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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Fatty acid, amino acid, mineral and antioxidant contents of acha (*Digitaria exilis*) grown on the Jos Plateau, Nigeria

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Digitaria exilis **(Kippist) Stapf (also known as acha, hungry rice) has been cultivated for millennia in the dry savannahs of West Africa, but much remains to be learned about its nutritional properties. Acha was collected in four villages in Northern Nigeria and analyzed for fatty acids, minerals, amino acids and antioxidant content. Fatty acids accounted for 1.91% of the dry weight, with 47.4% linoleic acid and 30.5% oleic acid. The content of the essential minerals, copper, magnesium, molybdenum, zinc and calcium averaged 4.88, 1060, 0.23, 23.0 and 172 µg/g, respectively. The protein content was 6.53% and the essential amino acid pattern, except for lysine, compared favorably to a World Health Organization (WHO) reference protein. The total polyphenolic content of methanolic extracts of acha matched that of common cereals (for example, maize, rice, wheat) and the extracts contained substantial amounts of free-radical scavenging substances. Thus, acha is a source of many nutrients critical to human health.**

Key words: Acha, fatty acids, minerals, amino acids, polyphenols.

INTRODUCTION

Cereals are an important source of many nutrients for populations in all regions of the world, but especially for people living in underdeveloped countries where the lack of protein-rich foods and economic constraints compel them to rely upon cereals such as maize, sorghum, millet or rice as the staple of their subsistence diets (Burkill, 1994; National Research Council, 1996; Protabase Record available at http:/database.prota.org/dbtw-wpd/exec/dbtwpub.dll?AC+QBE_QUERY&BU=http://atabase; Coda et al., 2010).

Acha (*Digitaria exilis* (Kippis) Stapf), also called pene, fonio, petit mil, fundi and hungry rice in different regions of Africa, is a member of the cereal family that includes maize and millet (Burkill, 1994; National Research Council, 1996; Jideani and Jideani, 2011; Philip and Itodo, 2006, 2012). The plant is grown from Cape Verde to Lake Chad and in other regions of sub-Saharan Africa where it provides dry-savannah populations with a cereal staple or major dietary component that compares favorably with rice, sorghum, maize and millet in terms of its content of protein, crude fat, carbohydrate and essential minerals (Leung et al., 1968; Burkill, 1994; National Research Council, 1996; Irving and Jideani, 1997; Jideani, 1999). Acha (funde) was imported into the New

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World from West Africa in the 15th Century and is cultivated and consumed by populations in the Dominican Republic (Dieve, 1974).

Acha is often referred to as “hungry rice” by the indigenous people of West Africa who consume this grain; however, this is a misleading term, implying it is a ‘famine food’ consumed only during times of food scarcity. In fact, acha is prized for its taste and plays important economic and cultural roles in West Africa (National Research Council, 1996). Acha is of considerable importance in Nigeria where it is commonly eaten, often in preference to other cereals, as many as three times a day as a porridge, couscous or non-alcoholic beverage (National Research Council, 1996; Jideani, 1999). It is also valued as a weaning food because of its low bulk and high caloric density. Acha requires minimal processing and can be cooked quickly. Other attributes of acha are that it grows even where rainfall and soil fertility are poor (Jideani, 1999) and can be stored in closed containers for many years without need of preservatives. Furthermore, contrary to the misconception mentioned above that some regard acha as a food only for poor populations, in Nigeria, it is widely regarded as a prestige food.

Although the literature contains reports of the content of certain nutrients in acha (Leung et al., 1968; Dieve, 1974; Temple and Bassa, 1991; Barikmo et al., 2004), it should be kept in mind that nutrient data for a particular plant food from one region of Africa may not necessarily be representative for the same plant grown in some other region where the climate, soil conditions and farming practices may be different.

Despite its popularity as a food item for millions in Africa, and the availability of informative reports of the nutrient composition of acha (Jideani et al., 1994; Philip and Itodo, 2006; Fogny-Fanou et al., 2009; Jideani and Jideani, 2011), there remains a need for additional information regarding its content of fatty acids, minerals and antioxidants. Furthermore, the nutrient content of a particular plant food can vary considerably between countries and even regions within a country due to variations in temperature, rainfall, fertilizer use and the nutrient content of the soil (Greenfield and Southgate, 1992). Chukwu and Abdul-Kadir (2008) recently conducted a study of the proximate chemical composition of acha in which they determined the content of 18 amino acids and 8 essential minerals in a sample of acha purchased in the Central Market in Minna, Nigeria. These investigators found that acha contained more of methionine and certain essential minerals, and trace elements (calcium, magnesium, iron and copper) than most cereals.

The main purpose of the present study was to replicate and extend the study by Chukwu and Abdul-Kadir (2008) by determining the fatty acid composition, amino acid content and the amounts of 13 minerals and trace elements in some specimens of acha purchased in markets in four different villages in Plateau State, Nigeria. In addition, we estimated the quantity of phenolic compounds in methanolic extracts of acha and the capacity of these extracts to scavenge free radicals.

MATERIALS AND METHODS

Collection of acha

Acha was purchased in 2009 from retailers in open-air markets located in four villages (Rantya, Bokkos, Ganawuri and Hoss) in Plateau State in north-central Nigeria. The approximate distances of these villages from the center of the city of Jos range from 10 km (Rantya) to 150 km (Bokkos). Botanical documentation of the identity of the four specimens of acha was provided by Dr. Timothy K. Lowrey, Curator of the University of New Mexico Herbarium Museum of Southwestern Biology, Albuquerque, New Mexico, USA. The grains were hulled and milled locally and then sun-dried and ground to powder with the aid of a mortar and pestle. Immediately prior to analyzing the four acha specimens for their content of fatty acids, minerals and trace elements, amino acids and antioxidants, they were dried to a constant weight as specified below. Each of the four acha specimens was analyzed in triplicate and results reported as mean ± standard deviation. All analyses were performed in accredited laboratories in Taiwan and the United States.

Lipid extraction and fatty acid analysis

Prior to lipid extraction, powdered acha was vacuum-dried for 12 h using an Eyela centrifugal evaporator CVE-1000 (Tokyo, Japan). Total lipids were extracted using the method of Folch and coworkers (1975), with minor modifications (Glew et al., 2009). The extracted lipid was then reconstituted to 0.5 mL with chloroform. To prepare fatty acid methyl esters, a 0.1 mL aliquot of the chloroform solution of the total lipid fraction was evaporated under a stream of nitrogen, and then reacted with 14% (w/v) boron trifluoride methanol complex (BF_3) (Morrison and Smith, 1964) for 20 min at 95°C. The fatty acid methyl esters were extracted with 1 mL of hexane. The profile of the fatty acids in the hexane extract (1 µL) was determined by gas chromatography (GC) as previously described (Glew et al., 2009).

Mineral analysis

Samples (0.2 g) of vacuum desiccator-dried and powdered acha were weighed into 125 mL Phillips beaker, covered with watch glass and digested at 150°C for 30 min with 10 mL of concentrated nitric acid and 0.6 mL of 60% perchloric acid. The watch-glass cover was then removed and the samples were brought to near dryness at 120°C. The digested samples were cooled to room temperature and brought to 10 mL with 4% nitric acid/1% perchloric acid. The digested samples were analyzed for metal content by inductively coupled plasma optical emission spectrometry (ICP-OES) as described earlier (Fernandez et al., 2003).

Amino acid analysis

Twenty milligrams of desiccator-dried acha were hydrolyzed in 6 N HCl containing 1.0% (w/v) phenol at 110°C for 24 h *in vacuo*, and the resultant amino acids were separated and quantified using a Hitachi Amino Acid Analyzer L8900 (Tokyo, Japan) according to the manufacturer’s instructions (Dionex Corporation, 2001) and previously described methods (Ozols, 1990; Clark et al., 1999; Jadnick et al., 1999). For the determination of methionine and cysteine, samples were oxidized with performic acid (Hirs, 1967) prior to acid hydrolysis. The coefficient of variation of the method ranged from 0.6 to 11% for the amino acids reported. Tryptophan

Table 1. Mass content (mg/g dry weight of sample) of fatty acids in four specimens of acha.

Fatty acid	Rantya	Bokkos	Ganawuri	Hoss	Mean
C16:0	2.82 (0.23)[a]	2.53 (0.39)	2.36 (0.26)	3.81 (0.08)	2.88 (0.24)
C16:1 n-7	0.04 (0.01)	0.05 (0.01)	0.04 (0.01)	0.06 (0.01)	0.05 (0.01)
C18:0	0.59 (0.05)	0.52 (0.13)	0.48 (0.05)	0.73 (0.01)	0.58 (0.06)
C18:1 n-9	5.69 (0.47)	5.37 (0.61)	4.45 (0.53)	7.87 (0.18)	5.85 (0.45)
C18:1 n-7	0.17 (0.00)	0.20 (0.06)	0.13 (0.03)	0.25 (0.01)	0.19 (0.02)
C18:2 n-6	8.78 (0.72)	8.22 (1.07)	7.14 (0.87)	12.2 (0.27)	9.08 (0.47)
C18:3 n-3	0.24 (0.02)	0.22 (0.03)	0.15 (0.02)	0.28 (0.01)	0.22 (0.02)
C20:0	0.15 (0.01)	0.16 (0.02)	0.13 (0.01)	0.21 (0.00)	0.16 (0.01)
C20:1	0.07 (0.00)	0.08 (0.02)	0.06 (0.01)	0.10 (0.01)	0.08 (0.01)
C22:0	0.08 (0.01)	0.06 (0.01)	0.09 (0.04)	0.11 (0.01)	0.06 (0.02)
Total	18.6	17.4	15.0	25.6	19.1 (3.1)

[a]The number in parentheses indicates one standard deviation.

was not measured because hydrolysates of acha were incompatible with the particular chromatography column used to resolve and quantify amino acids.

Determination of total phenolic compounds

A one gram sample of acha was extracted three successive times for 24 h at 25°C with 20 ml of methanol in a shaker incubator at 125 rpm and then centrifuged at 1,000 × *g* for 10 min. The supernatants were combined, filtered through type 5A filter paper (Advantec, Tokyo, Japan) and evaporated using a vacuum concentrator. Finally, the acha extract was lyophilized and the dry weight of the extract determined. The total phenolic acid content of the methanol extract obtained above was estimated using the Folin-Ciocalteu colorimetric assay (Singleton et al., 1999). Gallic acid was used as the standard (0 to 40 mg/L), and results were expressed as mg of gallic acid equivalent (GAE) per gram dry weight of original acha specimen. These measurements were performed in triplicate.

The 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay

The ability of the methanol extracts of acha to neutralize the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Chemical Co.) was determined using the method of Yu et al. (2002). The lyophilized methanol extract was dissolved in distilled water and diluted to the appropriate concentration. One hundred microliters of the diluted solutions were aliquoted into the wells of a 96-well plate and mixed with 0.1 mL of 0.2 mM DPPH reagent. Distilled water served as control, and DPPH was replaced with distilled water to provide the blank. The plate was left to stand in the dark at 25°C for 30 min after which the absorbance was measured at 517 nm with the aid of an Anthos Zenyth 3100 Enzyme-linked immunosorbent assay (ELISA) reader (Anthos Labtec Instruments, Saltzburg, Austria). The DPPH scavenging activity was calculated as: DPPH scavenging (%) = [1 – (sample absorbance / control absorbance)] × 100%. The EC_{50} is the concentration (mg/mL) of the methanolic extract of acha required to scavenge 50% of the DPPH radicals in the assay.

Statistical analyses

Data were analyzed by analysis of variance (ANOVA) and Duncan's multiple range test using Number Cruncher statistical software (version 6, 2004, Kaysville, UT, USA). A p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Fatty acid composition and content

Since the fatty acid percentages for the acha specimens gathered at the four different locations were statistically indistinguishable, the four data sets were averaged (Table 1). Fatty acid accounted for 15.0 to 25.6 mg/g of the total dry weight of the four acha specimens and averaged 1.91% of the dry weight (Table 2). The acha from Hoss contained the highest amount of total fatty acid (25.6 mg/g dry weight) and that from Ganawuri contained the lowest amount (15.0 mg/g dry weight). Saturated fatty acids (mainly palmitic acid and stearic acid) represented, on average, only 18.1% of the total fatty acids. Noteworthy was the finding that, on the basis of percentage or absolute amount, the essential omega-6 fatty acid linoleic acid was the most abundant fatty acid in all four of the acha specimens. The acha contained very little α-linolenic acid. Furthermore, the average omega-6/omega-3 ratio for the four specimens was 41/1.

Acha, like most whitened cereals, does not contain very much fatty acid (Table 2) (mean, 1.91% of dry weight). The daily recommended intakes of linoleic acid and α-linolenic for an adult are 13 to 17 g and 1.1 to 1.6 g, respectively (World Health Organization, 2007). Fifty grams dry weight of acha would provide only about 0.4 g of linoleic acid and 0.01 g of α-linolenic acid. The quantity of α-linolenic acid in acha represents 2% or less of the daily requirement for an adult (World Health Organization, 1985). Furthermore, the fatty acid profile of acha is disadvantageous in respect of human nutrition because the ratio of linoleic acid/α-linolenic acid is relatively high at 41/1.

To put this ratio into perspective, consider that the linoleic/α-linolenic acid ratio in many western diets is in the range of 10/1 to 20/1. A linoleic acid/α-linolenic acid

Table 2. Mass percentage of fatty acids in four specimens of acha.

Fatty acid	Rantya	Bokkos	Ganawuri	Hoss	Mean
C16:0	15.1 (0.06)[a]	14.5 (0.66)	15.7 (0.24)	14.9 (0.05)	15.1 (0.25)
C16:1 n-7	0.23 (0.03)	0.27 (0.02)	0.24 (0.02)	0.25 (0.03)	0.25 (0.03)
C18:0	3.15 (0.02)	2.95 (0.36)	3.17 (0.08)	2.84 (0.02)	3.03 (0.12)
C18:1 n-9	30.6 (0.09)	30.9 (0.69)	29.6 (0.11)	30.8 (0.10)	30.5 (0.25)
C18:1 n-7	0.94 (0.08)	1.17 (0.40)	0.88 (0.06)	0.96 (0.04)	0.99 (0.15)
C18:2 n-6	47.2 (0.07)	47.2 (0.83)	47.5 (0.05)	47.6 (0.03)	47.4 (0.25)
C18:3 n-3	1.30 (0.01)	1.25 (0.07)	0.99 (0.02)	1.08 (0.03)	1.16 (0.03)
C20:0	0.79 (0.02)	0.92 (0.01)	0.88 (0.05)	0.81 (0.02)	0.85 (0.02)
C20:1	0.35 (0.02)	0.44 (0.10)	0.40 (0.04)	0.38 (0.03)	0.39 (0.05)
C22:0	0.43 (0.07)	0.34 (0.03)	0.60 (0.16)	0.43 (0.02)	0.45 (0.07)

[a]The number in parentheses indicates one standard deviation.

ratio greater than 5/1 is widely regarded as pro-inflammatory and generally unhealthful (Simopoulos et al., 1999; Simopoulos, 2002), especially with regard to cardiovascular disease and certain cancers (Ozols, 1990; Jadnick et al., 1999). In contrast to acha, the linoleic acid/α-linolenic acid ratio of black finger millet is 5.5/1 (Masum-Akond et al., 2002). To our knowledge, there are no fatty acid data in the literature for acha against which we might compare our data; however, Jideani (1999) reported that acha contains 2.5% crude fat, a value that is consistent with our finding of an average fatty acid content of 1.91% (Table 1). Others (National Research Council, 1996) have reported a fat content of 1.8% for acha.

Mineral and trace element content

The mean calcium content of the four acha samples was 172 µg/g dry weight and the mean copper content was 4.88 µg/g (Table 3). The magnesium content of the four acha samples ranged from 826 µg/g in the acha from Ganaweri to 1520 µg/g in Hoss. There was a very wide range (11.9 to 121 µg/g) in the iron content of acha. The mean manganese content was 14.8 µg/g. All four acha specimens contained high levels of zinc (20 to 26 µg/g) while selenium (a component of glutathione peroxidase known to defend the body against oxidative stress related diseases) was not detected in any of the four samples of acha. As for unhealthful elements, all of the plants contained significant amount of strontium (0.70 to 1.35 µg/g) but lead was not detectable (data not shown). We were interested in estimating the extent to which acha grown on the Jos Plateau might contribute to satisfying the calcium and iron requirements of humans. The recommended daily intakes of calcium (Bhatia, 2008) and iron (Andrews, 1999) for an adult male are 1,000 and 10 mg, respectively. From the data in Table 3, one can estimate that 50 g dry weight of acha would provide only about 10 mg of calcium or 1% of the daily recommended amount but 2.5 mg or 25% of an individual's daily iron need. Acha could also contribute significantly to an adult's daily needs of copper, magnesium and zinc. As for minerals and trace elements, acha obtained in Minna and that purchased in Plateau State contained similar amounts of calcium and magnesium (Chukwu and Abdul-kadir, 2008); however, the acha gathered in Minna contained one-fourth less copper but 3 to 4-fold more iron than the acha from the Jos Plateau that we analyzed in the present study.

Amino acid composition and content

As shown in Table 4, the protein content (obtained by summing all the amino acids together) of the four acha specimens ranged from 4.65 to 8.02% (mean, 6.53%). Temple and Bassa (1991), using a direct method for estimating protein, reported that acha contains 7% crude protein. To assess the quality of the protein in the acha specimens, we compared their proportions of essential amino acid (except tryptophan) to the proportions of the same amino acids in a WHO reference protein (World Health Organization, 1985). The data in Table 5 show that acha scored above 100 for five of seven essential amino acid categories. The scores for lysine and threonine were 42 and 90%, respectively, of the corresponding amino acids in the WHO reference protein. The sulfur amino acid (that is, cysteine plus methionine) score for acha was 231, which is high relative to that for other West Africa staple cereals (rice, maize, millet and sorghum). With regard to the other essential amino acids or amino acid pairs; isoleucine, leucine, threonine, valine and phenlyalanine plus tyrosine, the essential amino acid scores for acha ranged between 106 and 150 (Table 5).

Thus, in terms of protein content and its pattern of amino acids relative to the WHO standard, the acha samples from four different villages on the Jos Plateau of North-central Nigeria contained moderate amounts of protein and, except for lysine, a pattern of essential amino

Table 3. Amino acid content (mg/100 mg dry weight) of acha grown in Nigeria.

Amino acid	Rantya	Bokkos	Ganawuri	Hoss	Mean
Aspartic acid	0.487 (0.040)[d]	0.511 (0.066)	0.325 (0.033)	0.405 (0.032)	0.432
Threonine	0.250 (0.015)	0.271 (0.023)	0.192 (0.018)	0.217 (0.023)	0.233
Serine	0.419 (0.040)	0.477 (0.064)	0.239 (0.024)	0.315 (0.023)	0.369
Glutamic acid	1.78 (0.14)	1.81 (0.02)	0.95 (0.11)	1.29 (0.11)	1.46
Proline	0.420 (0.044)	0.480 (0.008)	0.248 (0.019)	0.342 (0.037)	0.373
Glycine	0.191 (0.009)	0.188 (0.008)	0.140 (0.014)	0.172 (0.013)	0.173
Alanine	0.694 (0.047)	0.751 (0.012)	0.413 (0.044)	0.525 (0.040)	0.596
Valine	0.417(0.018)	0.413 (0.030)	0.247(0.024)	0.301(0.029)	0.345
Isoleucine	0.322(0.097)	0.332 (0.060)	0.173 (0.019)	0.301(0.029)	0.282
Leucine	0.737 (0.025)	0.910 (0.019)	0.444 (0.054)	0.554 (0.054)	0.536
Tyrosine	0.200 (0.021)	0.231 (0.020)	0.124 (0.009)	0.160 (0.037)	0.179
Phenylanine	0.463 (0.012)	0.555 (0.016)	0.263 (0.021)	0.355 (0.034)	0.409
Histidine	0.170 (0.010)	0.179 (0.014)	0.098 (0.012)	0.131 (0.010)	0.145
Lysine	0.160 (0.006)	0.149 (0.019)	0.121 (0.019)	0.159 (0.014)	0.147
Arginine	0.160 (0.037)	0.262 (0.038)	0.178 (0.034)	0.239 (0.029)	0.211
Cysteine[e]	0.180	0.163	0.146	0.168	0.164
Methionine[e]	0.384	0.342	0.342	0.378	0.362
Total	7.54[a]	8.02[a]	4.65[b]	5.93[c]	6.53

[a]Different from groups 3 and 4, [b]different from groups 1, 2 and 4, [c]different from groups I, 2 and 3, [d]the number in parentheses is one standard deviation, [e]only a single determination was made.

Table 4. Comparison of the mineral content (µg/g dry weight) of four specimens of acha and black finger millet.

Mineral	Acha specimen				Mean	Black finger millet[a]
	Rantya	Bokkos	Ganawuri	Hoss		
Calcium	187	86	100	314	172 (105)[b]	4010
Chromium	n.d.	0.19	0.22	0.32	0.21 (0.09)	2.23
Copper	4.57	5.44	4.12	5.39	4.88 (0.64)	7.47
Iron	19.0	40.9	11.9	121	48.2 (50.1)	182
Potassium	2430	2200	2250	3070	2490 (401)	5410
Magnesium	1010	826	894	1520	1060 (314)	1740
Manganese	22.9	10.6	8.95	16.6	14.8 (6.30)	292
Molybdenum	0.16	0.30	<0.11[c]	<0.11[c]	-	0.15
Sodium	<41[c]	<41[c]	<41[c]	<41[c]	-	148
Phosphorus	2180	1950	2040	3310	2370 (634)	2760
Zinc	24	21	20	26	23 (3)	27.1

[a]Data from Glew et al. (2008), [b]the number in parentheses indicates one standard deviation, [c]lower limit of detection. Selenium was not detected (<4.2 µg/g dry weight).n.d = no determination.

amino acids that compared favorably to a WHO reference protein (World Health Organization, 1985). The amounts and proportions of amino acids in the acha obtained in four different villages in Plateau State are similar to values reported by Chukwu and Adbul-kadir (2008), Temple and Bassa (1991), and others (National Research Council, 1996) for acha. As others have noted previously (World Health Orgnaization, 1985; Chukwu and Abdul-kadir, 2008; Carbenier et al., 1960; Vodouhe et al., 2003), it was also found in this study that acha protein contains relatively high proportions of the sulfur amino acids (methionine and cysteine), and glycine, glutamate/glutamine, proline and leucine (Table 3).

The protein in a typical serving of acha could satisfy about 10% of the daily needs of an adult for all of the essential amino acids except lysine which scored only 42% relative to the WHO standard protein (Table 5). For example, consumption of 50 g dry weight of acha (about 65 g of freshly-harvested acha) with a protein content of 7% would provide 3.5 g of protein, or a little less than

Table 5. Comparison of the amino acid composition of acha to black finger millet and the WHO reference protein (World Health Organization, 1985)

Amino acid	WHO ideal[a] (% of total)	Black finger millet[b] (% of total)	Acha[c] (% of total)	Acha × 100/ WHO ideal
Isoleucine	4.0	4.8	4.3	108
Leucine	7.0	10.4	8.2	117
Lysine	5.5	2.6	2.3	42
Methionine plus cysteine	3.5	6.2	8.1	231
Phenylalanine plus tyrosine	6.0	6.7	9.0	150
Threonine	4.0	3.9	3.6	90
Valine	5.0	6.8	5.3	106

[a] WHO (World Health Organization, 1985), [b] data from Glew et al. (2008), [c] averages from Table 4 of this report.

populations relying heavily on this grain should be advised to consume other food sources such as soy beans that would supplement their lysine intake.

Polyphenol content

The mean polyphenol content of the acha specimens from the four villages was 2.0 (0.01) mg/g dry weight, which is similar to the polyphenol content of 2.2 to 3.2 mg/g reported for rice (Chotimarkon et al., 2008), 1.29 mg/g for wheat (Masum-Akond et al., 2002) and 0.8 to 1.4 mg/g for oats (Adom and Liu, 2002). The DPPH radical-scavenging activity of the methanol extracts of the four acha specimens, expressed as the EC_{50} value, was 0.51 units/mL which is similar to values reported for several varieties of winter wheat (Kähkönen et al., 1999). The total phenolic content of methanolic acha extracts, estimated as gallic acid equivalents (GAEs), is in the range of values reported for other cereals, including rice, wheat, maize and oats (Adom and Liu, 2002; Masum-Akond et al., 2002; Chotimarkon et al., 2008). However, although the ability of methanolic extracts of our four acha specimens to neutralize free radicals was poor relative to similar extracts of green leafy vegetables, the antioxidant content of acha was similar to that of other grains which, in general, are not rich in these types of substances.

The overall result of this study was the finding that acha grown on the Jos Plateau contains useful amounts of the essential fatty acid linoleic acid (Table 2), a number of essential minerals and trace elements, including copper, iron, magnesium, molybdenum and zinc (Table 3), and nutritionally-significant amounts of good-quality protein (Tables 4 and 5). However, compared to another common cereal grown on the Jos Plateau, namely black finger millet (Glew et al., 2008), acha appears to be a poor to moderate source of α-linolenic acid, calcium, chromium, manganese and lysine. It has long been recognized that acha is a poor source of lysine (Food and Agriculture Organization, 1970; Chukwu and Abdul-kadir, 2008).

The wide variation we found for the iron content of the four acha specimens could be due to contamination introduced by the tools used by the local farmers to gather and mill acha. In contrast, the percentages of individual fatty acids between the four different specimens of acha analyzed in the present study was small (less than 16%) relative to the large variations in the content of various minerals which ranged from 13 to 117% (Table 3). This observation indicates that the fatty acid composition of acha grown in different locations on the Jos Plateau was much less dependent on soil conditions and climate than the mineral content of acha. Nevertheless, it would be useful to test under controlled conditions the effects of light, temperature, rainfall and fertilizer use on the nutrient composition of acha.

In light of the relatively high incidence of malnutrition (for example, stunting, underweight, wasting) and deficiencies of zinc and other micronutrients in Nigeria and many other regions of West Africa (Carbenier et al., 1960; Thacher et al., 1999 ; Agyei-Frempong et al., 2001; Lutter and Rivera, 2003; Vodouhe et al., 2003; Adu-Afarwuch et al., 2008), the information in this study should serve to both underscore the significant contributions acha can make to the human diet; however, it also points out certain nutritional limitations of this cereal grain, in particular, its low content of lysine, the minerals and trace elements calcium, manganese, selenium and the essential fatty acid (α-linolenic acid).

Future studies should be aimed at identifying means and growing conditions that would increase the content of these particular nutrients in acha. There is also a need for studies of the bioavailability of various nutrients in acha and on the potential thyroid toxicity of flavonoids contained in the seed (Startelet et al., 1996). Furthermore, the suggestion by Jideani (1999) that acha might be a useful component of special diets for individuals with diabetes remains to be evaluated. Finally, since the Jos University Teaching Hospital in Jos, Nigeria (Fernandez et al., 2002; Bond et al., 2005) and institutions elsewhere in West Africa (Agbon et al., 2009) have a long-standing interest in developing cereal-based weaning foods and

complimentary foods for malnourished children and adults, the information provided herein regarding the nutrient content of acha should be helpful to dieticians in Nigeria and elsewhere in the world, including the Caribbean region where acha is also grown (Leung et al., 1968), who are interested in developing the next generation of complementary foods.

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Full Length Research Paper

Effect of soya bean (*Glycine max*) on coagulation profile of New Zealand white rabbits

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The effect of differently cooked soya bean (Glycine max.) on some blood coagulation parameters was studied using forty (40) New Zealand white rabbits. The animals were grouped into four (A-D) groups of ten (10) rabbits each and fed 100% growers mash and water adlibitum for three (3) weeks. Group A (control) animals were maintained on the standard pelletized feed while group B, C and D Rabbits had 25% of their feed substituted with 'fermented and boiled', 'roasted and boiled' and 'roasted' soya bean respectively for two weeks. The percentage of diet substituted with soya was increased to 50% and 75% each for a period of two weeks, after which the rabbits were returned to 100% growers mash for three weeks to observe recovery form effects. Blood was collected from the marginal ear vein at the end of each stage of the research to determine prothrombin time, activated partial thrombin time, serum calcium concentration and platelet counts. The result show that soya bean prolonged prothrombin time and activated partial thrombin time but decreased serum calcium and blood platelet concentration. Soya bean intake prolonged blood coagulation. These effects were dependent on the method of processing and percentage composition of soya bean in the feed. Soya bean is rich in protein but contain numerous antinutritional factors which are not completely eliminated in traditional methods of cooking.

Keywords: Soya bean, prothrombin, cooking, platelets, coagulation.

INTRODUCTION

Soya bean is the yellow skin seed of *G. max*, a plant belonging to the Fabaceae family. The protein in soya bean is of high quality, consisting of most of the essential amino acids. Soya bean is also rich in minerals and vitamins such as iron, zinc, copper, thiamine, riboflavin, niacin and panthothenic acid. Most of these are well-known haematinics and are essential in the formation of red cells (McArthur et al., 1988). High protein intake affects normal hemostasis, fluid balance and organ growth (Kung-Chi et al., 1993).

Since the beginning of 1970, there has been a great breakthrough in the popularization of soya bean based food in Nigeria and in many parts of the developing world. As a result, soya bean products have been incorporated into many traditional Nigerian foods (Theodore, 1998). High protein foods of animal origin such as meat, fish, milk and eggs are very expensive especially to the low income earners who are the majority in the population of West African sub region (Belloque et al., 2002). Poverty has therefore aggravated the incidence of protein energy malnutrition in the West African sub region and particularly in Nigeria. In an attempt to combat the ill-effects of protein deficiency, soya bean consumption has been promoted rapidly as a cheap alternative source of protein for low income earners.

Chemical or physical methods are employed in the processing of soya bean. The applications vary in industrial processing or kitchens for human or animal

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consumption. They include roasting, cooking, sprouting, fermentation, use of proteolytic enzymes, infra red heating, alkali and or acid treatment. Steaming at 100°C inactivates the anti-nutritional factors in raw soya flour, thus rendering a maximum protein efficiency ratio. The objective of processing soya bean is to detoxify haemagglutinin and other harmful substances present in the bean while preserving the nutrients (Norman, 2000). According to Nwokolo (1996) the more the degree of processing of soya bean the higher the digestibility.

Consumption of soya beans has been suggested to be beneficial in different health aspects such as cancers, osteoporosis and menopausal symptoms, diabetes, besides exerting a cholesterol lowering and anti-viral activity. It is weakly diaphoretic and stomachic. It is also used in the treatment of colds, fevers and headaches, insomania, irritability and stuffy sensation in the chest. These health effects are mainly attributable to the presence of saponins and isoflavones in soya beans (Vincken et al., 2008). Nevertheless, despite the good properties associated to soya bean proteins, there are many countries in which the addition of these proteins is forbidden or in which the addition of soya bean proteins is allowed up to a certain extent (Belloque et al., 2002).

Raw soya bean contains numerous anti-nutrients. Although processing can reduce them, it does not completely eliminate them (Perez, 2006). The anti-nutrients in soya bean include phytates, protease inhibitors, trypsin inhibitors, lectins, oxalates and goitrogens. Soya bean consumption has also been associated with cases of infantile leukaemia, increased osmotic fragility, rashes (allergy) and has carcinogenic/mutagenic activity. It is also rich in haemagglutinin, which promotes the clumping of blood cells (Kyaala, 2005).

Phytic acid blocks the uptake of essential minerals – calcium, magnesium, iron and zinc in the intestinal tract (Katz, 1987). These minerals play an essential role in blood coagulation and the maintenance of haemostasis. Hence, a deficiency in these factors will create disequilibrium in the blood coagulation process. Tagnon and Soulier (1946) reported that crystallized soya bean trypsin inhibitor prevents the formation of thrombin from a mixture of prothrombin and blood thrombokinase. The crystallized soya bean trypsin inhibitor had marked anticoagulant activity on whole blood.

Crystallized soya bean trypsin inhibitor, at a concentration of 100 mg/mL, suppresses the production of thrombin from a mixture of prothrombin and blood thrombokinase (Tagnon and Soulier, 1946). Reduced thrombin formation will result in prolongation of blood clotting time.

However, Kung-Chi et al. (1993) opined that a high protein intake caused rapid coagulation of blood in rats without affecting the activity of clotting factors, the diet sensitized rats to factors that initiate clotting *in vivo*.

The broad objective of this work is to assess the effect(s) of *G. max* dietary preparations on some blood coagulation components of New Zealand White Rabbits. Measures of prothrombin time (PT), activated partial thromboplastin time (APTT), platelet and serum calcium levels in tests and control animals were compared.

MATERIALS AND METHODS

Animals

Forty (40) New Zealand White (Oryctolagus cuniculus) rabbits were selected for use in this study. The rabbits were housed in collective cages (2 rabbits per cage) measuring 37.0 x 31.0 x 16.0 cm, under controlled temperature conditions (22°C) and with a 12 h light-dark cycle (lights on at 6:00 AM). All experiments involving animals were approved by the Committee on Animal House / Ethics at Igbinedion University, Okada. Edo state.

Experimental design and groups

Soya bean was weighed dried, cooked according to the method of processing indicated and mixed with commercial feed. 5 g/100 g body weight/day of feed and water *ad libitum* was given to all the animals.

Preparation of soya bean diet

1. Roasted soya bean: Soya bean was handpicked to remove chaff. Aluminium plate was placed on a red- hot electric heater. Soya bean was poured into the plate and stirred intermittently. Roasting continued until the coat appeared golden brown with visible cracking. This was blend into fine particles using a dry electric grinding machine.
2. Boiled soya bean: Soya bean was handpicked to remove chaff. Aluminium plate was placed on a red- hot electric heater. Soya bean was poured into the plate and stirred intermittently. Roasting continued until the coat appeared golden brown with visible cracking. This was blend into fine particles using a dry electric grinding machine. Water was boiled to 120°C. Roasted, grated soya bean was added and stirred while heating for 5 min to obtain a smooth paste.
3. Fermented and boiled soya bean: Soya bean was handpicked to remove chaff. This was blend into fine particles using a dry electric grinding machine. Soya powder was soaked in the dark for 2 days. This was heated to 120°C for 5 min.

Sample collection and analysis

Two mL of blood were collected via the marginal ear vein into sodium citrate and lithium heparin anticoagulant tubes, first after three weeks and subsequently at two-week intervals throughout the study. PT, APTT, platelets counts and serum calcium estimation were carried out on the samples following the manual methods described by Dacie and Lewis (1991).

Statistical analysis

The data obtained was expressed as mean ± SEM (Standard Error of Means of ten observations) and statistically by application of the Statistical Package for Social Science (SPSS) version 11. P-values < 0.05 were considered to be significant. All experimental results were first evaluated to establish the necessity for using parametric

Table 1. Coagulation profiles of rabbit fed soya bean with respect to processing methods.

Coagulation parameters	Group A (Control)	Group B (Roasted)	Group C (Roasted and Boiled)	Group D (Fermented and Boiled)	Concentration
PT	14.8±2.1	13.9±2.1	14.8±1.6	13.7±1.1	Baseline data
APTT	22.6±1.9	22.7±1.9	22.8±1.8	22.8±1.6	
Platelets	4.4±0.4	4.5±0.4	4.6±0.5	4.8±0.6	
Calcium	2.8±0.3	2.9±0.1	3.0±0.1	3.0±0.2	
PT	13.8±1.3	20.2±1.5	21.3±3.5	28.7±3.9	25% Soya bean
APTT	22.8±1.9	29.8±2.6	26.6±1.2	27.8±1.9	
Platelets	4.5±0.4	4.2±0.3	4.1±0.3	4.1±0.4	
Calcium	2.7±0.2	1.8±0.6	1.6±1.3	1.8±0.9	
PT	14.5±1.4	43.1±1.7	31.3±3.7	37.8±5.7	50% Soya bean
APTT	23.0±1.9	30.8±3.1	26.0±2.0	28.6±1.9	
Platelets	4.5±0.4	3.9±0.2	3.8±0.3	3.4±0.4	
Calcium	2.6±0.3	2.0±0.1	2.1±0.2	2.0±0.3	
PT	14.5±2.3	46.8±4.5	38.1±3.8	44.8±6.2	75% Soya bean
APTT	22.8±1.5	29.6±3.7	29.0±1.7	29.8±2.9	
Platelets	4.6±0.4	3.6±0.3	3.0±0.3	2.9±0.6	
Calcium	2.5±0.3	2.1±0.2	2.3±0.1	2.4±0.1	
PT	15.7±2.9	19.5±2.7	17.4±3.1	19.7±2.1	Recovery
APTT	25.5±2.1	25.5±2.1	25.5±2.1	25.5±2.0	
Platelets	4.5±0.4	3.2±0.2	3.2±0.3	3.7±0.4	
Calcium	2.7±0.2	2.7±0.1	3.3±0.2	2.5±0.3	

statistics. The data were determined to have a normal distribution

RESULTS

PTT and APTT

There were significant increases in PTT and APTT of all the groups compared to control with few exceptions (Table 1). Increase in blood clotting times (PTT and APTT) was dose related (Table 1, Figures 1 and 2). The values obtained at 75% soya bean concentration were higher than those at 50% which were also higher than values for 25% soya bean concentration i.e. 75% (PTT and APTT) > 50% (PTT and APTT) >25% (PTT and APTT)> baseline (PTT and APTT). F&B soya bean prolonged blood clotting time than R&B soya bean, which recorded higher values than RST soya bean.

Serum Calcium and Platelets

Ca+ and Platelet counts recorded were lower in the treatment group (Table 1; Figure 3-4). The effects of soya bean on these variables were also dose related. The pattern was similar for all the methods of cooking employed.

DISCUSSION

Soya bean is a widely used inexpensive source of protein both for human and animal consumption. It also has other applications in health and industries. There have been growing concerns on the dietary utilization of soya bean because of the anti-nutritional factors highly present in it. In the present study, it is confirmed that the components of soya bean elicit variable effect on blood coagulation parameters.

Blood coagulation profile of rabbits fed soya bean deviated from the values obtained at baseline and recovery period. The differences between the values were dependent on the method of cooking employed and the percentage composition of soya bean in the diet (Figures 1 to 4).

Fermentation of soya bean resulted in a marked extension of prothrombin time and activated partial thrombin time compared to values obtained from roasted and boiled soya or roasted soya bean (Figures 1 and 2). Isolated trypsin inhibitor from soya bean has been proven

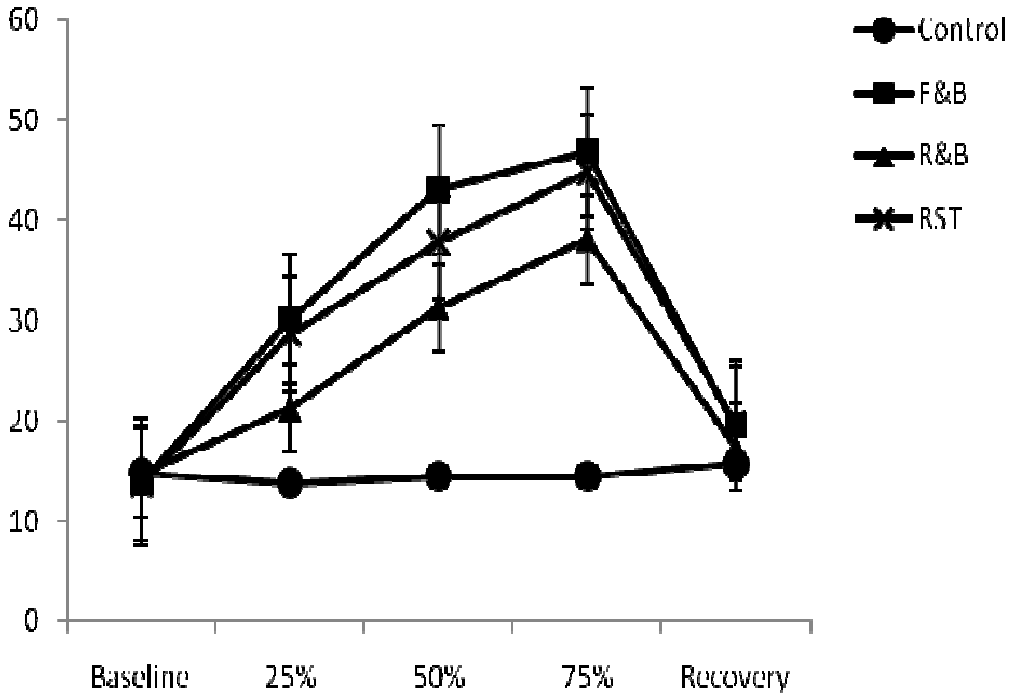

Figure 1. PT of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B- fermented and boiled soya bean; R&B – roasted and boiled soya bean; RST – roasted soya bean.

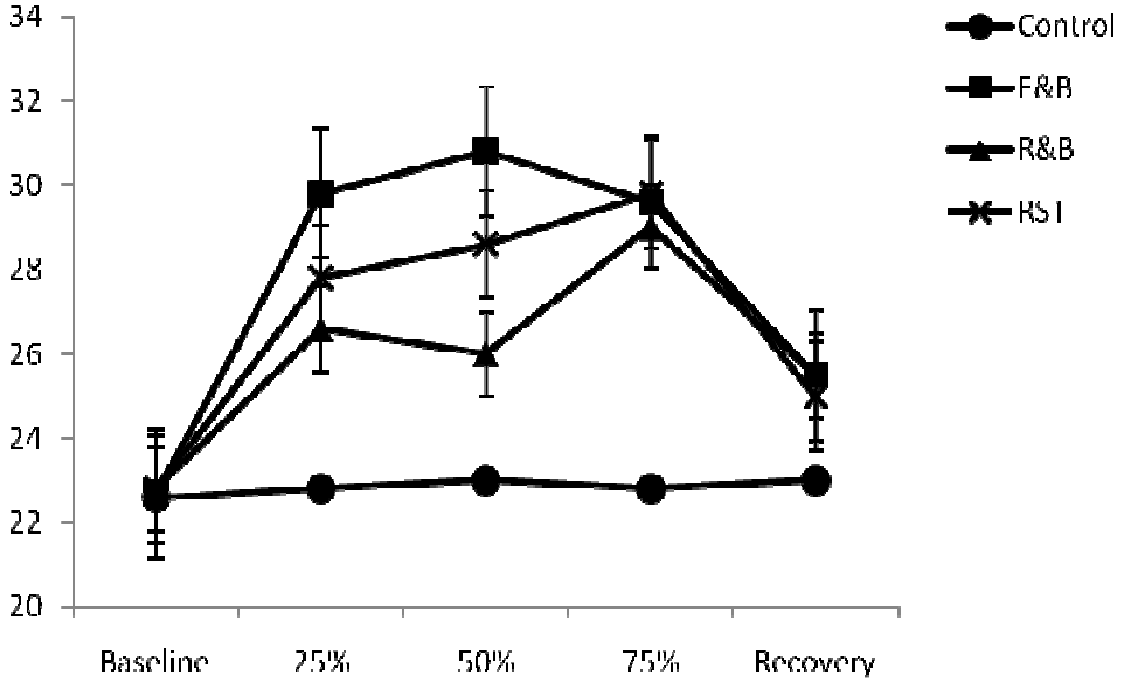

Figure 2. APTT of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B- fermented and boiled soya bean; R&B – roasted and boiled soya bean; RST – roasted soya bean

to be capable of delaying the coagulation of blood. Previous studies by six investigators have shown that it inhibits the first phase of coagulation and has no effect on the activity of thrombin (Tagnon and Soulier, 1946;

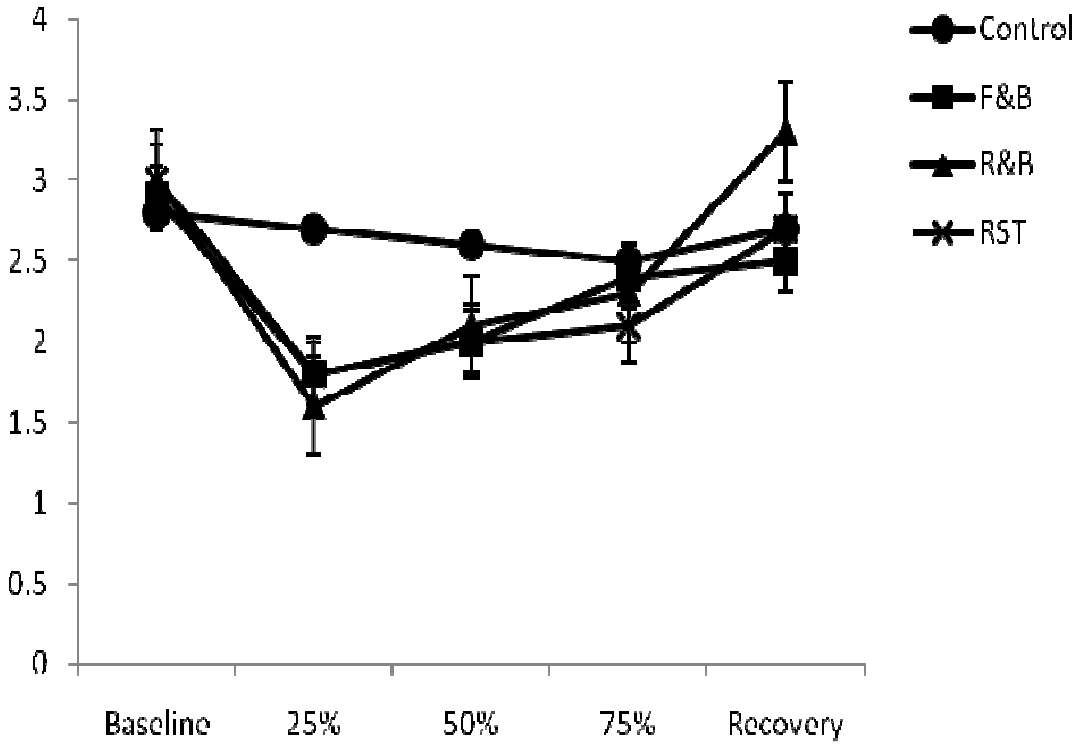

Figure 3. Serum calcium of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B- fermented and boiled soya bean; R&B – roasted and boiled soya bean; RST – roasted soya bean.

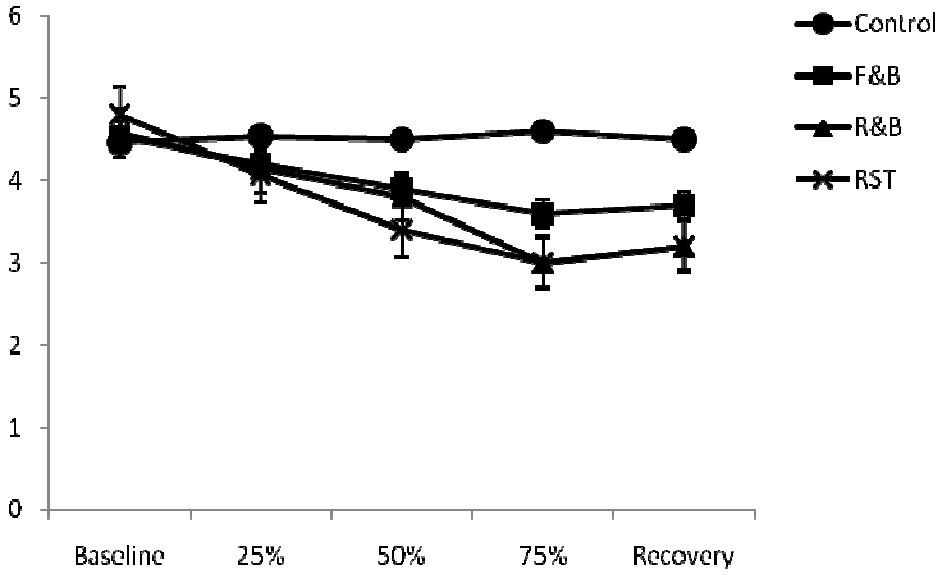

Figure 4. Platelet count of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B- fermented and boiled soya bean; R&B – roasted and boiled soya bean; RST – roasted soya bean.

Macfarlane et al., 1946; Croxatto, 1946; Glazko, 1947; Macfarlane, 1947; Guest and Nelson, 1949; Glendening and Page, 1951a).

The site of inhibition of blood clotting by soya bean trypsin inhibitor was first identified by Glendening and Page (1951b). They opined that soya bean trypsin

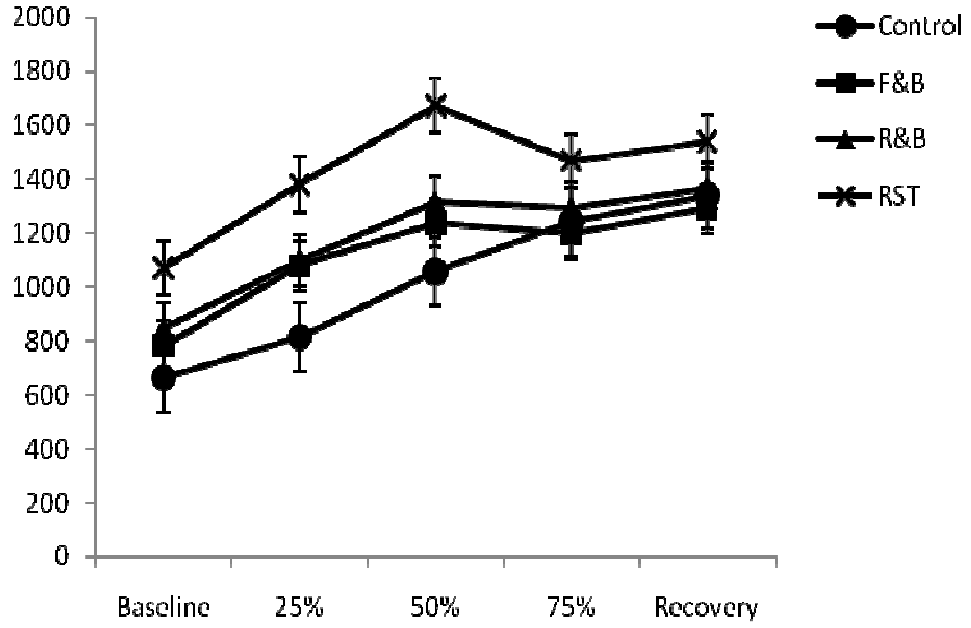

Figure 5. Weight of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B – fermented and boiled; R&B – roasted and boiled; RST - roasted

inhibitor delays the coagulation of blood by a mechanism unique among other biological inhibitions. It apparently forms a dissociable complex with the substrate, prothrombin or a dertivative of the substrate. The inhibitor does not exert its effect upon the enzyme of the first phase (thromboplastin), nor upon the cofactor (accelerator globulin) nor upon the product (thrombin). It would interfere with the conversion of prothrombin to thrombin in 25% sodium citrate when all other factors are absent.

Soya bean trypsin has anti-thrombin properties (Tagnon and Soulier, 1946). Since thrombin is required for the formation of blood clot, it is not surprising that dietary soya bean prolonged blood clotting (Figures 1 and 2) in this study. Heat treatment which is employed in most traditional cooking was applied, but does not completely destroy trypsin inhibitors present in Soya bean. The role of trypsin inhibitor in the coagulation process was confirmed in the report of Tagnon and Soulier (1948) in which soya bean preparation containing a trypsin inhibitor was injected intravenously into 2 dogs and 3 rabbits producing the following effects: prolongation of the clotting time and of the prothrombin time, and increase in the antiproteolytic activity of the blood plasma or serum.

Serum calcium concentration was lower (Figure 3) in all the rabbits fed soya bean at 25, 50 and 75% concentration. Phytic acid inhibits the absorption of calcium in the gastrointestinal tract (Katz, 1987). Both calcium and phytic acid are highly present in separate fractions of soya bean. Calcium is required in the initial and final stages of blood clotting. Therefore, a reduction in plasma calcium will further prolong blood coagulation *in vivo* in addition to the effect of trypsin inhibitors on thrombin.

Platelet count reduced (Figure 4) in all the rabbits administered soya bean diet irrespective of the processing method employed.

Heat-labile trypsin inhibitors are not the only toxic factors in the beans. Other factors identified include haemagglutinin activity, cyanide production, amylase inhibition, and urease activity (Ekpenyong and Borchers, 1981) which essentially affects growth or weight gain rather than coagulation. Weight gain pattern of control and treatment groups was similar from the onset to the period the rabbits were fed 50% soya bean (Figure 5). The rabbits began to record significant weight loss when the proportion of soya in the diet was increased to 75% and recovered slowly when returned to 100% CF. Kiers et al. (2003) reported similar effects of soya bean on weight in weaned piglets.

Weight gain was observed at lower concentration of soya bean. Gupta (2009) obtained similar results after addition of soya bean oil to standard diet of rabbits. The reduction in weight gain as the soya bean concentration increased may result from the presence of genistein in soya bean which promotes weight loss (Naaz, 2003).

In summary, Soya bean diet affects coagulation profiles in rabbits. The effects observed were dependent on the method of processing and the proportion of soya bean in the diet. Soya bean diet had a significant reduction effect on serum calcium concentration and platelet counts ($P<0.05$). The anti-nutritional factors present in soya bean are not completely eliminated by the methods of

processing employed and are responsible for these effects.

When the consumption of soya bean is inevitable because of its cost effectiveness as a source of protein, fermentation or pre-soaking of whole soya bean is recommended. Soya rich diet should be supplemented with dietary calcium. The consumption of soya bean should be with caution in people who have abnormal tendency to bleed, thrombocytopenia or other blood clotting problems. There is need for further research into soya bean and health in infants, women and experimental models.

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Appendix. Experimental design and groups.

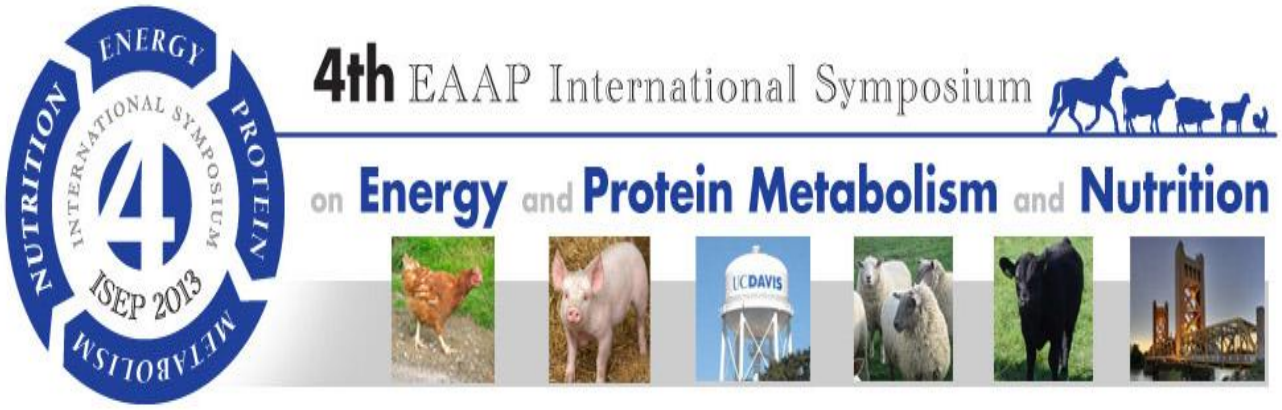
Duration (weeks)	Group A (Ctrl) n=10	Group B (F&B) n=10	Group C (R&B) n=10	Group D (RST) n=10
3 WK	100% CF	100% CF	100% CF	100% CF
2 WK	100% CF	75% CF; 25% SOY	75% CF; 25% SOY	75% CF; 25% SOY
2 WK	100% CF	50% CF; 50% SOY	50% CF; 50% SOY	50% CF; 50% SOY
2 WK	100% CF	25% CF; 75% SOY	25% CF; 75% SOY	25% CF; 75% SOY
3 WK	100% CF	100% CF	100% CF	100% CF

* CTRL – control, F&B – fermented and boiled, R&B- roasted and boiled, RST – roasted, CF-commercial feed, SOY- soya bean.

UPCOMING CONFERENCES

ICFSN 2013 : International Conference on Food Science and Nutrition
London, UK
July 08-09, 2013

4th International Symposium on Energy and Protein Metabolism and Nutrition, California, USA, 9 Sep 2013



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