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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Digestibility of Neutral Lipids and Phospholipids from Isocaloric Leguminous Based Diets by Rats

A.A. Odutuga[1], F.A. Bamisaye[1], A.A. Amballi[2] and G.B. Adebayo[3]
[1]Department of Biochemistry, College of Natural Sciences,
Joseph Ayo Babalola University, Ikeji-Arakeji, P.M.B. 5006, Ilesa, Osun State, Nigeria
[2]Department of Chemical Pathology, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria
[3]Department of Chemistry, Kwara State Polytechnic, Ilorin, Kwara State, Nigeria

Abstract: The effects of three isocaloric leguminous based diets on the digestibility of neutral lipids and phospholipids in albino rats were investigated. Proximate analyses of each of the beans and formulated diets were carried out to determine crude protein, crude fat, crude fibre, total ash, moisture and carbohydrate contents. A total of thirty (30) albino rats (Rattus norvegicus) were used for the study. The rats were divided into three groups of ten rats each and maintained on Soyabean-Based Diet (SBD) (Control), Cowpea-Based Diet (CBD) and Hyacinth Bean-based Diet (HBD) for a period of five weeks. At the end of the experiment, the brain, liver, heart and the kidney of the rats were isolated and the neutral lipids and phospholipids contents of each organ were estimated. Rats maintained on Cowpea-Based Diet (CBD) had the lowest weight gain when compared with those fed on the HBD and SBD respectively. The results revealed that the neutral lipids and phospholipids of CBD show a significantly reduced ($p<0.05$) digestibility than HBD and SBD. The significantly reduced ($p<0.05$) digestibility of lipids in CBD may be considered to be partly responsible for the lower growth rate of rats maintained on this diet.

Key words: Isocaloric, leguminous, neutral lipids, phospholipids, soybean, cowpea and hyacinth bean

Introduction

Nutrition contributes significantly to the well-being of a populace by innovative discovery of new food sources that can supply the essential nutrients in the diet. One of such nutrients is lipid. Lipids supply essential fatty acids that are necessary for the maintenance of cellular membrane integrity and the various biochemical functions associated with it (Oyeleke, 1984). Lipids are agents that, in situ or via the circulation, exert a broad spectrum of physiological and pharmacological effects on many target tissues (Odutuga *et al*., 1997).

The essential fatty acids are usually limited in a diet and must be taken in from sources which are especially rich in them. Fat content of most pulses is low (1 to 2%). Malnutrition is usually prevalent where too few different plant foods are available, most especially in poor homes which results from irregular and unbalanced diet (Olaofe *et al*., 1998).

At present, the developing countries of the world are advocating for a breakthrough in science and technology; therefore any research on the locally available materials will be an advantage. It has been advocated that the problem of balanced diet in poor families is primary and research should be centered on nutrient composition in locally available food materials (Fetuga *et al*., 1983).

It has been increasingly recognized in recent years that the production of high yielding and high quality varieties of pulses would therefore, offer the least expensive and most immediate practical way of diminishing the threat of high cost of the few available ones. However, not much work had been done on the breeding and selection of the tropical pulse crops; although the local cultivators must have carried out some selection for palatability and adaptation to their local environments over the many countries in which they have been grown (Purseglove, 1988). These nutritional limitations due to lipids of low nutritional qualities prompted this investigation.

Materials and Methods

Source of the diets: Soyabean (Glycine max), Cowpea (Vigna sinensis), Groundnut oil, Maize for corn starch and Maize husk (source of cellulose) were purchased at the Ipata Market, Ilorin, Kwara State, Nigeria. Hyacinth bean (Dolichos lablab) were obtained from Government Secondary School farm, Ilorin, Nigeria. The vitamin/mineral mixture and DL-Methionine were purchased at Affcom Live Stock Feeds, Kulende Junction, Ilorin, Nigeria and they are products of Nomados International Feed Industries, 6090 A,A, Halclen, Holland.

Preparation of the diet: Each of the three bean samples was cooked, sun-dried, grounded into power and sieved. The samples were then used to formulate 3 different diets: Soyabean-Based Diet (SBD) Cowpea-Based Diet (CBD) and Hyacinth Bean-based Diet (HBD)

Table 1: Proximate Analysis of Cooked Beans (%)

	Beans		
	Soyabean	Cowpea	Hyacinth bean
Crude protein	28.03±0.44	24.72±0.68	21.43±0.42
Crude Fat	10.00±0.06	3.50±0.12	0.50±0.25
Crude Fibre	7.00±0.06	2.00±0.14	7.50±0.07
Total ash	3.50±0.05	3.00±0.04	2.75±0.02
Moisture	18.70±0.05	17.30±0.04	13.25±0.05
Carbohydrate	32.77±0.05	50.48±0.64	54.57±0.62

The results are the mean values of 5 analyses±SEM

Table 2: Composition of the Formulated Diet (G/kg)

	CSBD	CCBD	CHBD
Corn Starch	516	387	406
CSB	250	-	-
CCB	-	379	-
CHB	-	-	360
DL-Methionine	4	4	4
Cellulose	40	40	40
Sucrose	60	60	60
Groundnutoil	80	80	80
Mineral/Vit.Mix	50	50	50

Mineral/Vitamin mixture contents (g/kg): Vitamin A (0.04); Vitamin D_3 (0.080); Vitamin E (0.04); Nicotine acid (0.02); Calcium pantothenate (0.20); Vitamin B_6 (0.048); Vitamin B_{12} (0.000013); Folic acid (0.04); Biotin (0.02); Vitamin C (0.009); Chloride (0.001); Managanese (2.290); Iron (1.000); Zinc (2.300); Iodine (0.80); Copper (0.80); Cobalt (0.01); Selenium (0.04)

Table 3: Proximate Analyses of Formulated Diets (%)

	Diets		
	SBD	CBD	HBD
Crude protein	18.57±0.05	18.59±0.02	18.60±0.02
Crude Fat	7.50±0.01	7.50±0.04	7.53±0.04
Crude Fibre	1.00±0.08	0.95±0.14	1.02±0.07
Total ash	5.50±0.02	5.45±0.02	5.30±0.08
Moisture	3.40±0.04	3.40±0.07	4.20±0.16
Carbohydrate	64.03±0.08	64.11±0.07	63.35±0.07

The results are the mean values of 5 analyses±SEM

Table 4: Growth Response and Lipids Excreted in Feces of Experimental Rats Fed Different Bean Diets

Diets	SBD	CBD	HBD
Initial body weight (g)	34.40±1.83^a	35.20±1.39^a	34.60±2.27^a
Final body weight (g)	53.82±3.59^a	44.09±2.18^c	46.27±4.83^b
Body weight gain (g/day)	0.55	0.25	0.33
Lipid in feces (%)	3.50±1.43	5.50±0.49	5.00±0.08
Feed intake (g/day)	3.85	3.31	3.59

The results are the mean values of 5 analyses±SEM

Table 5: The Percentage Digestibility of Lipids in the Rats Fed on Soyabean-based Diet (Sbd), Cowpea-based Diet (Cbd) and Hyacinthbean-based Diet (Hbd) for Five Weeks

	Diets		
	SBD	CBD	HBD
Total lipid	53.33±2.01^a	26.67±1.08^b	33.60±1.24^c
Phospholipids	23.20±1.32^a	8.93±0.82^b	14.21±1.02^c
Neutral lipids	29.87±1.51^a	17.73±1.14^b	19.39±1.33^b

a,b,c column values with different superscripts are significantly different ($p<0.05$)

following the standard procedures of Odutuga and Oloyede (1982). The proximate analysis was carried out on them using standard procedures of Oyeleke (1984) and A.O.A.C. (1990). The results obtained from the proximate analysis of the cooked beans (Table 1) were used to compose the isocaloric diets (Table 2).

Experimental animals: Thirty weanling albino rats (Rattus norvegicus) 21 days old, were used for the experiment by randomly distributing the rats into three groups, each containing 10 rats. They were then placed on the formulated diets, namely: Cooked Soyabean-Based Diet (CSBD) as the control, Cooked Cowpea-Based Diet (CCBD) and Cooked Hyacinthbean-Based Diet (CHBD).

The diets and water were given to the rats ad libitum. The animals were weighed weekly for five weeks. Within each diet, the collected feces, pooled from 2 rats to give 5 groups per diet were enlist for total neutral lipids and total phospholipids using A.O.A.C. methods (1990). The digestibility of the total lipids and total phospholipids was determined as described by Odutuga and Oloyede (1982). The growth rate was also determined (Odutuga and Oloyede, 1982).

The rats were killed at 5 weeks and the brain liver, kidney and heart removed and weighed. The lipid content of these organs was also analyzed as previously described (Odutuga, 1982).

Analyses of variance were carried out to determine the statistical significance of results.

Results and Discussion

Table 1 shows the proximate analysis of cooked beans while Table 2 and 3 show the compositions of the formulated diets and their proximate analyses respectively. Soya Bean Diet (SBD), Cowpea Bean Diet (CBD) and Hyacinth Bean Diet (HBD) contain approximately the same percentage of crude oil.

The growth response and lipids excreted in feces of the rats is shown in Table 4. Compared with the SBD, rats fed on the hyacinth bean based diet had significantly ($p<0.05$) higher fat contents in their feces. The rats fed with CBD had significantly ($p<0.05$) higher crude fat contents in their feces than with SBD. The observed higher faecal excretion of lipid in rats fed on CBD and HBD is considered to be partly due to decrease in percentage digestibility of lipids in them. Further more, the higher faecal excretion of lipids in rats fed on CBD and HBD compared with SBD may imply that the amount of Leucine, phenylalanine and tyrosine which are changed to lipids and digested is more in the SBD as earlier reported that about 40% of the protein follows the fatty acid path (Mottram, 1982). All the diets used for this study were made isocaloric, hence, the only source of difference in the percentage of lipid in these rats may be from the quality of proteins from these different diets.

Table 6: Lipid Content of Organs of Rats Fed Different Bean Diets

		Diets		
	Organs	SBD	CBD	HBD
Brain	Dry weight (g)	0.92±0.02	0.80±0.04	0.85±0.03
	Lipid (% dry weight of tissue)	50.00±0.04[a]	48.00±0.07[b]	49.20±0.06[b]
Liver	Dry weight (g)	1.20±0.02	1.25±0.26	1.40±0.18
	Lipid (% dry weight of tissue)	46.30±0.31[a]	43.80±0.51[b]	44.70±0.25[b]
Kidney	Dry weight (g)	0.31± 0.04	0.18±0.06	0.25±0.02
	Lipid (% dry weight of tissue)	49.00±0.07[a]	47.27±0.12[b]	47.10±0.05[b]
Heart	Dry weight (g)	0.96±0.02	0.70±0.02	0.90±0.01
	Lipid (% dry weight of tissue)	46.00±0.05[a]	44.21±0.04[b]	44.90±0.02[b]

The results are the mean values of 5 analyses±SEM; a, b, c, column values with different superscripts are significantly different ($p<0.05$), Results are expressed as percentage dry weight of organs

Table 4 also, shows that rats maintained on SBD have considerably higher final body weight (53.82g±3.59) when compared with those fed with the cowpea-based diet (44.09g+2.18) and hyacinth bean based diet (46.27g+4.83). The body weight gains are in the order SBD>HBD>CBD. This may be due to the quality of lipids in the different beans used to compose the diets as observed in previous findings (Odutuga, 1982; Odutuga *et al.*, 1997).

Table 5 depicts the percentage digestibility of both the neutral lipids and phospholipids in rats fed with different diets. The percentage digestibility of both the neutral lipids and phospholipids in rats fed with the SBD were significantly ($p<0.05$) higher than those fed with the CBD and HBD. The percentage digestibility of both the neutral lipids and phospholipids in rats fed with the CBD and HBD did not show any considerable difference. Animals fed with SBD had significantly ($p<0.05$) higher digestibility of the neutral lipids by 40.64% and 35.09% for CBD and HBD respectively. Similarly, the values for the phospholipids were 61.51% and 38.75% for CBD and HBD respectively.

It is evident from these results that animals maintained on the soyabean based diet had higher percentage digestibility of the neutral lipids and phospholipids than those fed with either the cowpea based diet or the hyacinth bean based diet. This also confirmed the observed highest amount of lipids present in the faecal excretion of rats fed with the CBD. The amount of lipids in the feces of rats fed with SBD was the lowest when compared with CBD and HBD. The decrease in percentage digestibility of lipid in rats fed with HBD and CBD is considered to be partly responsible for the higher faecal excretion in these rats. The result showed that the neutral lipids had higher percentage digestibility than the phospholipids. This may probably indicate that the phospholipids were not being digested to the same extent as the neutral lipids. Furthermore, the higher percentage digestibility of neutral lipids than phospholipids in rats fed with SBD has been observed earlier (Odutuga and Oloyede, 1982; Odutuga and Abe, 1999).

The higher percentage digestibility of neutral lipids than phospholipids in rats fed with SBD and other experimental diets observed in this study may be due to the fact that phospholipids may be complexing with the antinutritional factors to a higher degree than the neutral lipids owing to their structural differences. This complex formation may therefore, probably, be responsible for the higher faecal excretion of phospholipids (Odutuga and Oloyede, 1982). It has also been reported that higher percentage digestibility of neutral lipids than phospholipids were observed for rats fed with raw and cooked African Locust bean seeds (Odutuga and Oloyede, 1982). Cooking procedure may also be responsible for the removal or destruction of a higher percentage of the anti-nutritional factors present in soyabean (Jaffe, 1975).

The results of this study therefore, indicate that the lower digestibility of lipids of bean diets is responsible in part for the lower growth rate of rats fed with CBD and HBD. Rats fed with CBD had the least growth. Soyabean based diet supported rat growth, better than cowpea or hyacinth bean based diets.

Table 6 shows that the weights of different organs studied were higher in animals fed with SBD than those fed with CBD and HBD. When compared on dry weight basis, brains of rats fed with SBD were significantly ($p<0.05$) higher than rats fed with CBD and HBD while brains of rats fed with SBD and HBD were significantly ($p<0.05$) higher than those fed CBD. There was a significant difference in the weight of the hearts of rats fed with SBD and those fed with HBD and CBD. But there was no significant difference ($p>0.05$) in the weights of the hearts of rats fed with HBD and CBD. The weight of the kidney shows the same trend as in the heart. The weight of the liver of rats fed with SBD were significantly ($p<0.05$) higher than those of the rats on the other experimental diets. There was no significant difference ($p>0.05$) in the weights of the liver of rats fed with HBD and CBD.

The higher percentage of lipids content observed in some organs of rats fed with SBD compared with CBD and HBD may imply that the amount (quantities) of leucine, phenylalanine and tyrosine which can be

changed to lipids are more in SBD compared with HBD and CBD.

Earlier report of Mottram (1982) indicated that the tissues can only build up their substance with amino acids in the presence of carbohydrate. Since all the diets used for this study were made isocaloric, then the source of these amino acids that may have channeled into fatty acid synthesis is most likely from the proteins of the diets, as the oil used in the diets composition is of the same origin. The only source of difference in the percentage of lipid in these organs may be from the qualities of proteins in the different formulated diets.

The present study has shown that the experimental rats fed with SBD had the higher digestibility of lipids than those rats fed with CBD and HBD and this probably further support their better growth rate reported.

The significance of this finding becomes apparent when one considers the role of phospholipids in membrane structure. Thus, some or all of the processes associated with membrane integrity might be impaired in rats with low phospholipid digestibility.

It will also be appreciated when one considers the fact that lipids are used for the synthesis of important materials in living organisms, such as sex hormones and eicosanoids. Low digestibility of these lipids may impair their synthesis.

References

A.O.A.C., 1990. Official Methods of Analysis Association of Official Analytical Chemists. Washington D.C.

Fetuga, B.L., G.M. Babatunde and V.A. Oyenuga, 1983. Protein quality of some Nigerian food stuffs, biological evaluation of protein quality. J. Sc. Ed. Agri., 24: 1515-1523.

Jaffe, W.G., 1975. Toxic Factors in beans. Their Practical importance in: Nutritional Aspects of Common beans and other legume seeds as animal and human foods (W.G. Jaffe, editor). Ribeirao Preto, S.P. Brazil, pp: 109.

Mottram, R.F., 1982. Human Nutrition. 3rd Ed. Pub. By Edward Arnold, pp: 27.

Odutuga, A.A., 1982. Effects of low zinc status and EFA deficiency on growth and lipid composition of rat brain. Clin. Exptal. Pharmacol. Physiol., 2: 213-221.

Odutuga, A.A. and O.B. Oloyede, 1982. Digestibility of neutral lipids and Phospholipids from raw and cooked African Locust bean seeds (*Parkia filicoidea*) by rats. Nut. Reports Int., 26: 429-438.

Odutuga, A.A., J.A. Obaleye and F.O. Ologan, 1997. Thermoxidized soyabean oil: Spectroscopic investigation and the effects on selected rat tissues. Biokemistri, 7: 45-57.

Odutuga, A.A. and O.O. Abe, 1999. Effects of dietary soyabean and coconut oils on the lipid and fatty acid composition of rat kidney. Nig. J. Pure Appl. Sci., 14: 847-854.

Olaofe, O., L.A. Arogundade, E.J. Adeleye and O.M. Falusi, 1998. Composition and food properties of the variegated grasshopper, Zonocerus variegates. Trop. Sci., 38: 233-237.

Oyeleke, O.A., 1984. Outlines of Food Analysis. Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Purseglove, L., 1988. Tropical Crops: Dicotyledons. Low-Priced ed. Pub. By. Eng. Lang. Book Society (ELBS)/Longman. Printed in Singapore, pp: 199-201, 273-277.