Nutritional and Physiological Effects of Gradual Fish Replacement by *Volvariella volvacea* Powder in Growing Rats

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Abstract— *This work aimed to evaluate the effect of substitution of fish proteins by powder of Volvariella volvacea, an edible mushroom of Côte d'Ivoire, in the growing rats. The gradual substitution of fish proteins by mushroom proteins leads to a decrease in the growth performance of rats. With 75 % and 100 % mushroom in diets, body weight gain, food efficiency and protein efficiency ratio are negative. These diets do not affect the average weight of some organs (heart, liver and spleen), except for the kidney average weight that increases as mushroom levels increase in diets. Likewise, the average weight of abdominal fat decreases and disappears as the mushroom incorporation rate increases. The incorporation of mushroom powder into the control diet provokes changes in the mean value of some serum metabolites and electrolytes. The popular belief that states that mushrooms proteins can substitute meat proteins is not valid. Mushrooms can be proposed as dietetic regime for obese people.*

Keywords— ***Rats, Volvariella volvacea, growth, metabolites.***

I. INTRODUCTION

Mushrooms are part of Non-Timber Forest Products (NTFPs). They have long time played an important role in the survival of both rural and urban populations in Africa [1]. Furthermore, edible mushroom are rich in nutrients such as proteins [2] with a good proportion of essential amino acids and minerals [3, 4, 5]. They can be consumed in addition to cereals to meet the protein needs of poor populations [5]. [6] have argued that *Agrocybe chaxingu* may be a food source for protein enrichment and can therefore be classified as high protein foods for both humans and livestock. [7] stated that edible mushroom proteins are affordable and less expensive and can be consumed as a supplement or alternative to fish or meat. This work is carried out in order to study the performance of fish proteins replacement by the proteins of *Volvariella volvacea*, a widely known edible mushroom in Côte d'Ivoire.

II. MATERIAL AND METHODS

2.1 Animals

Growing male rats (*Rattus norvegicus*, *Muridae*, L.1753) of Wistar strain, aging between 50 and 60 days, weighing between 60 g and 70 g , 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 animals used was 24 at the rate of 6 rats per group. They were housed in individual metabolism cages [8] with 12 hours of light and 12 hours of darkness. Animals received tap water *ad libitum*, and were fed every day, between 7 am and 8 am, and weighed every three days. The feed were weighed and served daily; the weight of the refused feed was used to determine the feed ingested. A scale from Denver (Germany), was used to determine weight of rats and feed (rats and feed).

2.2 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 [9], with modifications (Table I). A total of 5 diets were tested during this experiment. A control diet (F) based on fish meal and four other diets containing different rates of *Volvariella volvacea* powder (25 %, 50 %, 75 % and 100 %) were formulated to provide 10 % of proteins to the rats. The preparation of diets consisted of mixing the different ingredients in a "Moulinex" brande 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 growth parameters were measured according to mathematical formulas (Table II). 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)				
	F	25 %	50 %	75 %	100 %
Fish powder (g)	140.26	105.20	70.13	35.07	0
Volvariella volvacea powder (g)	0	209.03	418.06	627.09	836.12
Cornstarch (g)	784.74	620.77	446.81	281.84	78.88
Sugar (g)	9	9	9	9	9
Premix (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

*Protein level in diets: 10.00%; 25%, 50%, 75%, 100%: different protein inclusion rates of **Volvariella volvacea**; Energy F: 4072.078 kcal / kg; Energy 25%: 3790.6915 kcal / kg; Energy 50%: 3313.30199 kcal / kg; Energy 75%: 2871.91549 kcal / kg; Energy 100%: 2122.526 kcal / kg ; F: fish-based diet;*

Source: [9] Garcin et al. (1984).

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
Protein intake (PI) g	DMI x % protein diet gold
Average weight gain (AWG) (g)	Final weight – Initial weight
Feed efficiency (FE)	AWG / DMI
Protein efficiency (PE)	AWG / PI

2.3 Sampling of organs and dosage of serum metabolites

At the end of the experiment, all animals were subjected to a 16 hours fasting. Then, they were sacrificed after anesthesia with ethyl urethane (20 %), the next day, in the morning. Blood was collected in dry tubes. These tubes were centrifuged in a refrigerated centrifuge (4 °C). 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 heart, liver, kidneys, Spleen and abdominal fat for biometry.

2.4 Expression and statistical analysis of results

The results presented here are in tabular forms and figures. 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

3.1 Effects of *V. volvacea* on growth characteristics of rats

The evolution of rat growth in this experiment is illustrated in Figure 1. It's shown that, as the rate of incorporation of mushroom increased (25 %, 50 %, 75 % and 100 %), the growth of mushroom-fed rats decreased, compared to that of fish-fed rats (diet F). The average values of the growth characteristics of the rats are shown in Table III. As the mushroom incorporation rate in the control diet increased (25 %, 50 %, 75 %, and 100 %), final weight, ingested dry matter (IDM), ingested protein values (IP), body weight gain (BWG) decreased in rats fed on these diets. The food efficiency (FE) and the protein efficiency ratio (PE) decrease proportionally. At 75 % of the mushroom incorporation rate, the FE and PE values were negative. Similarly, for 75 % of incorporation the average values of FE and PE were negative. For each nutritional characteristic, there were significant differences ($p \leq 0.05$) between the rats subjected to the five diets.

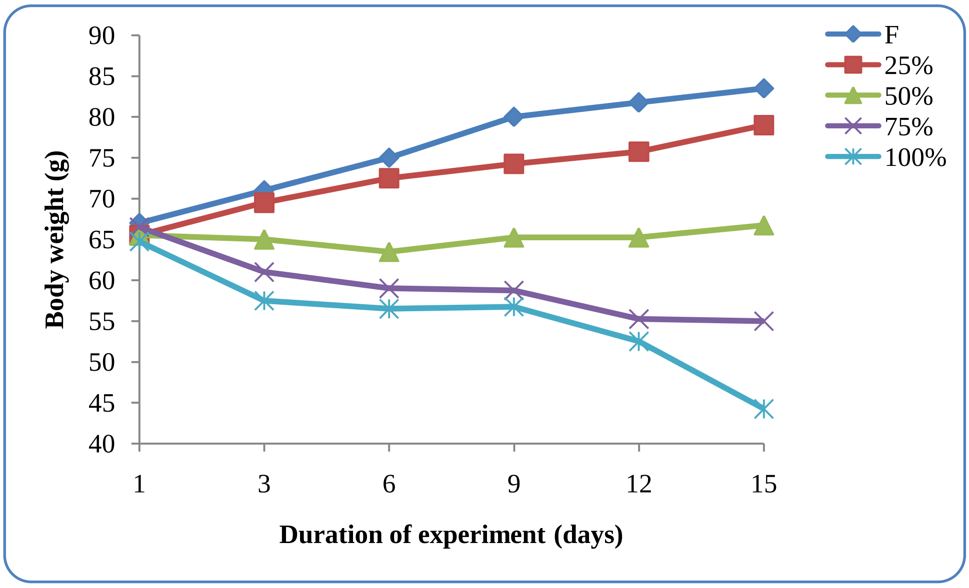

FIGURE 1: Growth of rats based on *V. volvacea* incorporation rate in the control diet

*n = 6): Number of rats per treatment. ANOVA followed by the Newman-Keuls multiple comparison test at the 5 %. F: fish-based diet; 25%, 50%, 75%, 100%: different protein inclusion rates of **Volvariella volvacea**.*

TABLE 3
AVERAGE VALUE OF GROWTH CHARACTERISTICS OF RATS

Parameters	Diet treatments				
	F (n=6)	25 % (n=6)	50 % (n=6)	75 % (n=6)	100 % (n=6)
Initial weight (g)	67.00±9.66[a]	65.50±7.54[a]	65.50±5,97[a]	66.50±3.41[a]	64.75±2.36[a]
final weight (g)	85.50±6.95[d]	79.00±8.98[d]	66.75±7.45[c]	55.00±4.83[b]	44.25±3.09[a]
DMI (g)	8.50	7.36	6.27	5.25	4.09
PI (g)	0.85	0.73	0.62	0.52	0.40
AWG (g)	1.10±0.40[d]	0.90±0.17[d]	0.08±0.11[c]	-0.76±0.17[b]	-1.36±0.31[a]
FE	0.03±0.01[d]	0.03±0.00[d]	0.00±0.00[c]	-0.03±0.00[b]	-0.08±0.01[a]
PE	0.32±0.12[d]	0.30±0.05[d]	0.03±0.04[c]	-0.36±0.08[b]	-0.83±0.18[a]

*(n): Number of rats per treatment. ANOVA, followed by the Newman-Keuls multiple comparison test at the 5%. On the same line, Means ± STD followed by different small letters within a row are significantly different ($P \leq 0.05$). F: fish-based diet; 25%, 50%, 75%, 100%: different inclusion rates of **Volvariella volvacea** proteins. ; DMI: dry matter ingested per day per rat; PI: protein ingested per day per rat; AWG: Awerage weight gain per day per rat; FE: food efficiency; PE: protein efficiency.*

3.2 Effects of *V. volvacea* on organs weight

The observation in Table IV indicates that, regardless of the rate of incorporation of mushroom in the control diet (fish), there is no significant difference ($p > 0.05$) between the mean weight of hearts and livers of the mushroom consuming rats, and those of the control fish fed rats. Table IV also shows that the average kidney weight increased significantly ($p \leq 0.05$) as the rate of mushroom incorporation increased in rat diets. In contrast, mean abdominal fat weights of rats were significantly reduced ($p \leq 0.05$) as the rate of mushroom incorporation increased in diets.

TABLE 4
EFFECTS OF EDIBLE *V. VOLVACEA* ON MEAN ORGAN WEIGHTS IN RATS

Parameters (% BW)	Diet treatments				
	F (n=6)	25 % (n=6)	50 % (n=6)	75 % (n=6)	100 % (n=6)
Heart	0,49±0,09	0,48±0,04[a]	0,65±0,25[a]	0,52±0,03[a]	0,65±0,10[a]
Liver	3,90±0,54[a]	4,49±0,45[a]	4,13±0,18[a]	4,07±0,24[a]	3,72±0,10[a]
Kidneys	0,78±0,04[a]	0,88±0,08[b]	0,99±0,03[c]	1,03±0,01[c]	1,29±0,05[d]
Spleen	0,23±0,04[a]	0,25±0,04[a]	0,26±0,03[a]	0,25±0,04[a]	0,24±0,03[a]
Abdominal fat	1,24±0,29[c]	0,77±0,53[b]	0,28±0,30[a]	0,00±0,00[a]	0,00±0,00[a]

(n = 6): Number of rats per treatment. ANOVA, followed by the Newman-Keuls multiple comparison test at the 5 %. On the same line, Means ± STD followed by different small letters within a row are significantly different (P ≤ 0.05). F: fish-based diet; 25 %, 50 %, 75 %, 100 %: different inclusion rates of ***Volvariella volvacea*** *proteins; PC: BW: body weight.*

3.3 Effects of *V. volvacea* on the level of serum metabolites

Table V shows that the intake of 75 % of mushroom proteins in the diet of the rats causes a significant increase (p ≤ 0.05) in the mean value of serum creatinine compared with rats that consume fish powder (P). Enrichment of rat diets with 25 % and 50 % resulted in a significant (p ≤ 0.05) increase in mean uric acid values in rats compared with fish-fed rats. At 75 % and 100 % incorporation of mushroom into the control diet (fish), there was a significant decrease (p ≤ 0.05) in the uric acid value in the rats. Incorporation of 25 % of mushroom proteins in the control diet (fish) resulted in a significant decrease (p ≤ 0.05) in the mean value of total and conjugated bilirubins. Likewise, with 100 % mushroom protein, the average value of conjugated bilirubin decreases. Still in Table V, it is noted that the averages values of glucose, triglycerides, total proteins, total cholesterol, HDL-cholesterol, LDL-cholesterol and urea are not different (p> 0.05) from a batch of rats to another.

TABLE 5
EFFECTS OF *V. VOLVACEA* ON SERUM METABOLITES IN RATS

Parameters	Diet treatments (1 kg of dry matter)				
	F (n=6)	25 % (n=6)	50 % (n=6)	75 % (n=6)	100 % (n=6)
Glucose (g/L)	0.73±0.10[a]	0.67±0.03[a]	0.64±0.02[a]	0.69±0.04[a]	0.70±0.02[a]
Triglycérides (g/L)	0.40±0.10[a]	0.43±0.11[a]	0.68±0.21[a]	0.60±0.32[a]	0.46±0.08[a]
Total protein (g/L)	68.75±5.73[a]	65.75±3.59[a]	70.75±4.34[a]	73.75±10.87[a]	68.00±4.08[a]
Total-Cholesterol (g/L)	1.42±0.21[a]	1.64±0.24[a]	1.75±0.16[a]	1.62±0.31[a]	1.50±0.12[a]
HDL-Cholestérol (g/L)	0.32±0.06[a]	0.35±0.08[a]	0.34±0.07[a]	0.34±0.07[a]	0.31±0.03[a]
LDL-Cholestérol (g/L)	1.02±0.15[a]	1.20±0.19[a]	1.25±0.15[a]	1.14±0.21[a]	1.10±0.14[a]
Urea (g/L)	0.14±0.04[a]	0.25±0.01[a]	0.26±0.18[a]	0.45±0.24[a]	0.26±0.26[a]
Creatinin (mg/L)	6.75±0.95[a]	9.50±0.57[ab]	9.50±3.78[ab]	14.25±4.34[b]	9.50±1.29[ab]
Uric acid (mg/L)	37.25±7.41[a]	39.00±8.86[a]	42.50±5.44[b]	33.85±14.59[b]	35.25±5.05[b]
Total Bilirubin (mg/L)	14.00±3.55[c]	6.75±0.95[a]	11.25±2.50[bc]	15.25±1.70[c]	8.50±1.29[ab]
Conjugated bilirubin (mg/L)	3.47±0.62[bc]	1.36±0.07[a]	2.85±0.47[b]	4.15±0.86[c]	1.40±0.40[a]

(n = 6): Number of rats per treatment. ANOVA, followed by the Newman-Keuls multiple comparison test at the 5 %. On the same line, Means ± STD followed by different small letters within a row are significantly different (P ≤ 0.05). F: fish-based diet; 25 %, 50 %, 75 %, 100 %: different inclusion rates of ***Volvariella volvacea*** *proteins.*

3.4 Effects of *V. volvacea* on the activity of serum enzymes

In Table VI, there is no significant difference (p > 0.05) between the mean values of the activity of enzymes such as alkaline phosphatase (ALP), aspartate amino transferase (ASAT), alanine amino transferase (ALAT) and gamma glutamyl transpeptidase (γGT) of rats belonging to the five groups of rats (0 %, 25 %, 50 %, 75 % and 100 %).

TABLE 6
V. EFFECTS OF *VOLVACEA* ACTIVITY ON THE MEAN ACTIVITY VALUE OF SERUM ENZYMES IN RATS

Parameters (UI/l)	Diet treatments				
	F (n=6)	25 % (n=6)	50 % (n=6)	75 % (n=6)	100 % (n=6)
ALP	188.50±91.54[a]	120.50±10.21[a]	137.75±40.72[a]	135.25±20.27[a]	132.25±24.17[a]
ASAT	51.75±55.45[a]	38.00±15.25[a]	34.75±22.91[a]	45.25±19.31[a]	41.50±9.53[a]
ALAT	36.50±26.60[a]	39.00±19.61[a]	42.00±15.53[a]	48.25±18.73[a]	40.25±9.74[a]
γGT	49.25±24.25[a]	33.50±11.67[a]	25.75±7.18[a]	34.75±7.27[a]	50.75±9.32[a]

(n = 6): Number of rats per treatment. ANOVA, followed by the Newman-Keuls multiple comparison test at the 5 %. On the same line, Means ± STD followed by different small letters within a row are significantly different (P≤0.05). F: fish-based diet; 25 %, 50 %, 75 %, 100 %: different inclusion rates of ***Volvariella volvacea*** *proteins*

3.5 Effects of *V. volvacea* on the level of serum electrolytes

The mean values of the serum electrolytes (phosphorus, calcium, magnesium, iron, sodium, potassium) are shown in Table VII. Similarly, the incorporation of 25 % and 50 % mushroom protein levels resulted in a significant increase ($p \leq 0.05$) in mean serum of iron compared with 75 % in fish-fed rats and 100 %. Finally, the different incorporation rates (25 %, 50 %, 75 % and 100 %) of the mushroom do not affect ($p > 0.05$) the different mean serum values of the rats (P^{5+}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and P^{5+}/Ca^{2+}).

TABLE 7
EFFECTS OF *V. VOLVACEA* ON SERUM ELECTROLYTES OF RATS

Parameters	Diet treatments				
	F (n=6)	25 % (n=6)	50 % (n=6)	75 % (n=6)	100 % (n=6)
P^{5+} (mg/L)	44.75±4.27[a]	46.25±6.39[a]	44.50±3.69[a]	48.00±2.94[a]	46.25±3.77[a]
Ca^{2+} (mg/L)	91.25±2.87[a]	91.25±5.12[a]	93.00±2.44[a]	92.25±4.34[a]	91.00±3.74[a]
Mg^{2+} (mg/L)	20.00±0.81[a]	20.75±0.95[a]	22.75±1.25[a]	21.50±1.29[a]	21.50±2.64[a]
Fe^{2+} (mg/L)	1.20±0.09[a]	1.61±0.31[b]	1.71±0.28[b]	1.19±0.11[a]	1.27±0.10[a]
Na^{+} (mEq/L)	139,25±2,62[a]	137,50±2,08[a]	139,50±3,10[a]	138,50±3,00[a]	140,25±1,70[a]
K^{+} (mEq/L)	4.17±0.30[a]	3.90±0.14[a]	4.30±0.60[a]	4.40±0.77[a]	3.87±0.09[a]
Ca^{2+}/P^{5+}	2.05±0.16[a]	1.99±0.25[a]	2.09±0.15[a]	1.92±0.16[a]	1.97±0.15[a]

(n = 6): Number of rats per treatment. ANOVA, followed by the Newman-Keuls multiple comparison test at the 5%. On the same line, Means ± STD followed by different small letters within a row are significantly different (P≤0.05). F: fish-based diet; 25%, 50%, 75%, 100%: different inclusion rates of ***Volvariella volvacea*** *proteins.*

IV. DISCUSSION

Evaluation of growth parameters such as final weight, ingested dry matter, ingested protein, body weight gain, feed efficiency and protein efficiency ratio shows that at the end of 15 days of experimentation, there is a significant difference between the control rats fed on fish and the rats that consumed supplemented mushroom diets. The performance of diets decrease when the rate of incorporation of mushroom (*Volvariella volvacea*) increase. Ingested dry matter and body weight gain decrease as the rate of incorporation of the mushroom increase. This decrease of intake diet resulted in a decrease of the average value of the proteins ingested. The dietary efficiency ratio and the protein efficiency ratio of the diets decrease significantly to reach negative values for diets with 75 % and 100 % incorporation of the mushroom. The dietary energy contribution decrease when the rate of insertion of mushrooms increase. This decline reflect in a decline of growth, due to an inefficiency of mushroom proteins to replace fish proteins [10]. Also, the increasing tannin of mushroom intake by rats would have inhibited protein efficiency [11, 12]. The gradual increase of the amount of mushroom in the diets of rats provokes an increase in the content of these diets in tannins and polyphenols [13, 14, 15, 16].

The average weight of the kidneys of the rats increase as the rate of insertion of the mushroom increase, and conversely, the average weight of rats abdominal fat is lower than that of rats consuming fish. This decrease in abdominal fat goes to a complete disappearance of abdominal fat at 75 % and 100 % incorporation rate of mushroom. The increase in the weight of the kidneys of rats is due to hyperactivity of the kidneys and the presence of antinutrients. On the other hand, the decrease of abdominal fat accumulation, or even its total disappearance in rats when the rate of incorporation of the mushroom increases energy would be the consequence of the low dietary energy intake of diets containing the mushroom.

The incorporation of mushroom provokes an increase in the serum mean value of uric acid and a decrease in the mean serum value of total and conjugated bilirubins. The decrease of the mean serum concentration of total and conjugated bilirubin is due to the presence of compounds such as flavonoids which, through their antioxidant effect, could play a protective role on liver cells [17, 18]. The increase of serum uric acid concentration think to be due to the catabolism of purine bases or nucleic acids. The increase of serum uric acid and creatinine would indicate renal failure in these rats [19]. The mean value of serum enzymes activity is not affected by the different treatments. The consumption of diets supplemented with the mushroom caused an increase in the average serum iron value. This lowering of the average iron value can be due to the disturbance of blood cell metabolism. The ratio of calcium serum to phosphoremia is close to 2. This ratio is an indicator of phosphocalcic metabolism. It would thus translate a good metabolism of these two minerals by hormones (parathyroid hormone and calcitonin). Nephrons would provide good control of plasma calcium and phosphorus levels [20, 21].

V. CONCLUSION

From this study, it’s concluded that mushrooms consumption provoked low feed ingestion and consequently low growth. The results indicated that edible mushroom proteins cannot 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 is noted on blood parameters.

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