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Effects of consumption of bambara groundnut (*Vigna subterranea* (L.) Verdcourt) seeds on nutritional characteristics and average values of serum metabolites in rat

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Abstract

Protein-energy malnutrition is a major public health problem for developing countries like Côte d'Ivoire. Promotion of local agricultural products, especially bambara groundnut seeds, could help to improve nutritional status of populations affected by this scourge. It is in this framework that current study was initiated in order to determine effects of consumption of powders from dried and germinated bambara groundnut seeds on growth and mean values of serum biochemical parameters in rats. A twenty-one day animal experiment was carried out with twenty-one growing male *wistar* rats, divided into three batches of seven rats. Animals were subjected to three diets of 10% protein, using herring fish powder for control diet (TP), dried seed powders (PBS) and germinated (PBG) of bambara groundnut. At the end of the experiment, body weight gain of rats subjected to PBS (0.85±0.32g) and PBG (1.66±0.67g) are lower than rats consuming fish powder (TP:2.40±0.35g). Likewise, dry matter ingested by rats under PBS (7.96±1.61g) and PBG (9.19±1.37g) are lower than control rats (10.20±1.82g). Compared to control rats (1.02±0.18g), total protein ingested by rats under PBS (0.80±0.16g) and PBG (0.92±0.14g) are also lower. Same goes for food and protein efficiency ratios. Concerning mean serum values of total proteins, rats fed with PBS (71±2.65g/L) and PBG (73.67±14.15g/L) have lower values than control rats (TP:79±3.61g/L). Those of triglycerides, total-cholesterol, HDL-cholesterol and LDL-cholesterol are significantly higher ($p \leq 0.05$). However, PBG diet has better values than PBS ($P \leq 0.05$). All results suggest that bambara groundnut seeds could be used as foods of good nutritional values.

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Introduction

The world's population, estimated at 7.35 billion people in 2015, is expected to reach nearly 9.8 billion by 2050 (ONU, 2017). According to the Food and Agriculture Organization of the United Nations (FAO), malnutrition affects more than one billion people worldwide, 90 % of whom are in developing countries (FAO, 2009). Indeed, the diets in these regions are mainly based on starchy food and cereals (Olapade and Aworh, 2012). Their low protein content has contributed to the onset of protein malnutrition. In addition, proteins of animal origin are still inaccessible to a large part of the world's populations, particularly in developing countries (Osho, 2003). Because of this large demography, it would be wise to seek new sources of protein in order to meet the nutritional needs of populations (Fouré *et al.*, 2013). Among them are legumes which, in addition to their high protein content, have the advantage of being widely cultivated by people in rural areas (Fasoyiro *et al.,* 2012). Among the many legumes species cultivated in Africa, and particularly in Côte d'Ivoire, there is the voandzou (*Vigna subterranea* (L.) Verdcourt) also called ground pea or bambara groundnut. The plant is cultivated for its seeds rich in proteins, carbohydrates, lipids and trace elements (Ndiang *et al.*, 2012). Despite these many advantages, voandzou is still one of the neglected and little used species in variety breeding programs. Many previous studies have focused on the various morphotypes, as well as on the nutritional characteristics of the seeds of this legume (Aremu and Ibrahim, 2014; Anhwange and Atoo, 2015). However, there is very little scientific information on the efficient use of bambara groundnut or voandzou seeds (*Vigna subterranea* (L.) Verdcourt) in food in Côte d'Ivoire. It is in order to respond to this concern that the present study was initiated.

Material and methods

Animal material

Young male rats of the *wistar* strain with an average weight of 67.30±0.66g, aged between six and eight weeks, were used for animal testing. These animals were bred at the higher normal school pet store in Abidjan, Côte d'Ivoire.

Technical materials

A scale was used to weigh animals and feed. A multifunctional apparatus was also used to measure serum metabolites, and a computer with graphpad program was used for statistical analyses.

Plant and food material

The dry seeds of *Vigna subterranea*, which vernacular name is ground peas or bambara groundnut, were bought at the cereal market, located in the commune of Adjamé, in the Abidjan District of Côte d'Ivoire. For this study, the dried seeds (PBS) and germinated seeds of bambara groundnut powders (PBG) were used. Herring fish was bought fresh at the fishing harbor of Abidjan. They were then smoked and powdered for use. Several other ingredients which come from the trade have been used in the formulation of different diets. These are cornstarch, vegetable sunflower oil, powdered cane sugar, a premix of vitamins and minerals supplement "vitaflash, France" and agar-agar.

Production of powders from dried and sprouted bambara groundnut seeds

Preparation of Dried Bambara groundnut Seed Powders (PBS): The dried seeds of bambara groundnut were bought at the Gouro market in Abidjan. They were sorted, cleaned and soaked for 6 hours in tap water. After the time has elapsed, the seeds have been drained and placed in an oven at 55 °C, for 48 hours, for drying. Then, they were ground using a micro-mill (Culatti type MFC, Germany) to obtain a fine powder of dried bambara groundnut seeds, which, once sifted, was packaged in hermetically sealed plastic boxes and kept in the refrigerator at 4 °C.

Germinated Bambara groundnut Seeds Powder (PBG): The dried bambara groundnut seeds were sorted, cleaned and then soaked in cold tap water for 24 hours. Then the seeds were drained and germinated in a clean cotton cloth for 72 to 96 hours. Once the seeds had germinated, they were dried in an oven at 55 °C for 72 hours before being finely ground, using a micro-mill (Culatti type MFC, Germany). The powder obtained was sieved, then packaged in plastic boxes, hermetically closed. Storage was done in the refrigerator at 4 °C.

Formulation and preparation of diets

The different diets were prepared according to the method described by Garcin *et al.* (1984) with modifications (Table 1). In the whole, three diets were formulated: a control diet based on "herring" fish powder (TP), a diet of dried bambara groundnut seed powder (PBS), and a diet of germinated bambara groundnut seeds powder (PBG). All formulated diets are iso-protein (10% protein) and iso-energy (4226 kcal/kg of dry matter). They have been enriched with vitamins and minerals with the vitamin supplement "vitaflash, France". Their carbohydrate content was provided by cornstarch, sunflower oil and cane sugar which were purchased commercially. Agar-agar has been used as a ballast in each diet. The preparation of the diets consisted in mixing the different ingredients of each experimental diet with one liter of water was added to it, in a blender brand "moulinex, France", in order to better homogenize all the different ingredients. Subsequently, each mixture was transferred to a saucepan, and the whole was subjected to cooking on an electric stove brand "IKAMAG, Germany" until it is solidified. The food is placed on a plate and after cooling, it is wrapped in aluminum foil and stored in the refrigerator at 4 °C. This preparation is renewed every four days.

Animal experimentation

A total of twenty one growing male rats, of *wistar* strain, whose age is between six and eight weeks, and weighing on average 67.30±0.66g, was used. There were seven rats per diet, located in individual metabolism cages, with wire mesh bottoms. These cages were placed in a room, with a temperature of 26 °C and relative humidity 70% to 80%. The room is lit for 12 hours and remains dark for 12 hours. This growth experiment took place over a period of twenty-one days, preceded by a period of adaptation of three days, during which, the rats received, ad libitum, a unique diet, based on fish powder with 10% protein.

The diets were distributed to each batch of rats, on a daily basis, between 7 am and 8 am for the duration of the experiment. Clean water, replaced every morning, and animal diets were served *ad libitum*. The dry matter of each diet served was determined daily on samples taken for this purpose.

The next day, before redistribution, the remains of the diets that had been served the previous day were collected and weighed.

The quantities of fresh food consumed were obtained by the difference between the quantities distributed and the quantities refused. These quantities are converted into total dry matter ingested (TDMI) by multiplying by the rate of dry matter and total protein ingested (TPI) by multiplying the amount of total dry matter ingested by the rate of protein contained in the diet. The animals were weighed at the start of the experiment, and then, every two days, until the end of the experimental period. The different expressions of the nutritional characteristics of the rats are recorded in Table 2.

Determination of mean values of serum metabolites of rats subjected to diet

At the end of the experiment, the rats were fasted for 16 hours. The next morning, they were sacrificed after anesthesia with ethyl urethane (Amoikon *et al.*, 2012). A quantity of 5 ml of blood was then taken from each rat and deposited in dry tubes without anticoagulant. These samples are sent to the laboratory to be centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge (Alresa Orto, Spain) at 4 °C. The serum obtained was collected in Ependhorf tubes and stored in the freezer at 0 °C, pending serum biochemical analyzes which were carried out in the medical biochemistry laboratory of the university hospital center of Treichville, Abidjan, Côte d'Ivoire. The biological constants were determined using an automatic multi-parameter analyzer (HITACHI 902, Germany).

Statistical analysis

Graphpad prism version 7.00 software was used for statistical analyzes, calculation of means and standard deviations. The analysis of variances (ANOVA) followed by the multiple comparison test of Newman-Keuls means were used to classify and compare the means.

The means are always followed by their standard deviations. Two or more means are significantly different if the probability resulting from the statistical tests is less than or equal to 0.05 ($P \leq 0.05$). On a same line, the means ± standard deviation followed by different letters a, b, c, etc., in super script, are significantly different ($p \leq 0.05$).

Results

Effects of consumption of powders of dried and germinated seeds of bambara groundnut on the nutritional characteristics of rats

The evolution of the growth of the rats is illustrated in Fig. 1. This figure shows an increase in the body weight of the rats subjected to the different diets during the experiment.

However, it should be noted that the growth of rats consuming bambara groundnut remains lower than that of the controls.

Table 1. Composition of experimental diets of rats.

Ingredients	Diets		
	TP	PBS	PBG
Fish powder (g)	140.25	-	-
PBS powder (g)	-	587.89	-
PBG powder (g)	-	-	529.66
Corn starch (g)	553.75	106.11	164.34
Sugar (g)	250	250	250
Premix	01	01	01
Agar (g)	05	05	05
Sunflower oil (mL)	50	50	50
Total dry matter (g)	1000	1000	1000
Water (mL)	1000	1000	1000
Energy (Kcal/Kg DM)	4226	4226	4226

The protein level in the diets is fixed at 10%; the energy value of each diet is equal to 4226 Kcal/Kg dry matter; TP: Control diet based on fish powder; PBS: Diet based on dried bambara groundnut seeds powder; PBG: Diet based on sprouted bambara groundnut seed powder. The raw energy of the diets was calculated by referring to the combustion values of the various nutrients on the basis of 4 kcal for 1 g of protein, 4 kcal for 1 g of carbohydrates and 9 kcal for 1 g of lipids.

The growth of rats fed on the PBG diet is more increased than that of rats on the PBS diet. Body weight gain of rats (Table 3) subjected to dry seeds of bambara groundnut (PBS: 0.85±0.32g) and germinated seeds of bambara groundnut (PBG: 1.66±0.67g) are lower than that of rats consuming fish powder (TP: 2.40±0.35g). Likewise, dry matter ingested by rats under PBS diet (7.96±1.61g) and PBG diet (9.19±1.37g) are lower than that of control rats (TP: 10.20±1.82g). Total protein ingested by rats under PBS regime (0.80±0.16g) and PBG (0.92±0.14g) are also decreased, compared to that control rats (TP: 1.02±0.18g). Likewise, food efficiency ratio and protein efficiency ratio are decreased by Bambara groundnut seeds diets (PBS, PBG). However, germination makes it possible to further improve these characteristics ($P \leq 0.05$).

Comparing the two diets PBS and PBG, it is noted that the rats having consumed the germinated bambara groundnut (PBG) have higher nutritional characteristics than those of the rats fed on PBS.

Table 2. Expression of nutritional parameters.

Nutritional parameters	Mathematical expressions
Total dry matter ingested (TDMI) :	$TDMI\ (g) = \frac{FI \times \%DM}{number\ of\ days}$
Total protein ingested (TPI) :	$TPI\ (g) = \frac{TDMI \times \%P}{number\ of\ days}$
Body weight gain (BWG) :	$BWG\ (g) = \frac{FW - IW}{number\ of\ days}$
Food efficiency ratio (FER) :	$FER = \frac{BWG}{TDMI}$
Protein efficiency ratio (PER) :	$PER - \frac{BWG}{TPI}$

Effects of consumption of powders of dried and germinated seeds of bambara groundnut on the average values of the serum metabolites of rats

Table 4 presents the results of the mean values of the serum metabolites of the rats. The mean values of uric acid, total and conjugated bilirubins, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol and alkaline phosphatase of rats subjected to the diets containing the seeds of Bambara groundnut (PBG, PBS) are significantly higher ($p \leq 0.05$) than those of control rats consuming fish powder (TP).

In contrast, the mean values of urea, creatinine, glucose, total proteins and activity of ALAT, ASAT, γGT of rats consuming bambara groundnut were statistically lower ($p \leq 0.05$) than those of control rats.

Table 3. Average value of nutritional parameters according to diet.

Nutritional parameters	Diets		
	TP	PBS	PBG
Initial weight (g)	67.81±7.08^{a}	66.99±6.40^{a}	66.51±5.35^{a}
Final weight (g)	118.30±4.40^{c}	84.79±8.58^{a}	101.30±16.48^{b}
Body weight gain (g/d)	2.40±0.35^{c}	0.85±0.32^{a}	1.66±0.67^{b}
total dry matter ingested (g/d)	10.20±1.82^{c}	7.96±1.61^{a}	9.19±1.37^{b}
total protein ingested (g/d)	1.02±0.18^{c}	0.80±0.16^{a}	0.92±0.14^{b}
Food efficiency ratio	0.27±0.04^{c}	0.11±0.02^{a}	0.17±0.03^{b}
Protein efficiency ratio	2.68±0.44^{c}	1.11±0.25^{a}	1.67±0.30^{b}

Analysis of variances followed by the Newman-Keuls multiple comparison test at the 5% threshold. On a same line, the means ± standard deviation followed by different letters a, b, c, etc. in super script are significantly different ($p \leq 0.05$). TP: fish powder diet; PBS: diet based on dry bambara groundnut seeds powder; PBG: diet based on powder of germinated bambara groundnut.

Discussion

The evaluation of the nutritional value of dried and germinated bambara groundnut seeds powders by animal experiments revealed significant differences ($p \leq 0.05$) in the growth characteristics. Rats fed with powders of bambara groundnut seeds (PBS, PBG) have lower nutritional characteristics than those of the controls. Ewuola *et al.* (2015) reported similar results using complementary diets prepared with mixtures of corn flour, powders of bambara groundnut and cowpea in weaned rats.

The presence of anti-nutritional factors in legumes may explain the lower intake amounts in rats consuming bambara groundnut powder. The differences in food intake and bodyweight gain recorded could also be explained by the source of protein and the organoleptic characteristics of the made-up diets. In this regard, the animals on the control diet, fed on fish proteins have a higher body weight gain. These results corroborate those reported by Pahane *et al.* (2017) working on the nutritional evaluation of milk and yoghurt from bambara groundnut seeds on albino rats. These authors claimed that the casein diet has the highest food consumption values compared to that of milk and bambara groundnut seeds yogurt. They observed that the food intake and growth rate of rats varied depending on the protein source.

Table 4. Average value of serum metabolites of rats.

Serum parameters	Diets		
	TP	PBS	PBG
Glucose (g/L)	0.86±0.06^{b}	0.81±0.05^{a}	0.87±0.02^{b}
Total Protéin (g/L)	79±3.61^{b}	71±2.65^{a}	73.67±14.15^{a}
Urea (mg/dL)	39±0.04^{b}	31±0.04^{a}	29±0.02^{a}
Créatinine (mg/dL)	1.4±0.1^{c}	0.96±0.11^{a}	1.0±0.2^{b}
Uric Acid (mg/dL)	1.44±0.32^{a}	5.3±0.43^{b}	4.73±0.66^{b}
Total Bilirubins (mg/dL)	0.63±0.15^{a}	1.03±0.37^{b}	0.96±0.11^{b}
Conjugated Bilirubins (mg/L)	0.11±0.03^{a}	0.22±0.05^{b}	0.29±0.03^{c}
Triglycerides (g/L)	0.74±0.03^{a}	1.47±0.32^{b}	1.56±0.32^{b}
Total cholesterol (g/L)	0.69±0.01^{a}	1.03±0.10^{b}	0.95±0.11^{b}
HDL-cholesterol (g/L)	0.16±0.02^{a}	0.25±0.03^{b}	0.24±0.06^{b}
LDL -cholesterol (g/L)	0.38±0.03^{a}	0.48±0.06^{b}	0.39±0.14^{a}
PAL (UI/L)	406.7±111.5^{a}	609.3±258.3^{c}	554±103.3^{b}
ALAT (UI/L)	160±43.59^{b}	155.3±26.27^{a}	174.7±6.80^{c}
ASAT (UI-L)	131.7±30.02^{c}	100.7±4.509^{a}	115.3±12.66^{b}
γGT (UI-L)	86.67±15.28^{b}	85.67±9.60^{b}	71.67±10.21^{a}

Analysis of variances followed by the Newman-Keuls multiple comparison test at the 5% threshold. On a same line, the means ± standard deviation followed by different letters a, b, c, etc. in super script are significantly different ($p \le 0.05$). TP: fish powder diet; PBS: diet based on dried bambara groundnut seed powder; PBG: diet based on germinated bambara groundnut seeds powder. PAL: alkaline phosphatase; ALAT: alanine amino transferase; ASAT: aspartate amino transferase; γGT: gamma glutamyl-transferase.

The food efficiency (FER) and protein (PER) ratios obtained with rats consuming the PBS and PBG diets are lower than those recorded with rats subjected to the control diet based on fish powder ($P \le 0.05$).

According to Bintu *et al.* (2017), the PER of a food reflects its biological value because the measured weight gain depends on the incorporation of dietary proteins in body tissues. Furthermore, FER could be influenced by the flavor, the essential amino acid contents of proteins.

Hence, food with high FER and PER values tend to promote weight gain, while low values tend to be used as energy rather than being stored in reserve (Laminu *et al.,* 2014). The determination of biochemical parameters is very important in nutritional studies because it makes it possible to detect certain functional pathologies of the regulatory organs of nutrition such as the kidney, the liver and the heart.

It is both an indirect way of exploring the state of these organs and also a way of exploring the metabolism of nutrients. The serum glucose and total protein values of rats on the PBS and PBG diets were lower ($p \le 0.05$) than those of rats on the control diet. These results are in agreement with those of Pasko *et al.* (2010), when they compared the groups of rats fed with quinoa to the group of the control diet fed with casein. They found that quinoa seeds significantly reduced the blood glucose levels of rats. While Olaiya *et al.* (2017) reported that a high value of serum protein indicates better protein quality of food consumed by animals.

The values of urea, creatinine of rats subjected to the PBS and PBG diets show a significant difference ($p \leq 0.05$), compared to those of rats consuming the control diet ($p \leq 0.05$). This significant depletion in the urea and creatinin values of rats subjected to the PBS and PBG diets compared to the control diet could be explained on one hand by a good quality of the proteins of the powders tested, and on the other hand, would express the beneficial effect of bambara groundnut proteins on the liver and kidneys of animals (decrease in uremia and creatinemia).

Similar observations have been reported by El-Sebeay *et al.* (2018) working on the chemical, sensory and biological evaluation of certain products formulated with quinoa for celiac disease.

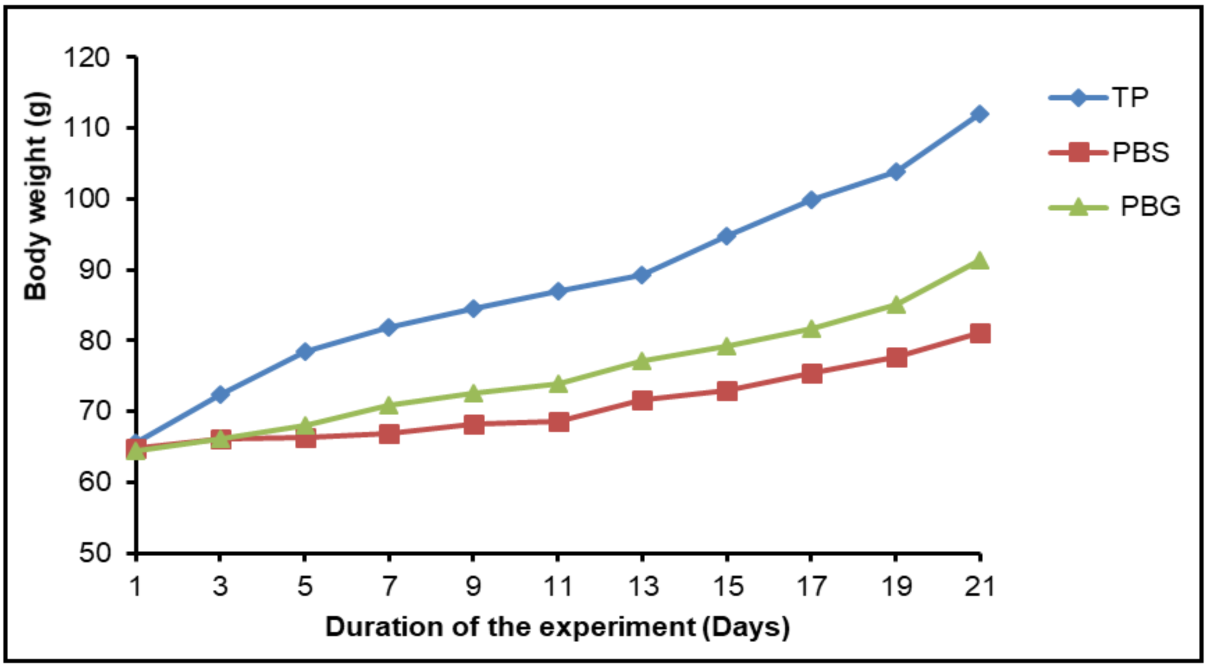

Fig. 1. Variation in body weight of rats according to diet.

TP: Control diet based on fish powder; PBS: Diet based on dried bambara groundnut seed powder; PBG: Diet based on sprouted bambara groundnut seed powder.

The triglycerides, total cholesterol, LDL and HDL cholesterol, total and conjugated bilirubins, uric acid and levels and PAL activity of animals fed the PBS and PBG diets are significantly higher ($p \leq 0.05$) than those of the rats on the control diet. The results of the PBS and PBG diets, although higher than those of the control diet, are within normal values and testify to the good nutritional value of the powders of bambara groundnut seeds on the organs. Takao *et al.* (2005) reported that the protein found in quinoa, which is also a legume, decreases the reabsorption of bile acids and the synthesis of cholesterol in the liver. Serum transaminases testing is done to assess the physiological condition of the liver and kidneys. The mean enzymatic activity values of the ASAT, ALAT and γGT transaminases recorded in animals fed the PBS and PBG diet show significant decrease, compared to those of rats subjected to the control diet.

These results demonstrate the negative effect of the bambara groundnut diets tested on the proper functioning of animal organs. Indeed, it should be noted that an abnormal decrease in the activities of these enzymes and more particularly those of ASAT and ALAT reflects a hepatic dysfunction, due to the transfer of these enzymes from the blood circulation to the cytosol of the liver (Lin and Huang, 2000).

Conclusion

It appeared from this study that the consumption of diets formulated from the powders of the dried and germinated seeds of bambara groundnut caused a considerable weight gain in the rats. The results obtained show that bambara groundnut seeds have a beneficial effect on the nutritional status and health of rats and could constitute a good food source of protein for animals and humans. In addition, it was observed that the germination of the seeds, with the reduction of the anti-nutritional factors, favored a better food consumption.

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