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Effect of Food Intake on Weight Gain, Liver Weight and Composition in Rats Fed Dehulled African Yam Bean and Bambara Groundnut Supplemented with Sorghum or Crayfish

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Abstract: Forty-eight adult Wistar albino male rats (75-128g) of age 8 weeks were used to study the effect of dehulled African yam bean (DYB) and dehulled soaked bambara groundnut combined with sorghum or crayfish on food intake, weight gain, liver weight and composition of rats fed for 10 days. Three out of the ten days were for adjustment and the rest for the balance period. Eight diets were formulated and fed to the rats. The mixed protein diets provided 10% protein daily for the entire study period. Casein (CA) served as a reference protein. Rats fed dehulled bambara groundnut brown (DBGB) and sorghum soaked for 18 hours (S₁₉) had the least food intake, weight gain, liver weight and liver nitrogen (N). Dehulled African yam bean (DYB) supplemented with sorghum soaked for 18 hours (S18) had the highest liver lipids which was significantly higher (P < 0.05) than those of the other test groups and control (Casein). DYB:S₁₈ also has the least liver moisture which was significantly lower (P < 0.05) than those of the other test groups and casein. Addition of crayfish (CR) to soaked brown bambara groundnut (SBGB) and sorghum soaked for 24 hours (S_{24}) produced significant increase (P < 0.05) in liver N (183.7g) but a significant decrease in liver lipids (3.2g). As judged by liver nutriture, SBGB: S_{24} : CR appears to be a better supplement than the test groups and control. This result suggested that different cultivars, varietal difference and treatment affected food intake, weight gain, liver weight and composition of rats fed dehulled African yam bean (DYB) and bambara groundnut supplemented with sorghum and/or crayfish.

Key words: Sorghum, bambara groundnut, rats, African yam bean

INTRODUCTION

In Nigeria, protein energy malnutrition is common. Presently, protein energy malnutrition is endemic and the situation is complicated by inflation, economic recession, insufficient local production of animal protein sources, insufficient system for the distribution of animal protein sources and above all the exorbitant cost which made it out of reach for the low socio-economic class (Okoh *et al.*, 1985).

The low income groups who constitute the bulk of the population are particularly at risk in this situation. They have no alternative other than to depend on cereals and legumes (Okoh *et al.*, 1985) which are cheaper and are easily affordable (Osagie and Eka, 1998). Cheap and nutritious mixed protein diets based on locally available and acceptable plant protein foods to serve as substitutes for animal proteins appear to be the best situation (Obizoba, 1985a).

Sorghum is a popular cereal in Nigeria, particularly in the northern part of the country where it is produced. It constitutes a major source of energy and protein in areas where it is a staple. The protein varies from 7.5 to 9.0% and like other cereal proteins, it is limited in amino acids, lysine, threonine tryptophan and methionine. Supplementation with other foods whose protein would complement the amino acid composition is necessary (Nnam, 2001). Cereal proteins are excellent sources of methionine while legume proteins contain a high level of lysine and could be of potential usefulness for enriching cereal based diets (Obizoba, 1985b).

In Nigeria, crayfish (*Atacus fluviatilis*) are generally used as a food condiment. In some parts of Nigeria, however, crayfish are used as a source of animal protein and other often form the main source of dietary protein. The crude protein content of crayfish has been shown to be 60-70% (Anyika, 2003).

Bambara groundnut is an important legume produced extensively in northern Nigeria. The legume is sweet and pleasant to eat either as dry or immature seeds. The protein content in the grains range from 20-25% (dry matter basis) (Arora, 1995). It has more methionine than is found in other grain legumes and would serve as a good supplement to sorghum protein (Nnam, 2001). African yam bean (AYB) is among the under-exploited

legumes in Nigeria. The stem produces a small underground tuber which is not consumed in Nigeria. The pods are long and about 25-30cm, with smooth, hard roundish seeds which may be white, brown, black or mottled. The dry seeds are considered delicious by many and are often preferred over other grain legumes (Okigbo *et al.*, 1997).

Legumes and cereals are inexpensive sources of protein, minerals and vitamins, but despite the nutritional value and increased production of legumes, consumption has not increased in rural and urban populations like cereal products. The reasons are that legumes take relatively longer time to cook and there is much inconvenience encountered in preparing them into local dishes. Another negative factor in the use of legumes is their undesirable cooking characteristics (Anyika, 2003). Utilization of legume proteins depends both on the kind and method of processing (Greevani and Theophilus, 1981). Soaking and dehulling has been reported to increase levels of minerals and vitamins in both cereals and legumes (Roa and Prabhavathi, 1982). The objective of this study was to evaluate the effect of food intake on maintenance body weight, liver weight and composition of rats fed dehulled African yam bean and bambara groundnut supplemented with sorghum and/or crayfish.

MATERIALS AND METHODS

A 10-day mineral balance study was conducted; the study involved a 3-day adjustment and a 7-day balance period. These short frames for both the adjustment and collection of metabolic wastes were used because 3-7 days or more are enough for a balance study.

Animals and housing: Forty-eight 8 week old Wistar albino rats (75-128g) (supplied by the Department of pathology, University of Nigeria, Nsukka) were divided into 8 groups of 6 rats each on the basis of body weight. The rats were weighed prior to access to the experimental diets and at the end of the study to determine maintenance of body weight. They were individually housed in stainless steel metabolic cages equipped to separate urine and feaces of animals. The rats were fed their respective diets and deionized water ad libitum for 10 days, at the end of which the final weight was recorded.

Diets: Black African yam bean, bambara groundnut (white and brown varieties) sorghum and crayfish used as sources of dietary protein were purchased from Obukpa market, a small town near Nsukka in Enugu State, Nigeria.

Black Africa yam bean and bambara groundnuts, with a hard seed coat, were carefully split into two halves with a machine locally designed by Engineer (P.O. Ngoddy of the Department of Food Science and Technology, University of Nigeria, Nsukka). The split seeds were soaked for about 1^1I_2 hours in warm water ($35\pm0.2^{\circ}$ C) for easier seed coat removal before manual dehulling. The African yam bean and other halves of both varieties of bambara groundnut were soaked for about 5 hours before cooking.

Sorghum grains were soaked for 18 or 24 hours (S₁₈) and (S₂₄). The grains, after treatment were cooked separately in 1:3 deionized water at 100±0.2°C for 45 mins until they were judged soft enough for human consumption. They were removed from the fire and were found to have little or no cooking water left in the cooking utensil. The cooked grains were dried separately to 96% dry matter in GallenKamp, size one, BS oven at 85°C for 8 hrs before being ground to a fine powder in a Wiley laboratory hammer mill (70mm mesh screen). The crayfish (CR) (Astacus spp.) was also dried in the Gallekamp, size one, BS oven at 85°C for 30 mins and then ground into a fine powder using the same machine and screen size as the grains. The flours from the grains and crayfish were stored in polythene bags and frozen in a thermocool refrigerator at -7°C until used for the formulation of the test diets.

Table 1 data indicate the ingredient composition of the experimental diets. The control diet contained only casein as the sole source of protein. The seven diets derived 70% of their nutrients from dehulled African yam bean (DYB), dehulled bambara groundnut white (DBGW), dehulled bambara groundnut brown (DBGB), soaked bambara groundnut white (SBGW) and soaked bambara groundnut brown (SBGB). Five diets had 30% of their remaining dietary protein either from sorghum soaked for 18 hours (S_{18}) or 24 hours (S_{24}). Two diets had 30% of their dietary protein from 15% crayfish (CR). Food intakes were recorded (7 days) and the data were used to know its effect on weight gain, liver weight and composition for each rat.

Laboratory analysis: At the end of the study, the animals were decapitated using a decapitator. The animals were dissected and the livers were removed and weighed. The livers were dried in the Gallenkamp, size one, BS oven at 85°C for 24 hours to determine the percentage moisture. The liver nitrogen was determined using AOAC (1990). Liver lipids were determined using Tecator-Soxtec apparatus (Pearson, 1976). The liver of rats fed the same diets were ground into a fine powder in a hammer mill (Thomas Wiley mill, Model ED-5) (70 mesh screen). The flours of the liver were stored in polythene bags and frozen in a thermocool refrigerator at -7°C until analyzed.

One way analysis of variance and Duncan's Multiple Range Test (Steel and Torrie, 1960) were used to determine significant difference of food intake, maintenance body weight, liver weight, nitrogen, lipids and moisture.

Table 1: Composition of diets (g) with varying levels of groundnut, sorghum and crayfish

	DYB:S ₁₈	DYB:S₁₅:CR	DBGW:S₁8	DBGB:S ₁₈	SBGW:S ₂₄	SBGB:S ₂₄	SBGB:S ₂₄ :CR	CA
Protein sources and ratios in diets	70:30	70:15:15	70:30	70:30	70:30	70:30	70:15:15	100
Dehulled African yam bean²	306.42	306.42	-	-	-	-	-	-
Dehulled BGW ²	-	-	355.36	-	-	-	_	-
Dehulled BGB ²	-	-	-	288.41	-	-	-	-
Soaked BGW²	-	-	-	-	347.29	-	-	-
Soaked BGB ²	-	-	-	-	-	305.73	305.73	-
Crayfish ² -	25.99	-	-	-	-	25.99	-	
Sorghum soaked (18 and 24 hours) ²	320.7	160.36	320.7	320.7	305.73	305.73	152.87	-
Groundnut oil ²	48.0	48.0	48.0	48.0	48.0	48.0	48.0	48.0
Vitamin mix1	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6
Mineral mix1	33.6	33.6	33.6	33.6	33.6	33.6	33.6	33.6
Sucrose ¹	120.84	188.0	96.37	129.85	107.89	128.67	192.2	284.4
Corn starch¹	120.84	188.0	96.37	129.84	107.89	128.67	192.1	284.4
Caesin ¹	-	-	-	-	-	-	-	300.0
	960.0	960.0	960.0	960.0	960.0	960.0	960.0	960.0

Purchased from Tekland Dawley Inc.; Box 8156, Madison, Wisconsin, 53708, USA. Purchased from Local Retailers: DYB = Dehulled African yam bean; BGW = Bambara groundnut white; BGB = Bambara groundnut brown; D = Dehulled; S = Soaked (18 or 24 h); CA = Casein

RESULTS AND DISCUSSION

Table 2 presents the food intake, weight gain, liver weight and composition of rats fed with dehulled African yam bean, dehulled bambara groundnut supplemented with sorghum or crayfish. The food intake of all groups varied. DYB: S₁₈ had significantly (P < 0.05) higher food intake than both the control (casein) and the test groups. Food intake can be influenced by palatability, source of N and essential amino acid (EAA) profile. The higher food intake of rats fed DYB:S18 might be due to a combination of these factors. The casein group had significantly (P < 0.05) lower food intake than any others except the groups fed the DYB:S₁₈:CR, DBGB:S₁₈ and SBGW:S₂₄ diets. The higher significant difference (P<0.05) between DYB:S₁₈ and DYB:S₁₈:CR groups indicates that CR has a negative influence on food intake. This made the rats not to eat much of DYB:S₁₈:CR group. The CR either imparted an unfavorable aroma or taste or both on the DYB:S₁₈:CR diet.

The body weight of all groups varied. Diets containing DBGW: S_{18} maintained body weight (36.3g) in rats better that did casein and other test diets. Casein diet maintained the least body weight (17.8g). This might be attributed to lower food intake. Animals are known to eat more food when it has good organoleptic appeal (Obizoba, 1985a).

Liver weights of rats receiving dehulled African yam bean, dehulled and soaked bambara groundnut diets were directly related to food intake and weight gain. The animals that ate the least of the test diets (DBGB:S $_{18}$ and SBGW:S $_{24}$) had the least liver weight (4.5g and 4.7g) respectively. Liver weight was very sensitive to the level of food intake. The higher the food intake and weight gain, the higher the liver weight and vice versa. The low liver weight of DYB:S $_{18}$:CR diet as compared with DYB:S $_{18}$ and those of SBGB:S $_{24}$:CR as compared with SBGB:S $_{24}$ diet was comparable to what Obizoba (1985b) observed when he compared the supplementary effect of CR and brown bean to rice protein.

The liver N for all groups varied. The variation was due to food intake, source of N, liver lipid and moisture. The DBGW:S₁₈ diet had significantly (P < 0.05) higher liver N than casein and other test diets. Addition of equal amounts of CR to replace those of S₁₈ or S₂₄ lowered liver N in DYB group and increased liver N in the SBGB:S₂₄:CR group. The decrease in liver N due to the substitution of CR for S_{18} in AYB confirms that CR was inferior to sorghum as a supplement to AYB. The higher significant increase (P < 0.05) in the addition of CR to BG as compared to the higher significant decrease (P < 0.05) in liver N of the group fed DYB:S₁₈:CR diet was due to type of legume. BG appears to have a much better mutual supplementation effect with CR as opposed to YB. Addition of equal amounts of CR to replace those of S₂₄ indicated a synergistic supplementation effect which presumably resulted in a better essential amino acid (EAA) pattern which produced better quality protein with SBGB. This high quality protein furnished the EAA which the animals used to synthesize the liver N. The supplementation of DYB:S18 diet with CR was not beneficial. This was not surprising. Obizoba (Obizoba, 1985b) found that above a certain supplementation, brown bean protein was superior to CR as a supplement to rice protein.

The liver lipids of all the diets differed. The DYB:S $_{18}$ group had the highest value which was significantly higher than those of the other groups (P < 0.05) except for the SBGB:S $_{24}$ group. The SBGB:S $_{24}$ CR diet had significantly lower (P < 0.05) liver lipids than both casein and the other test diets. The liver lipids of animals fed DYB:S $_{18}$ diet was significantly higher (P < 0.05) than for all the groups. The significantly lower (P < 0.05) value between SBGB:S $_{24}$:CR and SBGB:S $_{24}$; DYB:S $_{18}$:CR and DYB:S $_{18}$ indicates that CR was a better supplement to both B.G. and D.Y.B. regardless of the treatments. Liver lipids is very sensitive to the level of protein in the liver (Waterloo, 1969). When the liver N is low, albumin synthesis in the liver decreases resulting in lower albumin in the plasma (Millward and Garlick, 1972; Ene-

Table 2: Food intake, weight gain, liver weight and composition of rats fed dehulled African yam bean and bambara groundnut supplemented with sorghum or cravfish

Food grains and crayfish	DYB:S ₁₈	DYB:S ₁₈ :CR	DBGW: S ₁₈	DBGB: S ₁₈ 70:30	
and their ratios in diets	70:30	70:15:15	70:30		
Food intake*, g	78.0°±5.0	69.3°±4.5 73.4°±1.9		65.7b±4.7	
Weight gain, g	26.2e±6.6	29.8°±5.0	36.3°±4.2	20.8b±5.2	
Liver weight ² , g	5.8°±0.5	5.1b±0.5	5.8°±0.7	4.5°±0.2	
Liver N ² , g	145.4°±12.5	79.4b±5.6	187.2g±18.4	70.0°±2.9	
Liver Lipids ² , %	14.7⁴±1.3	9.7e±0.7	6.5°±0.6	5.9b±0.2	
Liver moisture ² , %	31.8°±1.5	34.7b±3.0	38.8°±2.7	36.9b±1.7	

Table 2: Continue

Food grains and crayfish	SBGW:S ₂₄	SBGB:S ₂₄	SBGB:S ₂₄ :CR	CA
and their ratios in diets	70:30	70:30	70:15:15	100
Food intake*, g	65.7°±5.3	72.8°±5.5	71.6°±5.9	54.8°±3.3
Weight gain, g	21.4°±3.7	31.2°±5.2	28.6°±4.9	17.8°±1.8
Liver weight2, g	4.7°±0.3	6.2°±0.5	5.3b±0.5	5.0b±0.1
Liver N2, g	126.6°±8.3	161.2°±51.4	183.7 ^f ±20.6	121.6°±3.7
Liver Lipids2, %	8.1 ^d ±0.5	13.6 ^f ±1.0	3.2°±0.4	9.3°±0.3
Liver moisture2, %	37.7°±2.4	36.8b±1.8	34.6 ^b ±2.3	35.0⁰±1.0

¹Mean±SEM (6 rats); *7 day food intake; 2 Dry weight basis. Means with different superscript letters in a row differ significantly (P < 0.05). DYB = Dehulled African yam bean; BGW = Bambara groundnut white; BGB = Bambara groundnut brown; D = Dehulled; S = Soaked (18 or 24h); CA = Casein.

Obong and Obizoba, 1995). This defect in protein synthesis results in decreased levels of protein needed to form very-low-density lipoproteins (VLDL) which the liver forms to export triglyceride to the periphery. Fatty liver observed in DYB:S $_{18}$ and SBGB:S $_{24}$ diets might be due to increased lipolysis of lipids and the inability of the liver to deal with the export of excess fat formed from this specific fat-producing diet.

There is no significant differences (P > 0.05) in the liver moisture for all groups of rats except for the DYB:S₁₈ group (P < 0.05). It is known that the higher the protein content of a given protein source, the lower is the moisture content. This makes for better protein stability. Hydrophobic bonding is of great importance in the stability of protein molecules and is the result of lack of attraction for water molecules. Proteins are generally folded so that the non-polar groups are on the inside of the molecule while the charged groups are exposed to water on the outside. The polar groups of proteins, especially ones of amino acids are chiefly responsible for binding of water (Ihekoronye and Ngoddy, 1985). Dehulling and soaking had little or no influence on liver moisture. The significantly lower (P < 0.05) liver moisture of the group fed the DYB:S18 diet as compared to the other groups except for the DBGW: S18 diet might be attributed to the type of lugume (YB vs BG).

Based on these observations, there is need to reemphasize that when legumes and cereals are appropriately combined, their protein quality may be as good as that of casein or other animal protein sources or better than them. Crayfish protein was a better supplement to soaked brown bambara groundnut with sorghum soaked for 24 hours than dehulled African yam bean with sorghum soaked for 18 hours. The practice of combining dehulled brown bambara groundnut with sorghum soaked for 18 hours and adding crayfish to dehulled African yam bean needs to be discouraged. African yam bean, which is an under-exploited legume has proved to be very nutritious. There is need to reintroduce this legume into the diet of the populace. This can be achieved by increased production and development of appropriate processing technology in order to further improve its nutritional quality and increase its incorporation into the traditional diets of children and adults. This might be attributed to lower food intake. Animals are known to eat more food when it has good organoleptic appeal (Nnam, 2001).

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