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Effects of Incorporation of Psathyrella Tuberculata Mushroom in a Herring Fish Powder Diet on Growth Characteristics, Organ Biometrics and Serum Parameters in Rats

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Abstract Original Research Article

The aim of this study is to determine the nutritional and physiological effects of the remplacement of fish protein by the proteins of wild edible mushroom Psathyrella tuberculata. The results showed stunting in the rats consuming diets with a 20 % fish replacement. This rate of incorporation has declined food efficiency (FE) and protein efficiency (PE). Also, when the rate of incorporation of *Psathyrella tuberculata* increases in the diet, there is a decrease in the accumulation of abdominal fat. These different levels of incorporation of *Psathyrella tuberculata* did not affect the serum metabolites, enzymes and electrolytes in rats. These results clearly show that the proteins contained in this type of fungus do not promote growth and that their consumption has slimming effects.

Keywords: Incorporation, diet, nutritional and physiological effects, rats, growth delay, serum.

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INTRODUCTION

Non-timber forest products such as mushrooms still contribute today to poverty reduction and food security for populations in forest regions (Moupela, 2011). Indeed, even if their exploitation is done on an artisanal scale, non-timber forest products have an influence on the life or survival of rural populations. They play an important role in dietary balance, the conservation of cultural identity and health. Their marketing on national and international markets provides income (Awono, 2008). Mushrooms have a nutritional value very close to vegetables and meat. They are considered essential for a healthy and balanced diet, therefore playing a fundamental role in human survival (De Kessel et al., 2001; Gnakri (1988). Johnsy et al., (2011) showed that some wild edible mushrooms are rich in carbohydrates, proteins, vitamins, minerals, lipids and fiber. Therefore, they could help solve the malnutrition problem in the world (Baros et al., 2008). Wild edible mushrooms and meat could have similar nutritional

values and are increasingly considered essential for good health and a balanced diet (Courtecuisse and Duhem, 1994; De Kessel et al., 2001). However, Zoho Bi et al., (2018) showed that substituting proteins from herring fish with proteins from the fungus Volvariella volvacea resulted in a Detrimental to growth in rats at substitution rates of 25, 50, 75, and 100%. This study was conducted to determine the performance threshold for protein substitution of fish proteins with proteins from the fungus Psathyrella tuberculata.

I. MATERIALS AND METHODS

Animal

The experiment was carried out on growing male rats (Rattus norvegicus, Muridae, L. 1753) of Wistar strain whose weight is 50±4.31 g. The rats come from the animal facility of the Pharmacology Laboratory, UFR of Pharmaceutical and Biological Sciences of the Félix HOUPHOUËT-BOIGNY University. Four days before the experiment, the rats are subjected to a single

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Biology

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diet in order to accustom them to the experimental diets. In iron cages covered with grid, in groups of five (5), the animals are housed during the growth experiment. The food is placed on the grid covers. The duration of the experiment is 15 days. Every morning, around 8 a.m. and 9 a.m., the rats are fed and the water in the bottles is renewed. The food is weighed and placed on the grid (cage cover). The following day, the remains are collected from the cage lids and then weighed to determine the quantity of food ingested (Bouafou *et al.*, 2007).

Plant material

The fungus *Psathyrella tuberculata* was ovendried (MEMMERT, 854 Schwabach W, Germany), then ground into powder using a microgrinder (Culatti type MFC, Germany) equipped with a 10 μ m mesh sieve and stored in a refrigerator at 4°C.

DIETARY TREATMENT

Isoprotein diets are prepared according to Garcin *et al.*, (1984), seen in Table I. A total of five (5) diets are formulated. A control diet (F) based on Herring

fish powder. Four other diets, which result from a gradual incorporation of mushroom powder into the fishbased diet. This incorporation is done on the basis of a replacement of fish powder by mushroom powder. A level of fish protein is removed, then this is replaced by mushroom protein. During this experiment, 5 diets were formulated (Table I): a control diet (F) containing 100% fish protein intake and four diets comprising 5%, 10%, 15% and 20% protein intake from Psathyrella tuberculata powder. Diet preparation involves mixing the various ingredients in a Moulinex blender (France), while referring to the quantities mentioned in Table I. The mixture is then transferred to a saucepan, homogenized in 1 L of water, and cooked on a maxi electric stove, brand IKAMAG (Germany), until it takes shape. The food is placed on plates and stored in the refrigerator at 4°C. The diet preparation is repeated after four (4) days. Approximately five (5) g of each food item are taken in duplicate and placed in an oven (MMM Medcenter Gmbh, D-82152, Germany) at 105°C for 4 hours. After weighing, the dry matter content is calculated according to the formula mentioned in Table

Table I: Composition of the different diets

Table 1: Composition of the uniterent diets						
Ingrédients	Diet treatments (1 kg of dry matter)					
	F	5%	10%	15%	20%	
Fish powder (g)	140.26	133.25	126.23	119.23	112.21	
Psathyrella tuberculata(g)	0	45.29	90.58	135.87	181.16	
Cornstarch (g)	784.74	746.46	708.19	669.9	631.63	
Sugar (g)	69	69	69	69	69	
Prémix (g)	1	1	1	1	1	
Agar-agar (g)	5	5	5	5	5	
Sunflower oil (ml)	50	50	50	50	50	
Water (ml)	1000	1000	1000	1000	1000	

Source: Garcin et al., (1984)

Proteins level of diet: 10,00 %; Energy level in diet F: 4468,078 kcal/kg; Energy level in diet 5 %: 4370,562 kcal/kg; Energy level in diet 10 %: 4273,044 kcal/kg; Energy level in diet 15 %: 4175,532 kcal/kg; Energy level in diet 20 %: 4078,013 kcal/kg; F: Fish diet; 5 %, 10 %, 15 %, 20 %: Different inclusion rates of *Psathyrella tuberculata* proteins.

Table II: Expressions of nutritional parameters

Nutritional parameters	Mathematical Expressions
Feed intake (FI)	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 per day (DMI) g	[(FI x DM) / 15 days] / 5
Protein Intake (PI)	DMI x % protein of diet
Average Weight Gain (AWG) g	Final Weight – Initial Weight
Average Weight Gain per day (AWG/d) g	AWG / 15 days
Feed Efficiency (FE)	AWG / DMI
Protein Efficiency (PE)	AWG / PI

BLOOD AND ORGAN SAMPLE COLLECTION

At the end of the experiment, precisely on day 16, the animals were fasted for 16 hours (between 4 p.m. and 8 a.m.). The following day, between 9 a.m. and 11 a.m., the rats were sacrificed by decapitation after ethyl urethane anesthesia (Amoikon *et al.*, 2012). Approximately 5 ml of blood was collected from each rat

during decapitation and placed in "GMT Vacuum, 4 ml" tubes, without anticoagulant. The blood tubes were then centrifuged at 3000 rpm for 10 min in a refrigerated centrifuge (Alresa Orto, Spain) at 4 °C (Bouafou *et al.*, 2007; Amoikon *et al.*, 2010). The serum from each tube is then collected and placed in hemolysis tubes to be

stored in the refrigerator (0°C), pending the determination of various biochemical parameters.

After sacrifice, the rats' blood is collected directly using a tube, and a longitudinal laparotomy is performed to isolate the heart, liver, kidneys, spleen, and abdominal fat. The organs are weighed and then stored in dishes containing formalin diluted 10-fold. The organ weights and their weights are converted to the percentage of body weight. Serum metabolic assays (glucose, triglycerides, total protein, total cholesterol, HDL cholesterol, LDL cholesterol, urea, creatinin, uric acid, conjugated bilirubin, ALP, ASAT, ALAT, yGT, phosphorus. calcium, magnesium, sodium, and potassium) were performed on serum samples using a HITACHI 902 autoanalyzer (Roche, Japan) in the Biochemistry Laboratory of Treichville University Hospital, Abidjan.

STATISTICAL PROCESSING AND ANALYSIS OF RESULTS

The results presented in this document are in tabular form. Statistica version 7.1 software was used for statistical analyses. Analysis of variance (ANOVA) followed by the Newman-Keuls test (at the 5% threshold) was used for the multiple comparison test of means. The Newman-Keuls test was used to classify means. Standard deviations always follow the means. Two means are significantly different if the probability

resulting from these statistical tests is less than or equal to 0.05 ($P \le 0.05$); otherwise, there are no significant differences (P > 0.05). The letters a, b, c, d, e, etc. in superscript follow the means resulting from the Newman-Keuls test in the tables. Means followed by different letters in the same row or column are significantly different.

II. RESULTS

Effects of the Gradual Incorporation of *Psathyrella* tuberculata Powder on Growth Characteristics

The evolution of the rats' body weights is illustrated in Figure 1. This figure shows a growth delay in rats fed the diet with 20% Psathyrella tuberculata mushroom incorporation compared to rats fed the fish powder diet and rats fed the 5%, 10%, and 15% diets. The mean values of the growth characteristics of rats are mentioned in Table III. This table shows that the final weight of rats fed with 20% diet is lower than the weight of rats fed with other diets. The dry matter and protein intake rate is higher in rats fed with 20% mushroom incorporation diet compared to the relevant values of rats fed with other diets. However, rats fed with 20% incorporation diet, body weight gain (BWG), feed efficiency (FE) and protein efficiency (PE), are respectively lower ($p \le 0.05$) than those of rats fed with fish powder diet.

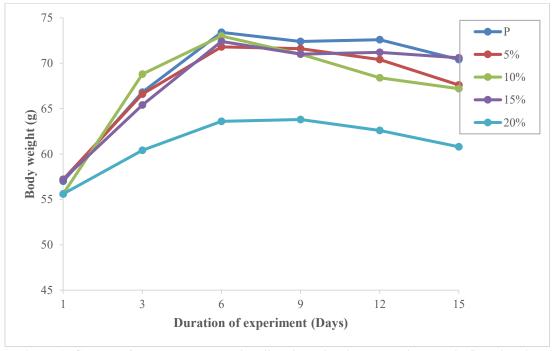


Figure 1: Growth of rats based on Psathyrella tuberculata incorporation rate in fish diet diet

(n=5): Number of rats per treatment. Analysis of variance followed by the Newman-Keuls multiple comparison test at the 5% threshold. F: fish powder diet; 5%, 10%, 15%, 20%: different incorporation rates of *Psathyrella tuberculata* protein.

Table III: Mean values of rat growth characteristics as a function of the incorporation rate of *Psathyrella tuberculata* in the control diet

control dict							
	Diets (1 kg of dry matter)						
Parameters	F	5 %	10 %	15 %	20 %		
	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)		
Initial weight (g)	57.00 ± 5.70^{a}	57.20 ± 3.63^{a}	55.60 ± 3.20^{a}	57.20 ± 3.63^{a}	55.60 ± 6.22^{a}		
Final weight (g)	70.40 ± 6.91^{a}	67.60 ± 5.85^{a}	67.20 ± 2.77^{a}	70.60 ± 9.07^{a}	60.80 ± 6.45^{a}		
DMI (g)	7.24	7.32	7.56	7.79	8.48		
PI (g)	0.72	0.73	0.75	0.77	0.84		
BWG (g)	0.89 ± 0.10^a	0.69 ± 0.29^a	0.77 ± 0.19^{a}	0.89 ± 0.61^a	0.34 ± 0.12^a		
FE	0.12 ± 0.01^{b}	0.09 ± 0.04^{ab}	0.10 ± 0.02^{ab}	0.11 ± 0.07^{ab}	0.04 ± 0.01^a		
PE	1.23 ± 0.13^{b}	0.94 ± 0.40^{ab}	1.02 ± 0.25^{ab}	1.14 ± 0.79^{ab}	0.40 ± 0.15^{a}		

(n): Number of rats per treatment. Analysis of variances followed by the Newman-Keuls multiple comparison test at the 5% threshold. On the same line, means followed by letters a, b, c, d, e, etc. in super different are significantly different ($p \le 0.05$). F: fish powder diet; 5%, 10%, 15%, 20%: different rates of incorporation of *Psathyrella tuberculata* proteins.; DMI: Dry Matter Ingested per day per rat; PI: protein ingested per day per rat; BWG: Body Weight Gain per day per rat; FE: Feed Efficiency; PE: protein efficiency

Effects of gradual intake of *Psathyrella tuberculata* powder on organ biometry

The results reveal that the mean weights (hearts, livers, kidneys, and spleens) of rats fed the five diets (P,

5%, 10%, 15%, 20%) are not significantly different (p > 0.05) (Table IV). However, the mean abdominal fat weight of rats decreases significantly (p \leq 0.05) as the mushroom incorporation rate increases in the rats' diets.

Table IV: Mean organ weights

Parameters	Diets (1 kg of dry matter)					
	F 5 %		10 %	15 %	20 %	
	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	
Heart	0.52 ± 0.06^{a}	0.53±0.04a	0.51±0.03 ^a	0.49 ± 0.04^{a}	0.53 ± 0.06^{a}	
Liver	3.05±0.62a	3.53±0.27 ^a	3.34±0.29a	3.40±0.22a	3.39±0.19a	
Kidneys	0.79 ± 0.07^{a}	0.78 ± 0.08^{a}	0.81±0.08 ^a	0.83 ± 0.07^{a}	0.83 ± 0.06^{a}	
Spleen	0.23±0.02a	0.25±0.04a	0.23±0.03a	0.27 ± 0.06^{a}	0.24±0.03a	
Abdominal fat	$0,16\pm0,04^{c}$	0,15±0,11°	$0,11\pm0,05^{b}$	$0,09\pm0,16^{b}$	$0,05\pm0,05^{a}$	

(n=5): Number of rats per treatment. Analysis of variances followed by the Newman-Keuls multiple comparison test at the 5% threshold. On the same line, the means followed by letters a, b, c, d, e, etc. in super different are significantly different ($p \le 0.05$). F: fish-based diet; 5%, 10%, 15%, 20%: different rates of incorporation of *Psathyrella tuberculata* proteins.

Effects of gradual intake of *Psathyrella tuberculata* powder on mean serum metabolite values

Observing the results reported in Table V confirms that rats consuming diets with 5 and 20% Psathyrella tuberculata powder incorporation have a mean blood glucose value that is higher (p \leq 0.05) than rats consuming the fish powder diet. Diets with 15 and 20% Psathyrella tuberculata powder incorporation lead

to a decrease (p \leq 0.05) in the mean triglyceride concentration in rats compared to those fed the fish powder diet. The differences observed between the mean values of other metabolites (glucose, triglycerides, total protein, total cholesterol, HDL cholesterol, LDL cholesterol, urea, creatinine, total bilirubin, and conjugated bilirubin) were not statistically significant (p < 0.05).

Table V: Effects of Psathyrella tuberculata on the mean values of serum metabolites in rats

Parameters	Diets (1 kg of dry matter)					
	P	5 %	10 %	15 %	20 %	
	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	
Glucose (g/L)	0.54±0.03a	0.67 ± 0.09^{b}	0.63 ± 0.07^{ab}	0.63 ± 0.05^{ab}	0.71 ± 0.04^{b}	
Triglycérides (g/L)	1.96±0.58 ^b	1.51±0.35ab	1.38±0.24ab	0.92 ± 0.3^{a}	1.16±0.3a	
Total Protein (g/L)	52.4±2.88 ^a	55.80±4.86a	51.00±5.43a	51.80±2.58a	54.20±2.68 ^a	
Total-Cholestérol (g/L)	1.04 ±0.13 ^b	0.96 ± 0.23^{b}	0.97 ± 0.17^{b}	0.61±0.21a	0.85±0.13 ^b	
HDL-Cholestérol (g/L)	0.25±0.03a	0.25±0.04a	0.27 ± 0.02^{a}	0.19 ± 0.05^{a}	0.22 ± 0.02^{a}	
LDL-Cholestérol (g/L)	0.39±0.13 ^a	0.39±0.26 ^a	0.42 ± 0.22^{a}	0.22 ± 0.09^{a}	0.39±0.11a	
Uréa (g/L)	0.31±0.05 ^a	0.34±0.07 ^a	$0.33{\pm}0.05^a$	0.31 ± 0.06^{a}	0.36±0.03a	
Creatinin (mg/L)	11.80±2.04a	11.60±2.40a	12.40±2.30 ^a	11.20±1.92a	13.00±2.34a	
Uric Acid (mg/L)	8.20±1.30 ^a	11.20±1.48 ^a	10.8±2.38 ^a	9.20±2.58 ^a	8.4±0.54 ^a	
Total Bilirubin (mg/L)	2.80±0.83a	3.40±1.14 ^a	3.80±1.30 ^a	3.20±0.83a	3.20±0.83a	
Conjugated Bilirubin (mg/L)	1.24±0.25a	1.24±0.56a	1.48±0.64a	1.66±0.25a	1.15±0.36 ^a	

(n): Number of rats per treatment. Analysis of variance followed by the Newman-Keuls multiple comparison test at the 5% threshold. On the same line, means followed by letters a, b, c, d, e, etc. in different superscript are significantly different ($p \le 0.05$). F: fish powder diet; 5%, 10%, 15%, 20%: different rates of *Psathyrella tuberculata* protein incorporation.;

Effects of gradual intake of *Psathyrella tuberculata* powder on mean serum enzyme activity in rats

The mean activities of the enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST),

alanine aminotransferase (ALT), and gamma glutamyltransferase (γ GT) in rats consuming the diets were not significantly different (p < 0.05) (Table VI).

Table VI: Effects of Psathyrella tuberculata on mean serum enzyme values in rats

Parameters	Diets (1 kg of dry matter)				
(UI/L)	F	5 %	10 %	15 %	20 %
	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
ALP	257.00±35.06a	246.60±15.66a	259.40±40.51a	275.40±66.64a	322.20±119.86a
ASAT	119.40±66.31a	99.00±39.92a	140.80±62.31a	203.00 ± 19.84^a	146.00±78.21a
ALAT	58.40±7.98 ^a	55.00±10.65a	63.20±8.04a	77.40±22.60 ^a	73.40±27.07 ^a
γGT	64.80±23.72a	75.20±12.29a	64.20±15.67a	68.60±12.77a	58.40±11.65a

(n): Number of rats per treatment. Analysis of variance followed by the Newman-Keuls multiple comparison test at the 5% threshold. On the same line, means followed by letters a, b, c, d, e, etc. in different super script are significantly different ($p \le 0.05$). F: fish powder diet; 5%, 10%, 15%, 20%: different rates of *Psathyrella tuberculata* protein incorporation.; ALP: Alkaline phosphatase; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; γ GT: gamma glutamine transferase

Effects of Gradual intake of *Psathyrella tuberculata* Powder on Mean Electrolyte Values in Rats

Mean electrolyte values are shown in Table VII. Different rates of mushroom incorporation into the fish

powder diet did not affect (p > 0.05) the different mean serum electrolyte values in rats.

Table VII: Effects of *Psathyrella tuberculata* on mean electrolyte values in rats

Parameters	Diets (1 kg of dry matter)					
	F	5 %	10 %	15 %	20 %	
	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	
P^{5+} (mg/L)	58.60±9.88a	53.40±5.12 ^a	59.40±8.67 ^a	53.40±8.08 ^a	54.20±4.54 ^a	
Ca ²⁺ (mg/L)	102.00±4.18 ^a	100.20±3.27 ^a	98.00±2.54a	98.40±4.33a	98.40±5.63 ^a	
Mg^{2+} (mg/L)	26.40±3.50 ^a	22.20±2.58 ^a	22.20±1.92a	22.40±2.07 ^a	22.20±3.27 ^a	
$Fe^{2+}(mg/L)$	1.48±0.07 ^a	1.60±0.17 ^a	1.58±0.22 ^a	1.84 ± 0.27^{a}	2.04±0.62 ^a	
Na^{2+} (mEq/L)	145.40±5.41a	143.20±3.70 ^a	150.80±6.01a	146.60±4.15a	149.40±6.76a	
K^+ (mEq/L)	4.92±0.81a	4.14±0.41a	4.82±0.43a	4.64 ± 0.50^{a}	4.96±0.33a	
Ca ²⁺ / P ⁵⁺	1.74±0.42a	1.87±0.63a	1.64±0.29a	1.84±0.53a	1.81±1.01a	

(n): Number of rats per treatment. Analysis of variance followed by the Newman-Keuls multiple comparison test at the 5% threshold. On the same line, means followed by letters a, b, c, d, e, etc. in superscript are significantly different ($p \le 0.05$). F: fish powder diet; 5%, 10%, 15%, 20%: different rates of *Psathyrella tuberculata* protein incorporation.

III. DISCUSSION

At the end of the 15-day experiment, rats fed the diet with 20% Psathyrella tuberculata mushroom powder incorporation showed delayed growth compared to those fed the fish diet. In this experiment, growth characteristics such as final weight, dry matter intake (DMI), protein intake (PI), body weight gain (BWG), feed efficiency (FE), and protein efficiency ratio (PE) were significantly different between rats fed fish-based diet (F) and rats fed mushroom-based diets ($p \le 0.05$). These growth characteristics are comparable to those found by Dabbour and Takruri (2002) who worked on four species of fungi proving that the proteins contained in mushrooms could not promote the growth of rats. Similarly, these results are similar to those of Zoho Bi et al., (2017) who worked on the fungi Psathyrella tuberculata, Volvariella volvacea and Termitomyces letestui. The growth retardation caused by the consumption of a diet containing 20% Psathyrella tuberculata mushroom is thought to be due to the high concentration of phenolic compounds. Tannins are known to retard growth by reducing the digestion and/or absorption of amino acids and minerals.

This decline manifests as a growth retardation due to the ineffectiveness of the proteins contained in this mushroom species to replace fish protein. Given this insufficient energy intake, the increasing supply of tannins from the mushroom appears to have inhibited the effectiveness of the proteins (OMS, 1965). Gradually increasing the amount of mushroom in the rats' diets leads to an increase in the tannin and polyphenol content in the diets. These are known to retard growth by reducing the digestion and/or absorption of amino acids and minerals (Miller, 1963; Hurell and Fiont, 1982; Laurena *et al.*, 1984; Da Damio and Thompson, 1992). Mushrooms are plants rich in polyphenol oxidases and phenolic compounds. Polyphenols are oxidized to quinones by polyphenol oxidases. These quinones can

complex proteins, leading to a decrease in available amino acids, particularly lysine (Hurell and Fiont, 1982; Da Damio and Thompson, 1992).

The results revealed a significant decrease in the accumulation of mean abdominal fat weight in rats as the rate of mushroom insertion in the diet increased. This decrease in abdominal fat is thought to be due to poor absorption of glucose and lipids caused by fungi in the intestine. These results are similar to those found by Zoho Bi *et al.*, (2018), who showed that consumption of diets with 75% and 100% mushroom powder in the diets caused a decrease and/or absence of abdominal fat accumulation.

IV. CONCLUSION

The gradual replacement of fish protein with Psathyrella tuberculata mushroom protein results in growth retardation in rats when the replacement rate is greater than 15%. The results reveal that the proteins in mushroom-based diets cannot ensure growth; however, mushroom proteins cannot replace those in fish. Average kidney weight increases as the level of fungi in the diets increases. The average weight of abdominal fat accumulation decreases as the level of mushroom incorporation increases. Diets incorporating 15 and 20% Psathyrella tuberculata mushroom powder into the diet lowered triglyceridemia. The proteins contained in the Psathyrella tuberculata mushroom did not affect mean serum electrolyte and enzyme concentrations. Mushroom proteins cannot replace those contained in fish, and the replacement efficiency rate must be less than 15% in the diet.

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