeks intervals for ten weeks. Proximate analysis revealed that the mushrooms contained 25.5% protein, 2.3% fat, 11.2% dietary fibre, 48.37% available carbohydrates and 12.67% water. The mushrooms were also found to contain 61 mg/100g of iron, 156 mg/100g of calcium and a number of other dietary minerals. Dietary inclusion of *Termitomyces microcarpus* mushrooms significantly reduced the feed intake and weight gain of the rats by up to 36.8 and 29.5%, respectively. The reduction increased with the proportion of dietary mushroom. Inclusion of mushrooms in the diets of rats also lowered their total serum cholesterol, LDL-cholesterol and triglycerides by up to 15.6, 28.3 and 29.9%, respectively. Reduction in serum lipids did not, however, show a clear relation to the quantity of mushrooms in the diet. The reduction in the total serum cholesterol, LDL- cholesterol and triglycerides may be attributed to the high quantities of fibre in the mushrooms. These results suggest that consumption of *T. microcarpus* mushrooms could contribute to reducing the prevalence of diseases linked to high blood lipids.

Key words: Serum cholesterol, mushroom, *Termitomyces microcarpus*, triglycerides

INTRODUCTION

It is generally accepted that lowering of the serum cholesterol and lipid levels plays a significant role in the prevention of arthrosclerosis and other nutrition-related diseases [1]. There is, therefore, need to identify natural substances with hypocholesterolemic effect. A number of mushroom species have been reported to exhibit hypocholesterolimic effects. This has been attributed to their high dietary fibre levels [2] and other components such as eritadenine, guanylic acid and ergosterol [3]. Mushrooms are low in calories and fat, but have higher protein, (19-35% of dry weight) than most vegetables and are good sources of vitamins, especially the B vitamins including riboflavin, niacin and folate [4].

Termitomyces microcarpus is a gilled fungi belonging to a group of organisms known as eukaryotes, subdivision Basidiomycota, class Basidiomycetes, tribe Termitomyeteae, Genus *Termitomyces* and species *microcarpus* [5]. *Termitomyces species* is a very important type of mushroom in Uganda [6]. These mushrooms grow basically in the wild usually in the rainy seasons, in places where termites are found.

MATERIALS AND METHODS

Mushrooms

The mushrooms (*Termitomyces microcarpus*) were collected from mushroom collectors during the late rains of 2005 (October-December) and early rains of 2006 (March-May). These mushrooms had been picked from the wild and semi-dried under the sun to a moisture content of about 45%. The mushrooms were collected from Kyenjojo district, which lies within the forest and Savannah ecological zone in the western region of Uganda [7].

The mushrooms were dried under sunlight for 48-96 hours to a stable moisture content of around 12% to extend their shelf life. The soil attached to the mushrooms was removed from the sun-dried mushrooms by rubbing onto the root of the mushrooms. The mushrooms (caps and stalks/stems) were then stored in airtight containers to avoid contamination awaiting milling into flour. Prior to grinding, the sun-dried mushrooms were further dried in an oven at 60 - 70^0C for 48 hours to ease the grinding.

Chemical analysis of *T. microcarpus* mushrooms and experimental diets

Proximate composition of the mushroom flour was determined by the Official Methods of the Association of Official Analytical Chemists [8]. Moisture content, total ash, crude fat, crude fibre, total carbohydrates and crude protein were determined by oven method, hot furnace, Soxhlet, Van Soest, spectrophotometric and Kjeldahl (N x 6.25) methods, respectively [8]. Total carbohydrates were determined by spectrophotometric method, based on reaction between glucose and anthrone, after hydrolysis. All the calculations were carried out on dry weight basis of the mushrooms. The ash obtained from the furnace during ash determination was digested using 50% HCl and used for determination of selected minerals. Calcium,

sodium and potassium were determined by Jenway flame photomer Model PFP7 (Jenway, Essex, UK). Copper and iron content were determined by atomic absorption spectrophotometry at 324.8 and 248.3nm [9].

Feed sourcing and analysis

The basal feeds were obtained commercially as rat pellets manufactured by Nuvita Animal Feed Company (Jinja, Uganda). The feed was then subjected to proximate analysis for crude protein, crude lipid, crude fibre and total ash using methods described above.

Feeding trials

Fifty weanling male albino rats purchased from a rat breeder in the Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda, with initial weight of approximately 36 g were used to study the effect of dietary inclusion of mushroom on feed intake, weight gain and the serum lipid profile**.** Efforts were made to treat the rats humanely and the protocol used was approved by the Makerere University Faculty of Agriculture Higher Research and Higher Degrees Committee. The rats were obtained at four weeks of age, the time at which they were weaned and separated from their mothers. Male rats were used to prevent hormonal interference associated with female rats at puberty. All animals were housed in individual escape proof cages with inside dimensions of 31 x 13 x 13 cm (length x width x breadth) with a wire mesh on the top to allow for free gaseous movement and light. The cages were cleaned with water and soap every day to prevent disease outbreak in the colony. Clean water was provided in clean drinkers, as recommended by the Animal Welfare Information Centre [10]. Each cage had a label that carried identification numbers for the individual and the feed. Each cage contained a feeder and drinker to facilitate easy collection of feed leftovers. Temperature in the animal houses where the cages were placed was between 21- 27^{o}C with relative humidity of 45-55%. The rats were divided into five groups of ten animals each by randomization.

Experimental design

The experiment was a Completely Randomized Design (CRD) in which animals in the five groups were fed for 10 weeks on diets that were formulated as indicated in Table 1. The diets included the control/basal diet, diet enriched with cholesterol and 3 diets containing mushrooms at levels of 25, 45 and 60%, with commercial feed making up the remaining proportion. The basal feed consisted of commercial rat pellets manufactured by Nuvita Animal Feed Company (Jinja, Uganda). All rats in the same group were subjected to the same conditions and were given the same amounts of feed. After an acclimatization period of 4 days, the feed intake, weight gain and feed efficiency ratio were monitored for six weeks while the serum lipids were monitored for 10 weeks.

Preparation of the experimental diets

Fruiting bodies of *Termitomyces microcarpus* were oven dried at 60-70^{o}C for 24 hours and pulverized to small size particles using a laboratory mill (Janke and Kunkel and company, model: 7921 Staufen, Germany). The commercially prepared rat pellets

were similarly milled in order to have the same consistence as the mushrooms, and this constituted the other portion of the experimental diets and was the only component of the control diet (basal diet). Cholesterol powder (cholesterol 95%; C3292-100G, ash-free, a product of SIGMA, Steinhein, Germany) was added to the diets of the rats except for the control diet. The mushroom flour and the flour from the pellets plus 0.5% cholesterol powder were mixed to constitute the diets. The diets were constituted as described in Table 1 and analyzed for crude protein, crude fat, crude fibre and ash using methods described above.

The basal diet (commercial rat pellets, diet 1) is a complete rat diet that is used by rat breeders to raise their rats. From analysis, they contain about 8.2% protein. In this experiment, part (40, 55 and 75%) of the basal feed was replaced with *T. microcapus* mushrooms to analyze the effect of the replacement on the different parameters. The proportion of mushroom and basal feed in the experimental diet of the rats was based on the recommended intake of protein for the rats which was 15-20% [11].

Determination of weight gain, feed intake and feed efficiency ratio of experimental rats

To determine the feed intake of the rats, the rats were each given 15-20g of feed per day in individual containers, and the leftovers, including spillages, were weighed the next day (after 24 hours) and subtracted from the amount offered in order to determine the actual intake for that day for each rat. The feed intake for each rat was determined and recorded everyday for six weeks.

The weight gain of the rats was determined by first weighing the rats before the beginning of the experiment (at four weeks of age) to ascertain their initial weights. Each of the rats was put in a tall weighing vessel and placed on an analytical balance and the weight was recorded to the nearest 0.1g. Thereafter, the experimental diets were introduced and the rats were weighed at intervals of 7 days for six weeks to determine their weight in grams. The effect of mushroom containing diets on growth of the rats as depicted by weight gain was determined from the weight gain data. The metabolic weight gain of the rats was also obtained from the expression;

$$\text{Metabolic weight gain} = \text{live weight gain to the power } 0.75$$

$$MWt = LWt^{0.75}$$

The feed efficiency ratio for the experimental rats was also determined during the time of study and the values were calculated from the expression:
Feed efficiency ratio = Total weight gain (g)/Total feed intake (g)

Determination of the effect of dietary intake of *T. microcarpus* on serum lipids

The effect of *T. microcarpus* mushrooms inclusion in the diet on serum total cholesterol, HDL-cholesterol, LDH-cholesterol and triglyceride profiles was analyzed as described below.

Collection of blood sample from rats
The blood was drawn from the rats for lipid profile analysis every two weeks using the tail sectioning method as recommended by Institutional Animal Care and Use Committee guidelines [12].

Tail Sectioning
The rats were restrained by holding both the hind and fore limbs together and the ears were also held firmly so as to restrain the head. The area over the tail vein was then cleaned with ethanol and with a sterile scalpel blade, before a transverse section through the long axis of the tail was made 2 mm from the base. A Pasteur pipette was then used to collect blood. The tail was massaged by passing the thumb and index finger from the base to the tip of the tail if blood flow was inadequate. Direct pressure was applied to the incision for 1-3 minutes to facilitate homeostasis. Repeated blood sampling was done at intervals of two weeks by sectioning the adjacent area of the tail.

The blood samples were then transferred from the Pasteur pipettes into 1 ml plastic vials without an anticoagulant. After the samples had stood at room temperature for 2 hours, serum was prepared by centrifugation (Centurion Scientific Limited UK) at a force of 12000x g for five minutes [13].

Determination of serum lipids
Serum total cholesterol, HDL-cholesterol, LDL- cholesterol and triglycerides were determined at intervals of two weeks using the serum collected from the rats. Colorimetric techniques using a visible light colorimeter (Model 6061, Jenway limited UK) were used.

Determination of total serum cholesterol of the rats
Total serum cholesterol was determined using an enzymatic colorimetric test for cholesterol with lipid clearing factor [14]. Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator red quinone was formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase.

The reagents and samples reagents were mixed in plastic cuvettes, vortexed and incubated for 5-10 minutes in a water bath at a temperature of 37^{o}C. The absorbances of the samples and standard were then measured at 520 nm using a colorimeter against the reagent blank within less than an hour. Cholesterol concentration was calculated from the relation:

Conc. of test = (Absorbance of test/ Absorbance of Standard) x Conc. of Standard

The standard used had a concentration of 200 mg/dl

Determination of serum triglycerides of the rats
This was done by enzymatic determination. The reagents the procedure and calculation were conducted as outlined for the determination of total serum cholesterol.

High density lipoprotein cholesterol (HDL)
The serum HDL-cholesterol concentration of the rats was determined using a homogenous enzymatic colorimetric test, in which the presence of magnesium sulphate in the reagent caused dextran sulphate to selectively form water soluble complexes with low-density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons, which were resistant to polyethylene glycol (PEG) derivative-modified enzymes. The cholesterol concentration of HDL-cholesterol was then determined enzymatically by cholesterol oxidase coupled with PEG to amino groups [15].

The mixture of the reagents and sample were then incubated for 5-10 minutes in a water bath at a temperature of 37^{o}C. Additional 250 µl of reagent were added to each of the cuvettes and then the contents were mixed properly and incubated again at 37^{o}C for 5-10 minutes. The absorbances were read at 600 nm. Serum HDL-cholesterol concentration was calculated as shown below and the concentration of the standard was 1.43 mmol/L.

HDL cholesterol = (Absorbance of test sample/ Absorbance of standard) x conc. of standard.

Low density lipoprotein cholesterol (LDL)
The serum LDL-cholesterol concentration of the rats was determined using a homogenous enzymatic colorimetric test [14]. The combination of a sugar compound with detergent in this method enabled the selective determination of LDL-cholesterol (low density lipoprotein) in the serum.

The procedure and calculation used for HDL-cholesterol (described above) were followed, save for the use of a different concentration of the standard (3.44 mmol/L).

Data analysis
Analysis of Variance (ANOVA) was run using the GenStat package to determine the statistical differences in the nutrient composition of mushrooms and experimental diets. The data for feed intake, weight gain and serum lipid levels were analyzed by repeated measures ANOVA using the LINEAR MIXED MODEL in SAS package and the results were reported as estimates for the interaction between diets and time and standard errors. Means differences were considered statistically significant at $p<0.05$.

RESULTS

Nutrient composition of mushrooms and experimental diets

Nutrient composition of *Termitomyces microcarpus*

Proximate analysis of *T. microcarpus* revealed that the *T. microcarpus* mushroom contained 25.48% protein, 2.32% crude lipid and 11.21%fibre (Table 2). The mushrooms were also found to contain variable amounts of minerals with potassium being the most abundant mineral in the mushrooms.

Nutrient composition of experimental diets

The nutrient composition of the five experimental diets showed significant differences (p=0.02) (Table 3). The protein content of the diets increased significantly with increase in mushroom content. The same was observed for the dietary fibre. The dietary fat on the other hand significantly reduced with increase in the amount of mushroom added.

Effect of dietary mushroom incorporation on feed intake, weight gain and feed utilization

Feed intake, live weight, metabolic weight gain and feed efficiency ratio reduced with increase in mushroom content of diets and were not affected by inclusion of cholesterol in their diet (Table 4). The feed efficiency ratio was, however, not affected by inclusion of mushrooms in the diet up to the level of 25%. At 60% mushroom, the feed efficiency ratio was significantly higher. The feed intake (Figure 1 and 2), weight gain (Figure 3) and feed efficiency ratio (Figure 4) did not seem to follow a clear pattern over time for the different diets.

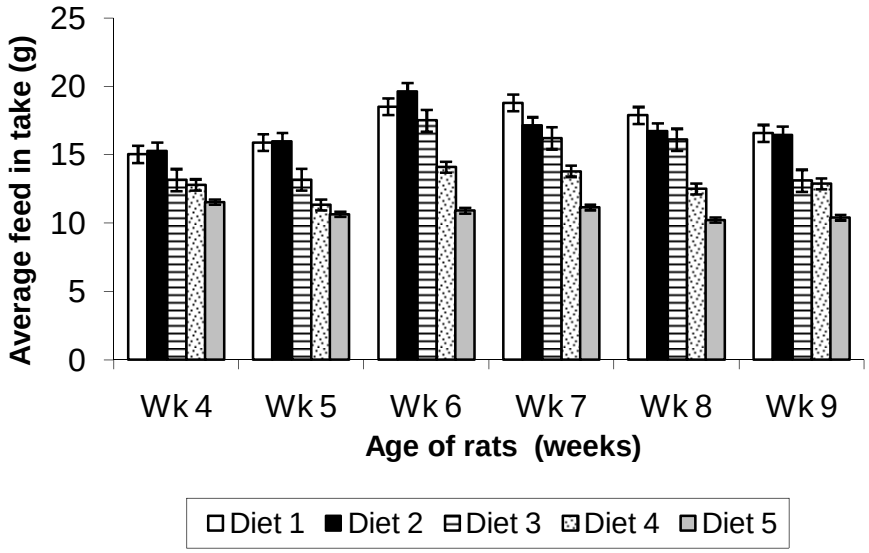

Figure 1: Average daily feed intake by the experimental rats

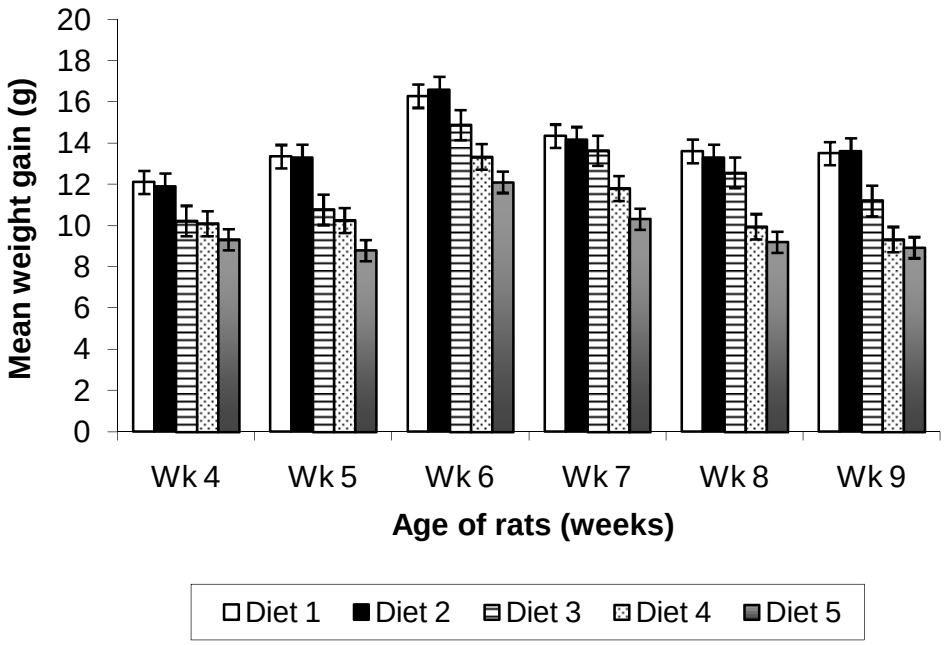

Figure 2: Average weekly weight gain of the experimental rats with age

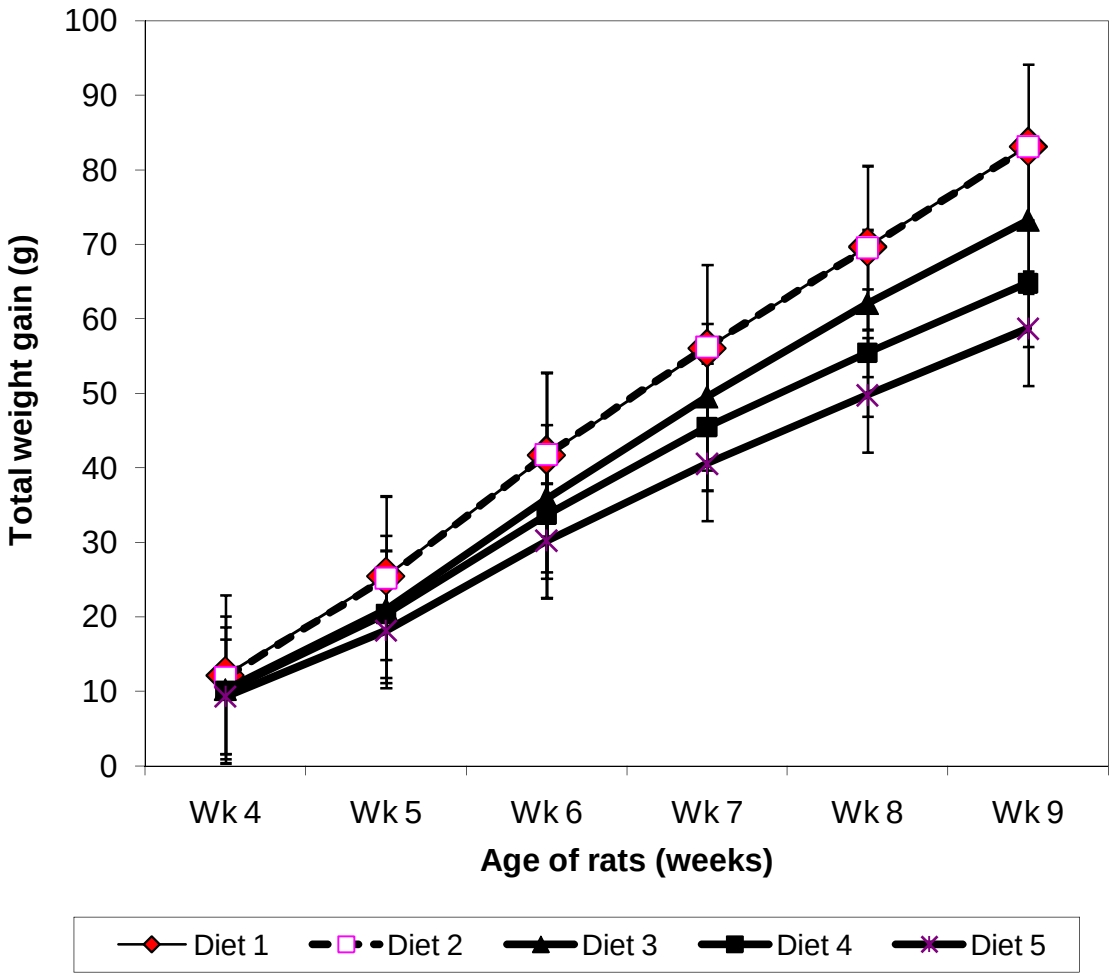

Figure 3: Changes in the total weight gain of rats with age

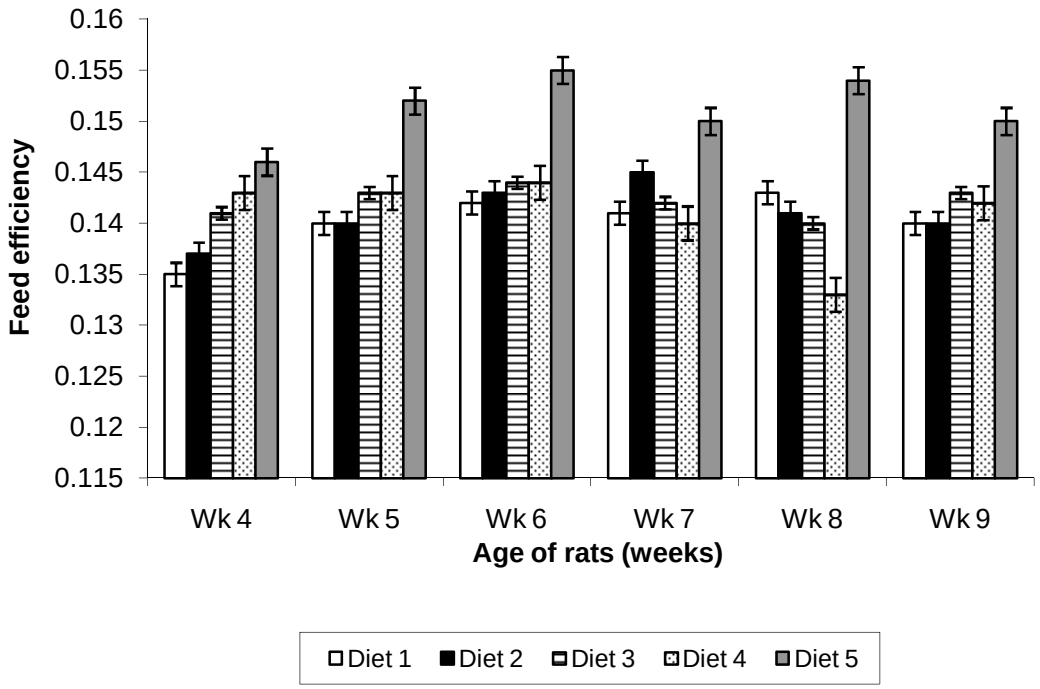

Figure 4: Change in mean feed efficiency ratio of rats with age

Effect of experimental diets on the serum lipid levels

Inclusion of mushroom in the diet of rats led to a reduction in total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides (Table 5). Inclusion of 0.5 % cholesterol in the diet was accompanied by a significant increase in serum triglycerides, an increase in total cholesterol and a rise in the LDL/HDL cholesterol ratio (Table 5). The hypocholestolemic effect of mushroom did not seem to increase with the proportion of mushroom in the diet (above 25%). There were also no clear trends in the serum level of the different lipid types over time (Figures 5, 6, 7 &8).

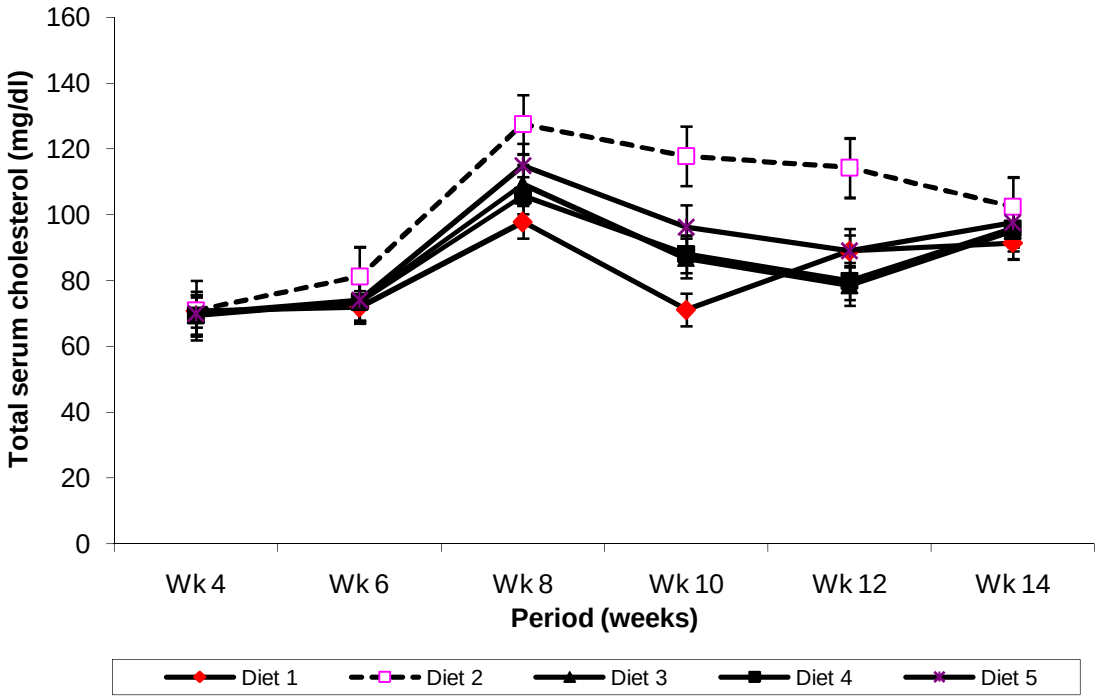

Figure 5: Changes in total serum cholesterol with age of rat

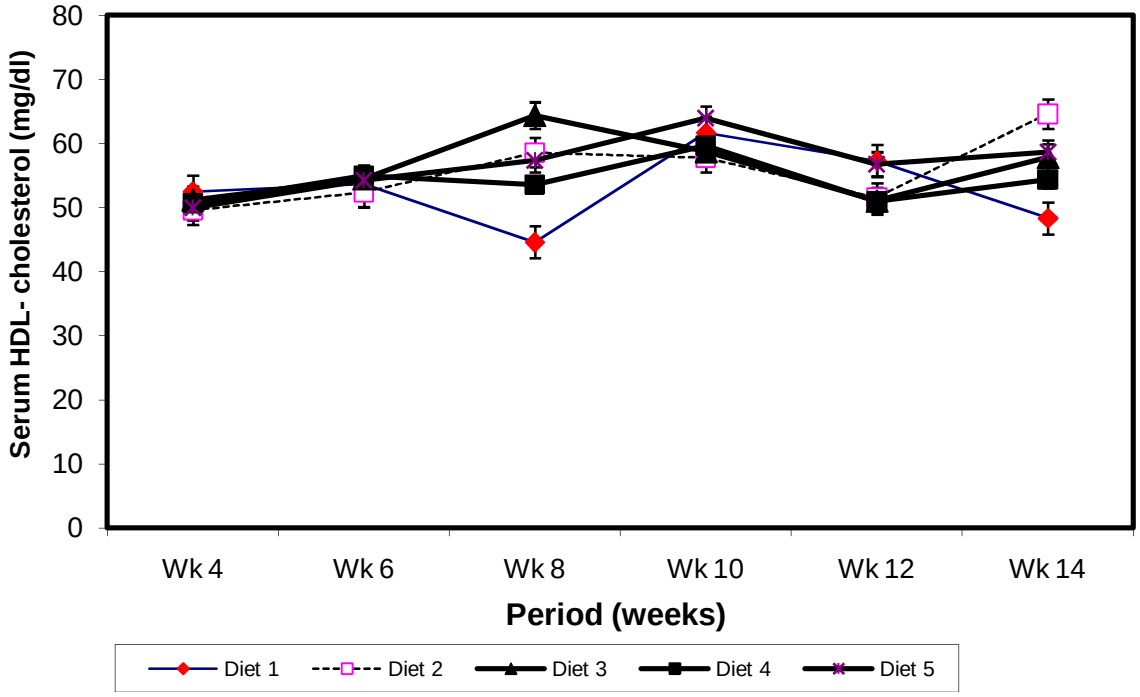

Figure 6: Changes of serum HDL-cholesterol with age of rats

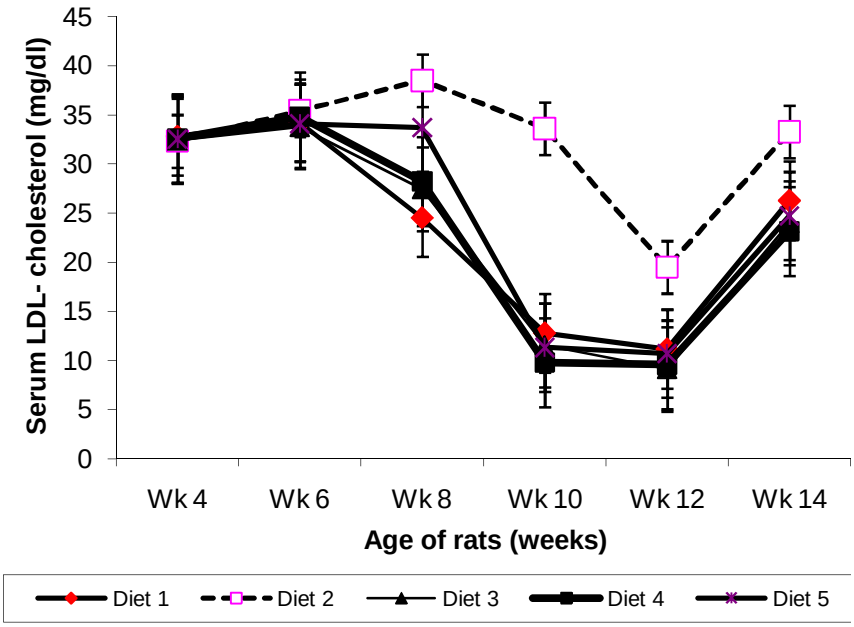

Figure 7: Changes of serum LDL-cholesterol with age of the rat

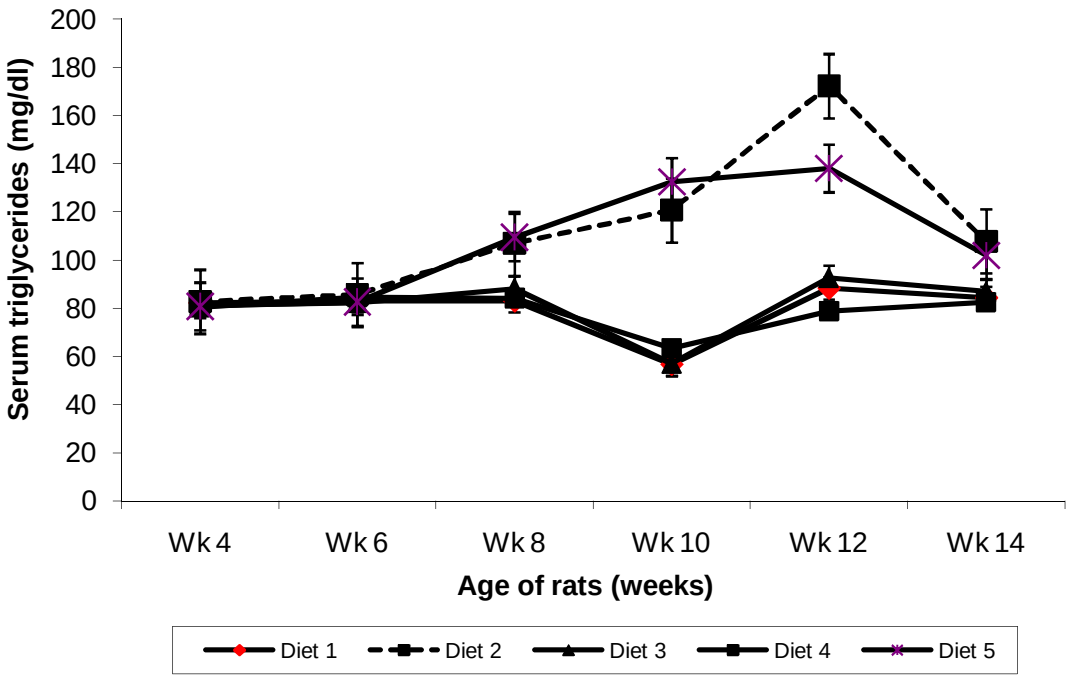

Figure 8: Changes of serum triglycerides with age of rats

DISCUSSION

The results of this study showed that *T. microcarpus* mushrooms are a good source of nutrients including protein, minerals and dietary fibre. This is in agreement with results from an earlier study on *T. microcarpus* in Tanzania, which reported that mushrooms contain between 23.9-49% protein, 2-10% fat and 11-23% ash [16]. Fibre content of 11.2 was reported for *T. microcarpus* mushrooms from the Khasi hills of Meghalay [17]. Amongst the minerals, *T. microcarpus* mushrooms used in this study were found to have higher mineral content than reported for other mushrooms [18]. The differences may be due to the high iron content of soils on which *T. microcarpus* grew.

The proximate composition of the experimental diets containing mushrooms showed that their protein and lipid contents met the recommendations (14% protein and 4% lipids) for the rats [19].

The lower feed intake of rats on diets with mushrooms than those on the commercial (control) diet could be attributed to the high fibre content of mushrooms and possibility reduced feed palatability. High dietary fibre content has been reported to lead to delayed release of chyme from the stomach into the intestines. This leads to slow the release of nutrients from the stomach. This effect creates a feeling of postprandial satiety as well as slowing down the digestion process and in effect decreasing feed intake [20]. Expectedly, the rats on diets containing mushroom also

exhibited lower weight gain. The markedly higher feed efficiency ration for diets containing 60% mushroom could be attributed to the reduced feed intake. With lower feed intake, animals tend to use the nutrients more efficiently. The higher total serum cholesterol for the rats fed on the diet containing 60% mushrooms may also be indicative of a tendency towards increased lipid deposition, at the expense of weight gain, with reduced feed intake.

The observation of reduced levels of total cholesterol, LDL-cholesterol and triglycerides in rats fed diets containing mushrooms is in agreement with results from other studies [21,22] that revealed that some edible mushrooms and their water-extract fraction have the capacity to lower the serum triglyceride concentration in spontaneously hypertensive rats and hyperlipidaemic rats. The results of this study also suggest that an increase in dietary cholesterol by even fairly low levels may be a pre-disposing factor for coronary heart problems.

Oyster [1] and *maitake* mushrooms [23] have been reported to exhibit serum lipid reducing effect in rats. This has been attributed to mushrooms' ability to alter lipid metabolism by inhibiting both the accumulation of liver lipids and the elevation of serum lipids. There are a number of mechanisms proposed for serum cholesterol reductions with increased fibre intake. It has been proposed [2, 24] that viscous polysaccharides act in the gastrointestinal tract to reduce blood cholesterol by decreasing absorption of cholesterol or fatty acids and decreasing absorption of biliary cholesterol or bile acids. Fibre may also cause altered serum concentration of hormones or short-chain fatty acids that affect lipid metabolism. ß-Glucan, the water-soluble fibre and pectin have been shown in animal models to be the active agent causing the altered cholesterol metabolism [25]. These also bind bile acids therefore reducing the formation of micelles and cholesterol absorption, causing reduction in the total serum cholesterol, LDL and HDL- cholesterol [26]. Higher excretion of bile acids induces a decrease in their enterohepatic circulation and, stimulates through a feedback mechanism, 7 alpha-hydroxylase, the rate limiting enzyme in the catabolism of cholesterol to bile acids [27,28]. Promotion of bile acid excretion due to deconjugation to produce free bile acids has been proposed as another mechanism for serum lipid improvement [29]. The glucans may also promote cholesterol clearance from the plasma via reverse cholesterol transport hence reducing its concentration in blood [30].

CONCLUSION

This study showed that dietary intake of *T. microcarpus* was associated with lower serum levels of total and LDL-cholesterol. Since high serum levels of total and LDL-cholesterol are predisposing factors for coronary heart diseases, it seems that incorporation of *T. microcarpus* could help to address this growing global health problem. However, currently, *T. microcarpus* only grows in the wild. For improved utilization, there is need to domesticate these mushrooms. Further studies are, however, needed to determine the benefits of lower doses of *T. microcarpus* mushrooms with respect to the reducing serum lipid levels.

Table 1: Experimental diet formulation

Diet	% of mushroom	%Basal diet (Nuvita Pellets)	% Cholesterol	Water
1	0	100	0	*Ad Libitum*
2	0	100	0.5	*Ad Libitum*
3	25	75	0.5	*Ad Libitum*
4	45	55	0.5	*Ad Libitum*
5	60	40	0.5	*Ad Libitum*

Table 2: Proximate composition of *Termitomyces microcarpus* mushrooms

Nutrient	Amount per 100g dry matter[1]
Crude protein [g]	25.48 ± 0.25
Crude lipid [g]	2.32 ± 0.16
Crude fiber [g]	11.21 ± 0.32
Total carbohydrates [g]	48.37 ± 5.06
Iron [mg]	61.60 ± 3.68
Potassium [mg]	1465 ± 14.00
Calcium [mg]	221.72 ± 9.50
Copper [mg]	1.99 ± 0.22
Phosphorous [mg]	156.53 ± 11.15
Sodium [mg]	906.47 ± 0.57

[1]Data presented are means of triplicate samples ± standard deviation of the means

Table 3: Proximate composition of the experimental diets

Diet	Crude protein	Crude lipid	Crude fibre	Total Ash
1	8.12[d]	6.18 [a]	8.57[a]	11.59[d]
2	8.11[d]	6.47 [a]	8.63[a]	11.51[d]
3	14.12[c]	6.17 [a]	10.42[b]	12.14[b]
4	17.53[b]	5.89 [c]	14.65[c]	11.81[c]
5	19.38[a]	6.01[b]	19.61[d]	12.55[a]

Diet 1 (control/ basal), diet 2 (basal feed plus 0.5% cholesterol), diet 3 (25% mushroom inclusion plus 0.5% cholesterol), diet 4 (45% mushroom inclusion plus 0.5% cholesterol) and diet 5 (60% mushroom inclusion plus 0.5% cholesterol). Data presented are means of triplicate samples.
Superscripts [a, b, c, d] mean values within a column with different superscripts were significantly different ($p<0.05$).

Table 4: Total feed intake, live weight gain, feed efficiency ratio and metabolic weight of the rats for the entire experimental period (6 weeks)

Diets	Feed intake [g]	Live weight gain [g]	Feed efficiency ratio	Metabolic weight
1	616.4 ± 0.29[a]	83.17 ± 4.26[a]	0.14 ± 0.32[a]	27.54 ± 1.54[a]
2	607.2 ± 0.56[a]	83.13 ± 1.28[a]	0.14 ± 0.44[a]	27.53 ± 1.65[a]
3	535.5 ± 0.91[b]	73.27 ± 0.59[b]	0.14 ± 0.43[a]	25.04 ± 2.09[b]
4	461.3 ± 0.48[c]	64.77 ± 1.64[c]	0.14 ±0.31[a]	22.83 ± 1.21[c]
5	389.6 ± 0.39[d]	58.67 ± 0.62[d]	0.15 ± 0.35[a]	21.20 ± 2.02[d]

Values are means ± SD, Diet 1 (control/ basal), diet 2 (basal feed plus 0.5% cholesterol), diet 3 (25% mushroom inclusion plus 0.5% cholesterol), diet 4 (45% mushroom inclusion plus 0.5% cholesterol) and diet 5 (60% mushroom inclusion plus 0.5% cholesterol).
Superscripts [a, b, c, d] mean values within a column with different superscripts were significantly different ($p<0.05$).

Table 5: Mean serum cholesterol and triglyceride values of the rats in mg/dl between week 4 and week 14 of age

Diets	Total cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides	LDL/HDL ratio
1	82.03[d]	53.00[d]	23.65[c]	79.56[d]	0.45
2	102.38[a]	57.42[a]	32.11[a]	112.67[a]	0.56
3	85.49[c]	56.27[b]	23.06[c]	81.38[c]	0.41
4	85.40[c]	54.05[c]	23.02[c]	78.99[d]	0.43
5	90.30[b]	56.81[ab]	25.75[b]	107.55[b]	0.45

Values are means of 20 measurements (10 rats and duplicate measurements per rat)

Superscripts [a, b, c, d] mean values within a column with different superscripts were significantly different ($p<0.05$).
Diet 1 (control/ basal), diet 2 (basal feed plus 0.5% cholesterol), diet 3 (25% mushroom inclusion plus 0.5% cholesterol), diet 4 (45% mushroom inclusion plus 0.5% cholesterol) and diet 5 (60% mushroom inclusion plus 0.5% cholesterol).

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