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Original Research Article

Nutritional and Physiological Effects of Consuming Legume Seed Powders (Glycine Max, Phaseolus Lunatus, Vigna Unguiculata, Cajanus Cajan) in Combination with Kponan Yam Powder (Dioscorea Alata) in Young Rats (Rattus Norvegicus)

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Abstract: The aim of this study is to assess the effects of consumption of Lima beans, angole peas and cowpeas on nutritional and physiological characteristics in growing rats. To do this, twenty-four growing male rats in four batches of six were fed for 21 days, with four diets. For example, a control diet based on soy powder (SAI), a Lima bean powder diet (PLI), an Angole pea powder diet (PAI) and a commercially purchased cowpea powder (NEI) diet were put into experimentation. The protein level of these diets is set at 10 %. At the end of the experiment, the results reveal that rats fed Lima bean powder diets experienced a loss of body mass from the beginning to the end of the experiment, while those fed with the other three diets (SAI, PAI and NEI) caused growth in rats. This study shows that there is no significant difference ($p>0.05$) between the values of triglycerides, HDL cholesterol and conjugated bilirubins of rats on the PLI, PAI and NEI diets compared to the control (SAI). Blood glucose levels in rats on the PAI and NEI diets are significantly higher ($p\leq0.05$) than control rats (SAI). Similarly, the hemoglobin values of rats on PAI and NEI diets show no significant ($p>0.05$) difference compard to those of controls. These results imply that soybeans, cowpeas and Angole Peas may contribute to the fight against children malnutrition unlike Lima beans.

Keyword: Rats, legumes, hematological parameters, serum metabolites, organ biometrics.

I INTRODUCTION

The lack of satisfaction of nutritional needs exposes a large proportion of the population of developing countries to undernutrition and often to malnutrition [1]. Economic difficulties or impoverishment are such that proteins of animal origin are still inaccessible to a large part of the population. Faced with this situation, it seems urgent to find other nutritional sources capable of satisfying the food needs of these populations. In the tropics, legumes, promising sources of vegetable proteins, such as cowpea (Vigna unguiculata), groundnut (Arachis hypogeae L.), soybean (Glycine max) are widely consumed, unlike other species such as lima bean (Plaseolus lunatus) and pigeon pea (Cajanus Cajan)) [2, 3]. Certainly, they are most valued in human nutrition for their rich source of macronutrients (carbohydrates, proteins, lipids and fiber) and micronutrients (vitamins and minerals) [4]. In order to better take advantage of the benefits of legumes, the 68th session of the United Nations General Assembly declared 2016 as the "International Year of Pulses" with a global production of 81 million tons [5]. However, very little research has been conducted so far on the nutritional potential of legumes. Thus, this study was initiated to evaluate the effects of consumption of these different legumes on the nutritional performance of consumers.

II MATERIAL AND METHODS

2.1 Animal material

Twenty-four male Wistar rats were used in the experiment. The animals had an average mass of 60-64 g and were 55-65 days old. The temperature of the animal house was 25-27±2°C. The humidity in the room is 70-80%, with 12 h of daylight and 12 h of darkness.

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2.2 Plant material

Dried legume seed powders (soybean, cowpea, pigeon pea and lima bean) and Kponan yam (Dioscorea alata) were used to make the different diets. Sunflower oil, cane sugar and Premix were used as food supplements. The legume powders were used as protein sources and the Kponan yam powder was used as a carbohydrate source. As for the premix purchased from Boivé laboratory, France, it was used as a source of vitamins and minerals

2.3 Processing of legume seeds and yam tuber

The legume seeds were sorted and washed. They were soaked in tap water for 8 hours. After soaking, the seeds were dried in an oven (MEMMERT 854. SCHWABACH W, Germany) for 24 h at 60 °C. They were ground to powder with a microgrinder and stored in a refrigerator at 4°C. The Kponan yam tuber was washed with tap water, peeled with a knife and cut into pieces. The resulting pieces were dried in an oven (MEMMERT 854. SCHWABACH W, Germany) for 48 h at 60 °C. After drying, the dehydrated yam pieces were ground into powder with a microgrinder and the powder obtained was stored in a refrigerator at 4 °C for further processing.

2.4 Preparation of the different diets

The different iso-protein diets were prepared according to the method of [6] with modifications. In total, four diets were formulated, one of which was a control diet (SAI) based on soybean powder and three experimental diets (PLI, PAI and NEI) based on lima bean, pigeon pea and cowpea powder, respectively, for this animal experiment. The preparation of the rats' food consisted in mixing the different ingredients of the same diet in a "Moulinex" brand blender (France), according to the proportions mentioned in Table I. These ingredients were transferred to a pan, and after homogenization in 1 liter of water to obtain a slurry. The slurry was subjected to cooking on an electric stove, brand "IKAMAG" (Germany), at 100 °C until it set. The food was placed on different plates and after cooling, it was kept in the refrigerator (4°C). This preparation was repeated every 4 days.

Table I: Proportions of Diet Ingredients

Ingrédients	**Diets**			
	SAI	**PLI**	**PAI**	**NEI**
Pigeon pea powder (g)	-	-	625,78	-
Lima bean powder (g)	-	478,24	-	-
Powpea powder (g)	-	-	-	577,034
Soy powder (g)	324,99	-	-	-
Yam powder (g)	378,02	282,09	285,77	274,83
Sugar (g)	295,53	179,21	34,71	93,27
Sunflower oil (mL)	0,46	59,46	52,74	53,86
Premix (g)	1	1	1	1
Total (g)	1000	1000	1000	1000
Water (mL)	1000	1000	1000	1000
Gross energy (Kcal/Kg)	3998,3	4293,3	4259,7	4265,3

2.5 Growth experience

A total of 24 rats were used and divided into groups of 6 to obtain 4 batches. A duplicate of 5 g of each feed was taken and placed in the oven (MMM Medcenter Gmbh, D82152, Germany) at 105°C for 4 hours. After the 4 h drying time, the feed samples were removed from the oven, weighed to determine the dry matter content. The rest of the feed was weighed in the mornings to determine the amount of feed ingested [7]. The animals were weighed every three days to assess the change in body weight.

2.6 Collection of blood samples

At the end of the animal experiment (21 days later), the rats were fasted for 16 hours [8]. The next day, between 8 and 10 am, approximately 5 mL of blood was collected from each rat in tubes containing an anticoagulant, ethyldiaminetetraacetic acid (EDTA) after anesthetizing the rats by inhalation of ether in a closed box until the animal was immobile, then occurs its sacrifice. The collected blood was centrifuged at 3000 rpm for 10 min in a refrigerated centrifuge (Alresa Orto, Spain) at 4°C [9, 7]. The serum was collected and deposited in hemolysis tubes for storage in a freezer (0°C), pending the determination of biochemical parameters. Serum metabolite assays (glucose, triglycerides, total protein, total cholesterol, HDL-cholesterol, LDL-cholesterol, urea, creatinine, uric acid, total bilirubin and conjugated bilirubin) were carried out on the serum samples, using a "HITACHI 902" autoanalyzer (Roche, Japan) at the Biochemistry Laboratory of the CHU of Cocody, Abidjan.

2.7 Statistical analysis of results

The results are presented in tables and figures. Statistica version 7.1 was used for the statistical analyses. Analysis of variance (ANOVA) followed by the Newman Keuls multiple comparison test at the 5% threshold was used to rank all means. The means are followed by their variances. Two means are different if the resulting probability is less than 5% ($p \leq 0.05$). The results were expressed as mean ± standard deviation. Comparisons of plasma biochemical and organ biometric parameter values were made using

STATISTICA software, version 7.0, by the 5% Newman Keuls test.

III RESULTS

3.1 Effects of legume consumption on rat growth

Figure 1 shows that the weight change curves of rats fed the soybean diet (SAI) and cowpea diet (NEI) are visibly above the curve of rats fed the pigeon pea diet, which is also above the curve of rats fed the lima bean diet (PAI). The curve for rats fed the Lima bean diet (PLI) has a downward trend from the beginning to the end of the experiment. Rats fed the soybean (SAI) and cowpea diets have relatively higher growth than rats on the pigeonpea (PAI) and lima bean diets. However, animals with the PAI diet have higher growth than those with the PLI (lima bean) diet. Table II shows the average values of the nutritional characteristics of the rats at the end of the animal experiment. The mean initial masses (M.I) of the 4 batches of rats were not statistically different ($p > 0.05$). The mean final mass of rats on the PLI (lima bean) diet (40.2 ± 5.22 g) was the lowest ($p \leq 0.05$). In contrast, the highest mean masses were observed in rats subjected to the control (SAI) (84.78 ± 7.33 g) and NEI (cowpea) diets (84.83 ± 5.03 g). The lowest body mass gain (BGM) (-1.02 ± 0.27 g) ($p \leq 0.05$) was attributed to the group of rats subjected to the PLI (lima bean) diet and the highest mass gain ($p \leq 0.05$) was obtained with those on the NEI (cowpea) (1.08 ± 0.42 g) and SAI (soybean) diets (1.1 ± 0.23 g). Regarding the ingested food (AI), rats on the PLI diet had the lowest mean value (12.86 ± 3.39 g). Rats on the control diet (SAI) (20.74 ± 4.12 g) and those on the NEI diet (21.39 ± 3.20 g) have the highest feed intake value. The highest dry matter intake (DMI) value (11.02 ± 1.58 g) was observed with the NEI diet and the lowest DMI value (5.59 ± 0.87 g) with the PLI diet. There were significant differences ($p \leq 0.05$) between the MSI values of the four groups of rats. The lowest level of ingested protein (IP) (0.55 ± 0.01 g) was observed with the PLI diet while the highest level (1.10 ± 0.03 g) was observed with the NEI diet. There were significant differences ($p \leq 0.05$) between the rates of (PI) in the four groups of rats. The lowest food efficiency coefficient (FEC) (-0.18 ± 0.00) was observed in rats on the PLI diet and the highest (0.11± 0.00) was observed in those on the SAI diet. Rats fed the PLI diet had the lowest protein efficiency ratio (PER) value (-1.85±0.03) while those fed SAI (soy) had the highest PER (1.18±0.00).

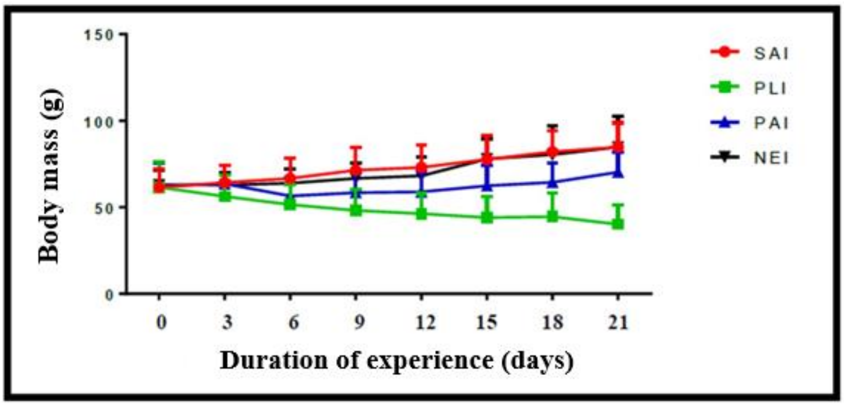

Figure 1: Variation in body mass of rats during the experiment

SAI: soy bean powder control diet; PLI: lima bean powder diet; PAI: pigeon pea powder diet; NEI: cowpea seed powder diet;

Table 2: **Average Value of The Nutritional Characteristics of The Rats at the End of The Experiment**

Parameters	Diets			
	SAI n = 6	PLI n = 6	PAI n = 6	NEI n = 6
M.I (g)	61,47 ± 5,31[a]	61,59 ± 4,75[a]	62,04 ± 5,03[a]	62,13 ± 5,12[a]
M.F (g)	84,78 ± 7,33[c]	40,2 ± 5,22[a]	70,29 ± 4,16[b]	84,83±5,03[c]
G.M.C (g)	1,1±0,23[c]	-1,02±0,27[a]	0,39±0,20[b]	1,08±0,42[c]
A.I (g)	20,74±4,12[c]	12,86±3,39[a]	15,47±2,83[b]	21,39±3,20[c]
M.S.I (g)	9,33±1,75[c]	5,59±0,87[a]	7,53±2,37[b]	11,02±1,58[d]
I.P (g)	0,93±0,03[c]	0,55±0,01[a]	0,75±0,03[b]	1,10 ± 0,03[d]
C.F.A	0,11±0,00[d]	-0,18±0,00[a]	0,05±0,00[b]	0,09±0,00[c]
C.E.P	1,18±0,00[d]	-1,85±0,03[a]	0,05±0,02[b]	0,98±0,02[c]

Experiment duration 21 days; n: number of rats per batch; SAI: soy bean powder control diet; PLI: lima bean powder diet; PAI: pigeon pea powder diet; NEI: cowpea seed powder diet; M. I: initial mass; M.F: final mass; GMC: body mass gain; AI: feed intake; I.P: protein intake; FEC: feed efficiency coefficient; FEC: feed efficiency coefficient; FEC: feed efficiency coefficient. Protein efficiency; g: Gram. Means are followed by letters (a, b, c and d) in super script. On the same line, means with different letters are significantly different ($p \leq 0.05$)

3.2 Effects of legume consumption on the mean value of serum parameters in rats

Table 3 shows the mean values of serum metabolites of rats at the end of the animal experiment. The lowest glucose level (0.59 ± 0.00 g/L) was observed in rats fed PAL and SAI diets, and the highest level is obtained with rats fed NEI diet (0.82 ± 0.23 g/L). The differences in glucose levels were significant (p ≤ 0.05) between the control and the other three batches of rats. Mean triglyceride values did not vary between the four batches of rats (p >0.05). The mean value of serum total protein in rats on the NEI diet (45.66 ± 5.13 g/L) was the lowest and that of animals on the PAL diet was higher (69.33 ± 6.65 g/L). The serum total cholesterol level in rats fed the PAI diet (0.76 ± 0.00 g/L) was lower while that in rats fed the NEI diet was higher (0.88 ± 0.04 g/L). The lowest mean serum urea value was obtained with PAI (0.17± 0.01 g/L) and the highest value was obtained with PAL (0.30 ± 0.00 g/L). The mean creatinine value of rats fed PAI (2.33 ± 0.5 mg/L) was statistically lower (p≤0.05) than those of the other three diets (SAI, PLI and NEI). Mean uric acid values ranged from 15.33 ±2.51 mg/L (NEI) to 40.00 ± 0.07 mg/L (PAI). The highest serum total bilirubin level (7.86 ± 0.4 mg/L) was observed with SAI while the lowest was observed with NEI rats (5.8 ± 0.88 mg/L). Serum conjugated bilirubin levels did not vary statistically (p >0.05) between the four batches of rats.

Table 3: Mean Value of Serum Parameters of Rats at the End of the Experiment

Parameters	Diets			
	SAI n = 6	**PLI n = 6**	**PAI n = 6**	**NEI n = 6**
Glucose (g/L)	0,59 ± 0,00[a]	0,59 ± 0,00[a]	0,68 ± 0,0[b]	0,82±0,23[c]
Triglyceride (g/L)	0,99±0,07[a]	0,97±0,03[a]	0,97±0,0[a]	1,05±0,01[a]
Total proteins (g/L)	65±2,64[b]	69,33±6,65[c]	65±3[b]	45,66±5,13[a]
Total cholesterol (g/L)	0,82±0,04[b]	0,81±0,02[b]	0,76±0,0[a]	0,88±0,04[c]
HDL-cholesterol (g/L)	0,18±0,02[a]	0,21±0,01[a]	0,20±0,01[a]	0,21±0,00 [a]
LDL-cholesterol (g/L)	0,44±0,04[a]	0,41±0,03[a]	0,65±0,04[b]	0,69±0,01[c]
Urea (g/L)	0,21±0,01[b]	0,30±0,00[c]	0,17±0,01[a]	0,21±0,01[b]
Creatinine (mg/L)	3±0,00[b]	3,33±0,6[b]	2,33±0,5[a]	3±0,00[b]
Uric acid (mg/L)	19±3,60[b]	29,66±1,52[c]	40±0,07[d]	15,33±2,51[a]
Conjugated bilirubins (mg/L)	7,86±0,49[b]	6,9±0,52[ab]	6,7±0,01[ab]	5,8±0,88[a]
Total bilirubins (mg/L)	1,06±0,15[a]	0,98±0,16[a]	1,04±0,14[a]	1±0,03[a]

Experiment duration: 21 days; n: number of rats per batch; SAI: soybean powder control diet; PLI: lima bean powder diet; PAI: pigeon pea powder diet; NEI: cowpea seed powder diet. The averages are followed by letters (a, b, c and d) in super script. On the same line, means with different letters are significantly different (p ≤ 0.05).

IV DISCUSSION

In this experiment, the soybean control diet (SAI) and the test diets (cowpea: NEI; pigeonpea: PAI) fed to the rats had positive effects on their growth while the formulated diet (lima bean: PLI) had a negative effect. This was reflected in the daily body weight gains observed in the animals on the four protein feeds. The final body weight values of rats on the soybean control diet and the cowpea diet showed no significant difference (P>0.05). This is because the amino acid profile of cowpea seeds is similar to that of soybean [10]. Their seeds have been successfully used by [11] in the feeding of local strain chicks at 10% and 15%, respectively during start-up and growth-finishing in Burkina-Faso. The body mass gain of the experimental animals is evidence of good utilization of the feed protein and good utilization of energy nutrients during the increase of adipose tissue. According to [12], the quality of the ingested protein depends on the amino acids and digestibility of the protein. Furthermore, this growth is more pronounced in rats fed the soybean and cowpea-based diets compared to the batch of rats consuming the pigeonpea seed diet, which has the lowest body mass gain. The loss of body mass of the animals on the Lima bean diet is a consequence of the low amount of dry matter ingested. The energy value of the control diet is lower than that of the Lima bean diet. When calorie demands are not met in the diet, the efficiency of protein utilisation is reduced. Thus, there is a threshold for energy intake below which the nutritional value of the protein consumed decreases. Similarly, in an undernourished individual, an increase in protein intake will only be fully effective if the diet provides the necessary amount of energy. This means that insufficient caloric intake is itself a cause of protein wastage [13]. The levels of protein ingested by rats consuming lima bean seeds are low compared to those of rats fed the soya diet. This is confirmed by the low value of the feed efficiency ratio and the protein efficiency ratio of the lima bean diet. This low value of the characteristics and the poor performance would be due to the presence of anti-nutritional factors (ANF), notably oxalates and phytates that the seeds of this legume would contain [14]. ANF could act at several levels by reducing protein assimilation, by deregulating pancreatic enzyme secretion and by limiting the assimilation of amino acids by clogging the intestinal mucosa with incompletely digested oligopeptides. The total blood cholesterol level for rats fed the control diet is lower than for rats fed the (NEI) diet. Incubation of saturated fatty acid in the diet causes hypercholesterolaemia in rats [15]. The consumption of these legumes does not affect the mean values of HDL-cholesterol, triglycerides and conjugated bilirubin in rats. The formulated diet (PLI) causes a decrease in the mean LDL-cholesterol value, while those of the diets (PAI and NEI) increase significantly compared to the control diet. The consumption of the diet (PLI) causes an increase in the mean value of urea, while that of the diet (PAI) decreases significantly. This is because creatinine is, like urea for routine assessment of renal function, a constituent of muscle protein, eliminated only by the kidneys. It is not affected by extra-renal factors [16, 17] has shown that an abnormal increase in serum creatinine occurs in dogs undergoing nephrectomy. These values are higher than those found by [18]. As for total protein values, they are normal in rats fed the diet (PAI). The lowest value is observed in rats fed the diet (NEI). Consumption of the pigeonpea powder diet caused a significant increase in mean uric acid compared to the control, PLI and NEI diets, while the total bilirubin value of rats fed the control diet was higher than those fed the other three formulated diets. These values are lower than those of [19]. Indeed, these authors found that consumption of soybean meal diets fortified with chromium tripicolinate resulted in an increase in the mean uric acid value in rats.

V CONCLUSION

At the end of this experiment, the results obtained indicate that the proteins present in pigeon pea and cowpea seeds, as well as in soybeans, promote the growth of rats. However, the consumption of lima bean powder led to a loss of body mass in the rats, which would be due to the low biological value of their proteins and a very low rate of ingested food. These effects would be justified by the presence of anti-nutritional substances contained in lima bean seeds, as lima beans are not palatable and digestible enough. Pulses would be recommended in the diets of malnourished children.

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