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EFFECTS OF THREE EDIBLE MUSHROOMS ON GROWTH CARACTERISTICS AND LEVEL OF SERUM PARAMETERS IN RATS

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Abstract

This work aimed to evaluate the effect of proteins of three edible mushrooms named Volvariella volvacea (VV), Termitomyces letestui (TL) and Psathyrella tuberculata (PT) of Côte d'Ivoire on the growth of rats. The results indicated that mushrooms treatments provoked lower growth, feed intake, and negative values of feed and protein efficiency, compared to those of fish diet. Mushroom-based diets consumption resulted in an increase of average weight of liver, kidneys, heart, and absence of abdominal fat. However, it did not affect the average weight of the spleen. Mushrooms did not modify the activity of ALAT, while increasing that of ALP. TL diet decreased the mean activity of ASAT; on the contrary, PT diet increased it. TL and PT diets increased the mean activity of γGT. These fungi had no effect on the mean value of most of serum metabolites and electrolytes. The negative effect of mushrooms on the growth of the rats is possibly due to their low energy content and the presence of polyphenols. Furthermore, Fungi are not palatable and digestible.

Key words : Rats, mushrooms, growth, biometry, metabolites

I. INTRODUCTION

Food resources, traditionally referred to non-timber forest products (NTFPs), including leaves, bark, broad-bush meat, caterpillars, insects, honeys and fungi. Bush meat is an exception, since its overexploitation causes protein deficiencies in some regions of Africa [1, 2]. [3, 4, 5] claimed that mushrooms are delicious food which are rich in proteins. Moreover, they possess most of the essential nutrients in great proportions. In times of food shortage or leaning, mushrooms are considered to be substitute for meat or fish, and are focus of attention. In most parts of Africa where people live, they are preferentially harvested by women and children [6]. [7] have shown that edible wild mushrooms are rich in carbohydrates, proteins, vitamins, lipids and fibers. In addition, they can be used to solve problems of malnutrition [8, 9]. In the present study, fungi served as sources of protein in diets to test the efficacy of their proteins.

II. MATERIALS AND METHODS

Animal experimentation. Growing male rats (*Rattus norvegicus*, *Muridae*, L.1753) of Wistar strain, aging between 50 and 60 days, were raised at the Laboratory of Nutrition and Pharmacology of the University Félix HOUPHOUET-BOIGNY. The experiment lasted 15 days, including three days of adaptation. The total number of rats used was 24, with 4 groups of 6 rats. They were housed in individual metabolism cages [10] with 12 hours of light and 12 hours of darkness. They received tap water *ad libitum* and were fed every day between 7 am and 8 am, and weighed every three days. The feed was weighed and served daily; the weight of the refused feed was used to determine the feed ingested. A scale (1/100 g), made in Denver (Germany), was used for different weighing (rats and feed).

Plant material. The following three fungi species *Volvariella volvacea (VV), Termitomyces letestui (TL)* and *Psathyrella tuberculata* (PT) were dried in an oven (MEMMERT, 854 Schwabach W, Germany), and then powdered, using a micromill (Culatti type MFC, Germany). A 10 µm sieve was used to purify the powder.

Dietary treatments. After weaning, the animals were fed with pellets for rabbits, manufactured by "IVOGRAIN" (Abidjan). For three days, the rats were all subjected to a unique diet, based on fish meal, in order to accustom them to experimental semi-synthetic diets. The different diets were prepared according to [11] with modifications (Table 1). A total of 4 diets were tested during this experiment. A control diet (FS) based on fish meal and three other diets containing mushroom powder (VV, TL and PT) were formulated to provide 10 % of proteins to the rats. The preparation of diets consisted of mixing the different ingredients in a "Moulinex" branded blender (France), according to the proportions mentioned in the table 1. These ingredients were then transferred into a saucepan, and after homogenization in 1 L of water, the liquid mixture was then subjected to baking, on an electric stove, marked "IKAMAG" (Germany), until it was set in bulk. This feed was placed on a plate and stored in a refrigerator (4 °C). The preparation was renewed every 4 days. The dry matter of the feed was determined on 5 g of feed sample at 104 °C for 4 hours. The effects of mushrooms consumption on growth parameters were measured (Table 2). Dry matter intake (DMI) and average weight gain (AWG) were estimated by day and by rat.

Table 1: Composition of diets

Ingrédients	**Diet treatments (1 kg of dry matter)**			
	FS	**VV**	**TL**	**PT**
Fish powder (g)	140.26			
Volvariella v. powder (g)		836.12		
Termitomyces l. powder (g)			732.60	
Psathyrella t. powder (g)				905.79
Cornstarch (g)	784.74	78.88	183.40	30
Sugar (g)	9	9	9	9
Premix (g)	1	1	1	1
Agar agar (g)	5	5	5	5
Sunflower oil (ml)	50	50	50	50
Water (ml)	1000	1000	1000	1000

FS: Control regime based on fish meal; VV: Diet based on powder of Volvariella volvacea; TL: diet based on Termitomyces letestui powder; PT: Diet based on Psathyrella tuberculata powder. Protein level in diets: 10 % ; Energy levels in diets: FS: 4072.078 kcal/kg; VV: 2122.526 kcal/kg; TL: 2426.895 kcal/kg; PT: 2164.932 kcal/kg; Premix: Premix of vitamins and minerals

Table 2 : Expression of nutritional parameters

Nutritionnal parameters	**Mathematical Expressions**
Feed intake (FI) (g)	Feed given – Feed refused
Material moisture content (MMC) %	[(Fresh Material - Dry Matter) / Fresh Material] x 100
Dry matter ratio (DM) %	100 – MMC
Dry matter intake (DMI) (g)	(FI x DM) / 21 days / 6 rats
Protein intake (PI) (g)	PI x % protein of diet
Average weight gain (AWG) (g)	(Final weight – Initial weight) / 21 days / 6 rats
Feed efficiency (FE)	AWG / DMI
Protein efficiency (PE)	AWG / PI

Sampling of organs and dosage of serum metabolites. At the end of the experiment, all animals were subjected to a 16 hours fasting. They were sacrificed after anesthesia with ethyl urethane (20 %) in the morning. The blood was collected in dry tubes. These tubes were centrifuged in a refrigerated centrifuge (4 °C), and the serum collected was used for the metabolites and electrolytes assays, with a HITACHI 902 autoanalyzer (Roche, Japan). Later on, a longitudinal

laparatomy was made on the rats, to isolate the heart, liver, kidneys, Spleen and abdominal fat for biometry. These different organs were weighed and then kept in formalin (10 %).

Expression and analysis of results. The results presented in this document are in tabular forms. Statistica version 7.1 was used for statistical analysis. The analysis of the variances (ANOVA), followed by the Newman-Keuls test (at the level of 5 %), was used respectively for the comparison of several means. The means are followed by their standard deviations. Two means are significantly different if the probability arising from the statistical tests is less than or equal to 0.05 ($P \leq 0.05$). Otherwise, these differences are not significant ($P > 0.05$). The letters a, b, c, d, e, etc., in super script, follow the means contrasts from Newman-Keuls tests in the tables. Means ± STD with different small letters within a row are significantly different ($P \leq 0.05$).

III. RESULTS

Effects of mushrooms on growth characteristics of rats. The values of the nutritional parameters are shown in Table 3. These results showed that the mushrooms proteins negatively affected the growth characteristics ($p \leq 0.05$) of rats, compared to rats fed with fish meal. Final weight, dry matter intake, protein intake of rats under mushrooms diets were lower than those of the control (fish meal) (96.66±7.84 g, 8.00±0.69 g, 0.80±0.06 g, respectively). Moreover, average weight gain, feed and protein efficiency values were negative for mushrooms diets. While those of fish diet were positive (1.33±0.27 g, 0.16±0.03 g, 1.66±0.33 g, respectively).

Table 3 : Effects of mushrooms on growth characteristics of rats

Parameters	Diet treatments			
	FS (n=6)	VV (n=6)	TL (n=6)	PT (n=6)
Initial weight (g)	76.66±4.45^{a}	76.16±4.16^{a}	75.83±4.21^{a}	75.66±4.45^{a}
Final weight (g)	96.66±7.84^{b}	64.50±6.47^{a}	67.66±3.82^{a}	67.83±5.26^{a}
DMI (g)	8.00±0.69^{b}	5.98±0.68^{a}	6.30±0.39^{a}	6.03±0,46^{a}
PI (g)	0.80±0.06^{b}	0.59±0.06^{a}	0.63±0.03^{a}	0.60±0.04^{a}
AWG (g)	1.33±0.27^{b}	-0.77±0.32^{a}	-0.54±0.18^{a}	-0.52±0.34^{a}
FE	0.16±0.03^{b}	-0.13±0.06^{a}	-0.08±0.03^{a}	-0.08±0.06^{a}
PE	1.66±0.33^{b}	-1.35±0.65^{a}	-0.87±0.33^{a}	-0.89±0.63^{a}

(n): Number of rats ; ANOVA is followed by the multiple comparison test of Newman-Keuls at the level of 5 % ; FS : fish diet; VV : Volvariella volvacea diet; TL : Termitomyces letestui diet; PT : Psathyrella tuberculata diet; DMI : dry matter intake ; PI: protein intake; AWG : average weight gain ; FE: feed efficiency ; PE: protein efficiency. Means ± STD followed by different small letters within a row are significantly different (P≤0.05).

Effects of mushrooms on organs weight. Table 4 shows the mean weight of the heart, liver, kidney, spleen and abdominal fat of the rats undergoing various treatments. Rats fed with the mushrooms diets (VV, TL and PT) had lower average weight of the heart than that of fish diet (0.45±0.01 %). The TL and PT diets provoked a greater average weight of the liver than that of fish diet (3.13±0.26 %). Rats fed with the VV and PT diets had a greater average weight of the kidneys of the rats than that of fish diet (0.84±0.04 %). The rats fed with mushroom-based diets had no abdominal fat (0.00 ± 0.00 %), while abdominal fat of rats nourished with fish meal represented 0.70±0.03 % of BW. On the contrary, Rats fed with the three mushrooms diets had no significant difference on the average weight of spleen, compared to that of fish diet (0.30± 0.09 %) ($p \geq 0.05$).

Table 4 : Effects of mushrooms on organs weight

Parameters (% of BW)	Diet treatments			
	FS (n=6)	VV (n=6)	TL (n=6)	PT (n=6)
Heart	0.45±0.01^{b}	0.39±0.03^{a}	0.40±0.02^{a}	0.38±0.04^{a}
Liver	3.13±0.26^{a}	3.43±0.18^{ab}	3.53±0.20^{b}	3.62±0.23^{b}
Kidneys	0.84±0.04^{a}	1.07±0.08^{b}	0.89±0.03^{a}	1.25±0.09^{c}
Spleen	0.30±0.09^{a}	0.27±0.05^{a}	0.29±0.04^{a}	0.31±0.06^{a}
Abdominal fat	0.70±0.03^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

(n): Number of rats ; ANOVA is followed by the multiple comparison test of Newman-Keuls at the level of 5 % ; FS : fish diet; VV : Volvariella volvacea diet; TL : Termitomyces letestui diet; PT : Psathyrella tuberculata diet; Means ± STD followed by different small letters within a row are significantly different (P≤0.05). BW: body weight

Effects of mushrooms on the level of serum metabolites. Concerning the average values of serum metabolites **(**Table 5), rats fed with fish and mushrooms diets had no significant difference ($p>0.05$). Except for the rats fed with the PT which had higher level (50.83±4.16 mg/l) of uric acid than that of fish diet (43.33±4.71 mg/l) ($p \leq 0.05$).

Table 5: Effects of mushrooms on the level of serum metabolites

Parameters	Diet treatments			
	FS (n=6)	VV (n=6)	TL (n=6)	PT (n=6)
Glucose (g/l)	0.77±0.06[a]	0.76±0.05[a]	0.79±0.07[a]	0.75±0.06[a]
Triglycerides (g/l)	1.28±0.09[a]	1.24±0.06[a]	1.29±0.07[a]	1.26±0.04[a]
Total proteins (g/l)	54.68±4.01[a]	57.58±3.45[a]	55.33±4.18[a]	55.16±4.40[a]
Total-Cholestérol (g/l)	0.87±0.06[a]	0.84±0.04[a]	0.85±0.06[a]	0.88±0.05[a]
HDL-Cholestérol (g/l)	0.38±0.03[a]	0.36±0.03[a]	0.39±0.01[a]	0.36±0.03[a]
LDL-Cholestérol (g/l)	0.26±0.04[a]	0.24±0.04[a]	0.22±0.02[a]	0.25±0.02[a]
Urea (g/l)	0.13±0.02[a]	0.13±0.02[a]	0.12±0.02[a]	0.11±0.01[a]
Creatinin (mg/l)	4.83±0.98[a]	4.83±1.16[a]	5.33±1.63[a]	5.50±1.04[a]
Uric Acid (mg/l)	43.33±4.71[a]	43.50±1.76[a]	48.33±2.06[ab]	50.83±4.16[b]
Total Bilirubin (mg/l)	4.78±0.93[a]	5.53±0.67[a]	5.08±1.22[a]	5.43±1.25[a]
Conjugated Bilirubin (mg/l)	1.63±0.44[a]	1.88±0.73[a]	1.95±0.55[a]	2.03±0.50[a]

(n): Number of rats ; ANOVA is followed by the multiple comparison test of Newman-Keuls at the level of 5 % ; FS : fish diet; VV : Volvariella volvacea diet; TL : Termitomyces letestui diet; PT : Psathyrella tuberculata diet; Means ± STD followed by different small letters within a row are significantly different (P≤0.05).

Effects of mushrooms on the activity of serum enzymes. In Table 6, the mean activities of the enzymes, in particular alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and gamma glutamine transpeptidase (γGT) are reported. The value of the ALP mean activity is higher in all rats fed with mushrooms diets than that of fish (80.33±3.01 IU/l) ($p \leq 0.05$). The rats fed with the TL diet had a lower mean activity of ASAT, while the PT diet had a greater mean activity of ASAT than that of fish diet (46.16±5.45 IU/l) ($p \leq 0.05$). The mean activity of γGT was greater in the rats fed with the TL and PT diets ($p \leq 0.05$) than that of fish diet (26.33±4.96 IU/l). The mean activity of ALAT was not significantly different ($p \geq 0.05$) in both groups of rats consuming fish (43.00±3.40 IU/l) and mushrooms.

Table 6 : Effects of mushrooms on the activity of serum enzymes

Paramètres (IU/l)	Diet treatments			
	FS (n=6)	VV (n=6)	TL (n=6)	PT (n=6)
ALP	80.33±3.01[a]	85.16±3.65[b]	88.33±4.41[b]	84.00±8.04[b]
ASAT	46.16±5.45[b]	48.16±5.45[b]	42.33±5.88[a]	55.16±5.07[c]
ALAT	43.00±3.40[a]	49.00±6.87[a]	48.66±3.26[a]	47.50±2.33[a]
γGT	26.33±4.96[ab]	24.66±3.55[a]	29.00±4.47[b]	29.66±5.16[b]

(n): Number of rats ; ANOVA is followed by the multiple comparison test of Newman-Keuls at the level 5 % ; FS : fish diet; VV : Volvariella volvacea diet; TL : Termitomyces letestui diet; PT : Psathyrella tuberculata diet; Means ± STD followed by different small letters within a row are significantly different (P≤0.05).

Effects of mushrooms on the level of serum electrolytes. The average level of the serum electrolytes are shown in Table 7. The rats fed with fish and mushrooms diets had no significant difference ($p > 0.05$) for the average value of P^{5+}, Ca^{2+}, Fe^{2+}, Ca^{2+}/P^{5+}. The mushrooms diets had a lower ($p \leq 0.05$) average value of Mg^{2+} than that of fish diet (17.88±1.11 mg/l). The TL and PT had lower ($p \leq 0.05$) average value of Na^{+} than that of fish diet (136.16±3.06 mEq/l). Rats fed with TL had lower average value of K^{+}, while the rats fed with PT had greater average value of K^{+} ($p \leq 0.05$) than that of fish diet (3.13±0.39 mEq/l).

Table 7: Effects of mushrooms on the level of serum electrolytes

Parameters	Diet treatments			
	FS (n=6)	VV (n=6)	TL (n=6)	PT (n=6)
P^{5+} (mg/l)	34.33±3.55^{a}	38.00±2.44^{a}	36.66±4.17^{a}	35.66±2.94^{a}
Ca^{2+} (mg/l)	78.16±2.85^{a}	78.16±2.85^{a}	80.66±2.06^{a}	80.16±2.04^{a}
Mg^{2+} (mg/l)	17.88±1.11^{b}	16.25±0.62^{a}	16.88±0.95^{ab}	16.95±0.38^{ab}
Fe^{2+} (mg/l)	3.23±0.50^{a}	3.12±0.46^{a}	3.20±0.24^{a}	2.93±0.36^{a}
Na^{+} (mEq/l)	136.16±3.06^{b}	134.00±3.40^{ab}	130.66±1.36^{a}	132.50±1.37^{a}
K^{+} (mEq/l)	3.13±0.39^{bc}	2.73±0.36^{ab}	2.49±0.54^{a}	3.31±0.18^{c}
Ca^{2+}/P^{5+}	2.29±0.21^{a}	2.06±0.13^{a}	2.22±0.24^{a}	2.26±0.19^{a}

(n): Number of rats ; ANOVA is followed by the multiple comparison test of Newman-Keuls at the level 5 % ; FS : fish diet; VV : Volvariella volvacea diet; TL : Termitomyces letestui diet; PT : Psathyrella tuberculata diet; Means ± STD followed by different small letters within a row are significantly different (P≤0.05).

IV. DISCUSSION

The body weight of rats fed with mushroom diets decreased all along the 21 days of experiment. The dry matter ingested of these rats was lower than that of rats consuming fish diet. This discrepancy could be due to the lack of palatability of the mushroom-based diets. The ingested protein evolved in the same direction as the dry matter ingested. Values of feed efficiency and protein efficiency of mushrooms fed rats were negative. Therefore, it is obvious that the proteins of fungi can not support growth, which resulted in a slimming of rats that consumed mushroom diets.

Tannins are known to impede animals and humans growth by reducing digestion and/or absorption of amino acids and minerals [12, 13]. They can be lethal in a few days when the food has a tannin concentration greater than 2 %. Mushrooms are good sources of several polyphenol oxidases (PPO) and phenolic compounds [5]. Polyphenols are oxidized to quinones by polyphenol oxidases. These quinones can complex the proteins. This leads to lower amino acids level, especially available lysine [14, 15]. The ingested dry matter of the fungi is lower. This is a proof of a lack of palatability that has a negative effect on the growth of rats, with poor quality of the proteins of the fungi tested [16, 17]. The need of rats in sulfur-containing amino acids, in particular methionine and cysteine, are higher than that of men [18], and any growth experiment with rats may therefore underestimate the quality of proteins, especially amino acids, as a limiting factor [17, 18]. In addition, high requirement for sulfur amino acids induce high requirement for amino acids such as histidine, isoleucine, threonine and valine in rats [18, 19].

When the calorie requirement is not satisfied by the diet, the effectiveness of the use of proteins is reduced. There is a critical level for energy intake below which the nutritional value of the protein consumed decreases. Consequently, in an undernourished subject, an increase in the protein intake will only be fully effective if the diet provides the necessary amount of energy. This means that insufficient calorie intake itself is a cause of protein wastage and thus tends to increase the insufficiency [20]. The average weight of the heart was decreased, with abdominal fat totally eliminated in rats which consumed the mushrooms. Hypertrophy was observed in mean liver weight of rats for *Termitomyces letestui* (TL) and *Psathyrella tuberculata* (PT) diets. The *Volvariella volvacea* (VV) and *Psathyrella tuberculata* (PT) diets provoked hypertrophy of the kidneys. On the other hand, the average weight of the spleen of the rats which consumed the fungi was not affected. This loss of the heart weight is due to a decline in cardiac activity; and the absence of abdominal fat in rats which consumed the fungi is due to deficit of energy intake and low dietary intake. The increase in liver weight would be the consequence of hepatic hyperactivity. This hyperactivity would be caused by the anti nutritional compounds of fungi. The absence of variation in the weight of the spleen which is a lymphoid organ shows that fungi do not have deleterious effects on the immune response of the body.

The effects of mushroom consumption were very low on the metabolites of rats. However, consumption of the PT diet provoked a significant increase in the mean value of uric acids. This increase is due to a catabolism of purine bases or nucleic acids. This disturbance would also reflect a renal failure in these rats [21]. In fact, these authors found that consumption of enriched protein diets with chromium tripicolinate resulted in an increased uric acid level in the rat serum. Mushrooms consumption had no effect on the mean value of the other metabolites (glucose, triglycerides, total proteins, total cholesterol, HDL-cholesterol, LDL-cholesterol, urea, creatinin, total bilirubin and conjugated).

The consumption of mushrooms diets provoked a decrease in magnesium level for the *Volvariella volvacea* VV diet, sodium and potassium for the PT and TL diets, then an increase of potassium level in the PT diet. Consumption of fungi does not affect serum concentrations of P^{5+}, Ca^{2+}, Fe^{2+}, and Ca^{2+}/P^{5+} ratio, which is about 2. The ratio of calcium to serum phosphorus is an indicator of phosphocalcic metabolism. This decrease in level of serum magnesium, sodium and potassium is due to the presence of oxalates and phytates in the fungi that would have chelated the magnesium, making it unavailable in the intestine. The decline of magnesium concentration would also be caused by the high presence of fibers in fungi that would prevent the bioavailability of sodium and potassium, which would largely pass into feces [22, 23, 24].

V. CONCLUSION

From this study, it's concluded that mushrooms consumption provoked low feed ingestion and consequently low growth, since fungi are not palatable and digestible enough. The results indicated that edible mushroom proteins can not support the growth of rats. So, the popular belief that states that mushrooms proteins can substitute meat proteins is not valid. Mushroom-based diets may have possible negative effects on heart, kidneys and liver function. The absence of abdominal fat suggests that mushrooms can be proposed as dietetic regime for obese people. However, no serious affection of these edible compounds was noted on blood parameters.

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