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Nutritional and bio-efficacy of acha (*D. iburua* & *D. exilis*) and soybean (*Glycine max*) flour blends in rats

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ADDITIONAL KEYWORDS
Acha-based infant foods.
Amino acid profiles.
Nutritional qualities.
Growth performance.

SUMMARY

The nutritional potentials of acha as alternative energy source to maize in complementary foods was evaluated with a view to increase the accessibility and affordability of complementary foods by the resource poor mothers. The formulated acha-based complementary foods (*D. iburua* (DIF); *D. exilis* (DEF)), maize-based complementary food (MCF) and commercial formula (CFF) were evaluated for chemical compositions and nutritional qualities. Data were statistically analysed. Crude protein (g/100g) and energy values (kcal.) of DEF and DIF were 15.28 - 15.69 and 409.6 - 405.3 respectively, and were comparable to MCF (15.40; 407.9) and commercial formula (CFF) (15.50; 415.0). Essential amino acids of DEF and DIF were 35.64 and 38.76 g/16N respectively. The Na/K and Ca/P molar ratios of DEF and DIF ranged from 0.59 – 0.60 and 1.67 – 1.71 respectively. Phytate, oxalate and tannin concentrations were within tolerable levels. Biological values and protein efficiency ratios of experimental foods were 87.21 - 87.81% and 2.94 - 3.12 respectively, and were higher than maize-based formula (84.32%; 2.02) and commercial formula (86.53%; 2.52). Growth performance of rats fed on experimental were comparable to those fed on commercial formula, but lower in production costs (USD = 0.24) than CFF (USD = 0.90). The acha-based complementary foods were characterized with essential nutrients, ability to support growth and development, affordable and accessible to poor resource mothers; hence, the food samples may be suitable as complementary foods.

Mezcla de harinas para ratas. Informe nutricional y bioeficacia de acha (*D. iburua* & *D. exilis*) y harina de soja (*Glycine max)*

PALABRAS CLAVE
Alimentos infantiles a base de acha.
Perfiles de aminoácidos.
Cualidades nutricionales.
Rendimiento de crecimiento.

RESUMEN

Los potenciales nutricionales de la acha como fuente de energía alternativa al maíz en alimentos complementarios se evaluaron con miras a aumentar la accesibilidad y la asequibilidad de los alimentos complementarios por parte de las madres con pobres recursos. Los alimentos complementarios formulados a base de acha (*D. iburua* (DIF); *D. exilis* (DEF)), alimentos complementarios a base de maíz (MCF) y fórmula comercial (CFF) fueron evaluados para composiciones químicas y cualidades nutricionales. Los datos se analizaron estadísticamente. Los valores de proteína bruta (g/100g) y energía (kcal.) de DEF y DIF fueron 15,28 - 15,69 y 409,6 - 405,3 respectivamente, y fueron comparables a MCF (15,40; 407,9) y a la fórmula comercial (CFF) (15,50; 415,0). Los aminoácidos esenciales de DEF y DIF fueron 35,64 y 38,76 g/16N respectivamente. Las relaciones molares Na/K y Ca/P de DEF y DIF oscilaron entre 0,59 y 0,60 y 1,67 a 1,71 respectivamente. Las concentraciones de phytato, oxalato y tanino estaban dentro de niveles tolerables. Los valores biológicos y las proporciones de eficiencia proteica de los alimentos experimentales fueron de 87,21 a 87,81% y 2,94 - 3,12 respectivamente, y fueron superiores a la fórmula a base de maíz (84,32%; 2,02) y a la fórmula comercial (86,53%; 2,52). Los resultados de crecimiento de las ratas alimentadas con CF experimental fueron comparables a los alimentados con fórmula comercial, pero menores en costos de producción (USD 0,24) que CFF (USD a 0,90 USD). Los alimentos complementarios a base de acha se caracterizaron por nutrientes esenciales, capacidad para apoyar el crecimiento y el desarrollo, asequibles y accesibles para las madres con bajos recursos; por lo tanto, las muestras de alimentos pueden ser adecuadas como alimentos complementarios.

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INTRODUCTION

Adequate nutrition during infancy and early childhood is essential for healthy growth and mental development of children (Kandala et al. 2011, p. 261). Poverty and poor child feeding practices have been implicated as the major causes of protein-energy malnutrition among children in developing countries (Hanif 2011, pp. 1-7). For instance, available evidences have shown that most nursing mothers in developing countries used un-supplemented cereal porridges made from maize, sorghum and millet as complementary foods due to their inability to afford high cost fortified nutritious proprietary complementary foods (Bruyeron et al. 2010, pp. S154-7; Muhimbula, Issa-Zacharia &

Kinabo 2011, pp. 26 - 1). The negative effects of this on the children however, cannot be underscored.

Recently, several efforts have been made to improve on local complementary foods through the combinations of cereals and or legumes, and other alternative locally available food resources. Such blends have been found to improve nutrient density and quality of the complementary foods with a concomitant reduction in malnutrition problems in pre-children (Beruk, Kebede & Esayas 2015, pp. 121-9; Achidi et al. 2016, pp. 21-1). However, information on the use of acha as alternative energy source of maize in complementary food formulation is scanty.

Acha (*Digitaria exilis* & *Digitaria iburua*), also known as fundi, hungry rice and Asian millet, is an underutilized and nutritious cereal mainly consumed as a whole grain, porridge and couscous, ground or mixed with other flours to make pastries by Africans and Asians (Ayo & Nkama 2006, pp.129-4; Chukwu & Abdul-kadir 2008, pp. 214-6). Acha protein is high in leucine, methionine and valine than other cereals (wheat, maize, millet, sorghum, barley, oat, etc.) (Princewill-Ogbonna 2015, pp. 213-8), and these nutritional potentials appeared not to have been harnessed in the formulation of complementary foods preparations. This gab therefore forms the major focus of this present study. The study therefore aimed to formulate complementary foods from acha supplemented with soybeans flour, and also, to provide information on the chemical compositions and nutritional efficacy of the formulated products.

MATERIALS AND METHODS

Processing of Food Materials into Flour

The food samples, that is, acha (Digitaria exilis and Digitaria iburua), maize kernels, soybean grains and commercial formula as control were obtained from local markets in Jos, Nigeria, and were processed into flours (Amankwah et al. 2009, pp. 1671-5; Famakin et al. 2016, pp.1-11). Each of the food materials was sorted, cleaned with double distilled water, oven dried at 60 °C (Plus11 Sanyo Gallenkamp PLC, UK) for 12 h, milled using Phillips laboratory blender (HR2811 model), sieved with 60 mm wire mesh (British standard) to obtain acha, maize and soybean flours respectively.

Formulations of food samples: Four experimental food samples, that is, basal diets (BSD, maize-based (MCF), Digitaria exilis-based (DEF) and Digitaria iburua based (DIF) were formulated with reference to recommended daily allowances (RDA) for energy and protein for 6 to 12 months old infant recommendations (WHO 1994). The flour samples were mixed in different proportions using Nutri-Survey Linear Programming software version 2007 as shown in **Table I**.

CHEMICAL ANALYSIS

Proximate Compositions Determination

Proximate compositions, that is, moisture content, ash, crude fiber, crude fat and crude protein content of experimental complementary food samples were determined using the standard methods (AOAC 2012). Carbohydrate content was determined by difference, i.e,

Carbohydrate (%) = 100-(%Moisture + %Fat + %Ash + % Crude fibre + %Crude protein

The gross energy values of the samples were determined (MJ/kg) by using Gallenkamp Adiabatic bomb calorimeter (Model CBB-330-01041; UK).

Mineral Compositions Determination

Calcium, magnesium, iron, copper and zinc were determined using Atomic Absorption Spectrophotometer (AAS Model SP9). Sodium and potassium were determined using flame emission photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) with NaCl and KCl as the standards (AOAC 2012). Phosphorus was determined as phosphate by the vanadium phosphomolybdate (vanadate) colorimetric method reported by Atasie, Akinhanmi and Ojiodu (2009, pp. 194-7).

The Na/K, Ca/P, Ca/Mg, [K/Ca+Mg], Phytate: Zn, Ca: Phytate and [Ca][Phytate]/[Zn] molar ratios were calculated as described by Ferguson et al. (1988, pp. 316-325).

Determination of Amino acid Compositions

The experimental samples were digested using 6N HCl for 24 h. Amino acids were determined using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter Inc., Fullerton, Calif., USA) employing sodium citrate buffers as step gradients with the cation exchange post-column ninhydrin derivatization method (Siswoyo et al. 2011, pp. 5648–6). The cysteine and methionine contents were determined after performic acid oxidation and tryptophan was determined as described by Pianesso et al. (2015, pp.172–83).

Determination of Anti-nutritional Factors

Phytate and tannin were determined according to the method described by Phillipson (2000, pp. 237 -48). The oxalate content of the samples was determined using the method described by AOAC (2005).

Nutritional Qualities of Experimental Food Samples in Wistar Albino Rats

Experimental design

The experimental layout was of completely randomized design (CRD). Eighty (40 males and 40 females) clinically healthy weanling Wistar albino rats (3 weeks old) were purchased from the Research rat colony, Federal University of Technology, Akure, Nigeria. The rats were divided into eight groups of 10 rats on the basis of initial weight and sex, and were individually housed in a metabolism cage (25 cm x 12 cm x 10 cm) locally designed with sufficient facilities to collect faeces and urine. The rats were fed on experimental food samples and water ad libitum for 28 days, weight changes and total food intake were measured. The collected faeces were oven dried at 60 °C, while urine voided was preserved in 10 mL of 10% sulfuric acid to eliminate microbial activities and prevention of nitrogen via ammonia evaporation, prior to nitrogen determination (Agbede & Aletor 2003, pp. 21-0). The feed efficiency, protein efficiency ratio, biological value and feed conversion ratio were computed (Pirman, Mari & Orenik 2007, pp. 95–2).

Table I. Gross compositions (g) of acha-based complementary foods (Composiciones brutas (g) de alimentos complementarios a base de acha).

Ingredients	BSD	MCF	DEF	DIF	CFF
Cassava starch	92.75	-	-	-	-
Maize	-	75.00	0.00	0.00	-
Acha Flour	-	0.00	75.00	75.00	-
Soybean Flour	-	7.65	7.65	7.65	-
Soy oil	-	4.00	4.00	4.00	-
Milk powder	-	10.00	10.00	10.00	-
Dicalcium phosphate	-	3.00	3.00	3.00	-
Vitamin Premix	2.45	0.25	0.25	0.25	-
Salt	0.3	0.10	0.10	0.10	-
Bine meal	4.5	-	-	-	-
Total	100	100.00	100.00	100.00	-

DIF = *D. iburua* food, DEF = food, NFE= nitrogen free extract. CFF = Commercial infant foods, MCF = Maize-based complementary food, BSD= Basal diet.

Biochemical Determination of Experimental Food Samples in Wistar Albino Rats

Blood and serum collection

At the end of experimental period (28 days), blood samples were collected following overnight fasting by cardiac puncture from chloroform anaesthetized rats into heparinised and non-heparinised tubes. The non-heparinised tubes were allowed to clot and were centrifuged at 3000 xg for 25 min to obtain the sera and stored in a deep freezer (-20 °C) prior to biochemical analysis (Agbede & Aletor 2003, pp. 21- 0).

Haematological evaluation

Packed cell volume was estimated by centrifuging about 75 Fl of each blood sample in heparinized capillary tubes in a haematocrit micro-centrifuge 3000 xg for 5 min. Haematological indices like red blood cells, white blood cells, platelets, and haemoglobin concentration were determined (Benson & Cales 1992, pp. 325–41). The mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, and mean corpuscular volume were calculated.

Biochemical Determination

Total blood protein, creatinine and serum albumin level concentration were estimated using Randox Diagnostic Kit (Pinnell & Northam 1978, pp. 80-6). Globulin level was determined by taking the difference between total protein and albumin (Omodanisi, Aboua & Oguntibeju 2017, pp.1-7). Plasma levels of alanine aminotransferase and aspartate aminotransferase activities were assayed (Varley, Gewenlock & Bell 1980, pp. 65–0), while cholesterol was determined using the methods described by Wieland and Seidel (1983, pp. 760-6).

Relative organ measurements

The heart, lungs, spleen, kidneys and liver were incised, blotted free of blood, weighed and subsequently expressed in g kg^{-1} body weight.

Ethical Approval

The study protocol was approved by the Animal Production and Health Ethical Committee of School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria with reference number: FUTA/APH-SAAT/2016/013. The experiments on animals were conducted in accordance with the force laws and regulations as regards care and use of laboratory animals.

STATISTICAL ANALYSIS

All data were expressed as mean ± standard error of mean (SEM) using the statistical analysis programme for social sciences (SPSS) (Edogbanya et al. 2016, pp.122-8). Mean values were considered statistically significant at $P<0.05$. Graphs were plotted using GraphPad Prism 6. Results were considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Nutrient (proximate, amino acids and minerals) Compositions of Complementary Foods

The proximate (g/100g) and energy values (kcal/100g) of acha (*D. exilis* and *D. iburua*) based complementary foods are presented in **Table II**. The moisture content of the formulated experimental food samples were 4.42 and 5.45 in DEF and DIF respectively, and these values were comparable to the value obtained for maize-based complementary food (MCF) (5.15), higher than CFF (a commercial food) (2.00), but within the range value of FAO/WHO (1991, p. 144) recommended value. The crude protein contents of DEF and DIF samples were 15.28 and 15.69 respectively, and were similar to the values obtained for maize-based complementary food (15.40), CFF (a commercial formula) (15.50) and FAO/WHO (1991, p. 144) recommended value (>15). The energy values of the experimental foods were 409.6 and 405.3 in DEF and DIF respectively, and were comparable to that of MCF (407.9) and CFF (415.0), but lower than FAO/WHO (1991, p. 144) recommended value (483.9). However, the energy values of these experimental complementary foods are desirable, because the diets (100g) may provide over 80% of daily energy requirements of young children (Belscak et al. 2009, pp. 707-16; Lettieri-Barbato et al. 2012, pp. 305-10). Comparatively, the protein and energy values of acha-based complementary foods in this present study were either similar or higher than what obtained by other researchers for complementary foods and cookies formulated from acha and legumes (Anuonye et al. 2010, pp. 680–91; Olapade & Aworh 2012, pp. 210-17; Dabels et al. 2016, pp. 401-6). The protein content of two varieties of acha kernels (9.72, 9.93 g/100g) were very low compared with the blends, this indicates that the consumption of acha alone may not produce the required level of protein in infants' complementary foods (Olapade & Aworh 2012, pp. 210-17). The improvement in nutritional qualities of food products from the blending of two or more food materials had been reported earlier by several studies (Ewuola, Ibironke & Fashakin 2015, pp.101- 5; Tufa et al. 2016, pp. 1-8).

The amino acid compositions (g/16N) of acha (*D. exilis* and *D. iburua*) and formulated complementary foods are presented in **Table III**. The amino acid compositions of acha-based complementary foods showed that glutamic acid had the highest concentration ranging from 13.34 g/16N in DEF to 13.35 g/16N in DIF, while cysteine had the lowest value ranging from 1.21 g/16N in DIF to 1.31 g/16N in DEF. This finding agreed with the report that glutamic acid usually had the highest concentration of amino acids in plant-based foods (Anuonye et al. 2010, pp. 680–91). The range values of total non-essential amino acids (TNEAA), conditionally essential amino acids (TCEAA) and total essential amino acids (TEAA) of acha-based complementary foods were 24.91 - 25.89, 17.93 - 17.95 and 35.64 - 38.76 g/16N respectively. The TEAA compositions of the formulated complementary foods were higher than FAO/WHO (1991, p. 144) recommended values for infants. Comparatively, the amino acid compositions of formulated food samples in this present study were similar to the report of other studies (Anuonye et al. 2010, pp. 680–91). The histidine (2.35 - 2.37 g/16N) and arginine (6.56 - 6.04 g/16N) in acha-based complementary foods were higher than WHO recommendations, that is, 1.9 and 2.0 g/day respectively, for infants. Histidine and arginine are essential amino acids for infants, because the guts (intestines) of infants cannot synthesized these amino acids. Besides, studies have shown that inadequate intake of arginine has been associated with many neonatal diseases, including persistent pulmonary hypertension (Vosatka, Kashyap & Trifiletti 1994, pp. 65– 0).

Minerals (mg/100g) and molar ratios of acha-based formulated complementary foods are shown in **Table IV**. The macro-element compositions of the acha-based complementary foods showed that magnesium had the lowest concentration, while calcium had the highest concentration with ranged values of 62.01 - 78.05 and 769.29 - 774.29 respectively. The micro-mineral elements had zinc as the lowest, while copper had the highest values which ranged from 6.11 - 6.21 and 188.81 - 200.23 respectively. Comparatively, the mineral compositions of acha-based complementary foods were higher when compared with other reports (Anigo et al. 2010, pp. 65- 2; Ijarotimi & Oluwalana 2013, pp.117-

Table II. Proximate compositions (g/100g) and energy values (kcal/100g) of acha (*D. exilis* and *D. iburua*) and formulated complementary foods (Composiciones aproximadas (g/100g) y valores energéticos (kcal/100g) de acha (*D. exilis* y *D. iburua*) y alimentos complementarios formulados**).**

Parameters	WDE	WDI	MCF	DEF	DIF	CFF	RV
Moisture	12.57 ±0.12^{b}	13.14 ±0.18^{a}	5.15 ±0.05^{d}	4.42 ±0.04^{e}	5.45 ±0.05^{c}	2.00 ±0.02^{f}	<5
Total ash	5.25 ±0.05^{b}	5.77 ±0.06^{a}	2.47 ±0.02^{cd}	2.39 ±0.02^{d}	2.55 ±0.03^{c}	2.50 ±0.02^{cd}	-
Crude protein	9.72 ±0.09^{b}	9.93 ±0.10^{b}	15.4 ±0.15^{a}	15.28 ±0.15^{a}	15.69 ±0.20^{a}	15.50 ±0.15^{a}	>15
Ether extract	4.07 ±0.04^{d}	4.33 ±0.04^{d}	10.25 ±0.10^{b}	10.70 ±0.11^{a}	10.11 ±0.10^{b}	7.30 ±0.07^{c}	10-25
Crude fibre	4.92 ±0.05^{a}	4.06 ±0.04^{b}	3.20 ±0.03^{c}	4.17 ±0.04^{b}	3.31 ±0.03^{c}	1.70 ±0.01^{d}	<5
NFE	63.47 ±0.63^{b}	62.77 ±0.60^{b}	63.53 ±0.81^{b}	63.04 ±1.20^{b}	62.89 ±0.71^{b}	71.00 ±0.71^{a}	64
Energy	139.9 ±1.39^{b}	140.4 ±1.43^{b}	407.9 ±4.40^{a}	409.6 ±4.09^{a}	405.3 ±4.05^{a}	415.0 ±4.15^{a}	483.9

Means (±SEM) with different superscripts in the same row show significant difference at P < 0.05.
Key: WDI = Whole *D. iburua*, WDE = Whole *D. exilis*, MCF= maize based food, DIF = *D. iburua* food, DEF = *D. exilis* food, NFE= nitrogen free extract. CFF= Commercial infant foods. *RV (Recommended value) by FAO/WHO [1991].

Table III. Amino acids profile (g/16N) of acha (*D. exilis* and *D. iburua*) and formulated complementary foods (Perfil de aminoácidos (g/16N) de acha (*D. exilis y D. iburua*) y alimentos complementarios formulados).

Amino acids	WDE	WDI	MCF	DEF	DIF	*RDA
		Total non-essential amino acids (TNEAA)				
Alanine	3.33 ± 0.17^{b}	3.22 ± 0.16^{c}	3.00 ± 0.02^{d}	3.98 ± 0.19^{a}	3.98 ± 0.20^{a}	-
Aspartic acid	2.94 ± 0.01^{d}	2.82 ± 0.00^{e}	4.31 ± 0.02^{c}	5.67 ± 0.03^{a}	4.67 ± 0.02^{b}	-
Glutamic acid	5.58 ± 0.03^{d}	6.53 ± 0.03^{c}	11.31 ± 0.06^{b}	13.34 ± 0.07^{a}	13.35 ± 0.06^{a}	-
Serine	2.38 ± 0.01^{b}	2.35 ± 0.00^{b}	2.01 ± 0.01^{c}	2.9 ± 0.02^{a}	2.91 ± 0.01^{a}	-
ΣNEAA	14.23 ± 0.07^{e}	14.92 ± 0.06^{d}	20.63 ± 0.10^{c}	25.89 ± 0.12^{a}	24.91 ± 0.13^{b}	
		Total conditionally essential amino acids (TCEAA)				
Arginine	3.63 ± 0.01^{e}	4.22 ± 0.02^{d}	5.11 ± 0.02^{c}	6.04 ± 0.03^{b}	6.56 ± 0.03^{a}	2
Cystine	1.42 ± 0.01^{a}	1.24 ± 0.00^{c}	0.90 ± 0.00^{e}	1.31 ± 0.01^{b}	1.21 ± 0.01^{d}	-
Glycine	3.31 ± 0.02^{d}	$3.490.02^{b}$	3.42 ± 0.02^{c}	3.84 ± 0.01^{a}	3.84 ± 0.02^{a}	-
Proline	2.55 ± 0.00^{c}	2.20 ± 0.01^{d}	2.00 ± 0.01^{e}	3.16 ± 0.02^{b}	3.36 ± 0.01^{a}	-
Tyrosine	2.70 ± 0.00^{c}	2.39 ± 0.01^{d}	2.08 ± 0.01^{e}	3.58 ± 0.02^{a}	2.98 ± 0.01^{b}	-
ΣCEAA	13.61 ± 0.06^{b}	13.54 ± 0.04^{b}	13.51 ± 0.06^{b}	17.93 ± 0.08^{a}	17.95 ± 0.07^{a}	
	Total essential amino acids (TEAA)					
Histidine	1.89 ± 0.00^{c}	1.81 ± 0.00^{d}	2.16 ± 0.01^{b}	2.35 ± 0.01^{a}	2.37 ± 0.01^{a}	1.9
Isoleucine	3.18 ± 0.02^{b}	2.98 ± 0.01^{c}	2.87 ± 0.02^{d}	2.56 ± 0.01^{e}	3.26 ± 0.02^{a}	2.8
Leucine	8.40 ± 0.04^{b}	9.16 ± 0.05^{a}	8.10 ± 0.04^{d}	9.22 ± 0.05^{a}	9.22 ± 0.05^{a}	6.6
Lysine	2.80 ± 0.01^{d}	3.14 ± 0.01^{c}	5.20 ± 0.02^{b}	5.72 ± 0.02^{a}	5.72 ± 0.01^{a}	5.8
Methionine	2.57 ± 0.01^{b}	2.03 ± 0.01^{d}	1.50 ± 0.00^{e}	2.50 ± 0.01^{c}	2.82 ± 0.00^{a}	2.2
Phenylalanine	3.78 ± 0.02^{c}	3.78 ± 0.02^{c}	3.96 ± 0.00^{b}	3.34 ± 0.01^{d}	4.14 ± 0.02^{a}	2.8
Threonine	3.78 ± 0.01^{b}	4.60 ± 0.02^{a}	2.81 ± 0.03^{d}	3.74 ± 0.02^{b}	3.44 ± 0.01^{c}	3.4
Tryptophan	0.60 ± 0.00^{c}	0.45 ± 0.00^{d}	1.58 ± 0.01^{b}	1.86 ± 0.01^{a}	1.86 ± 0.01^{a}	1.1
Valine	4.17 ± 0.02^{d}	5.02 ± 0.02^{b}	3.38 ± 0.01^{e}	4.35 ± 0.02^{c}	5.95 ± 0.02^{a}	3.5
ΣEAA	31.17 ± 0.16^{d}	32.97 ± 0.21^{c}	31.56 ± 0.15^{d}	35.64 ± 0.18^{b}	38.76 ± 1.01^{a}	30.1

Means (±SEM) with different superscripts in the same row show significant difference at $P < 0.05$. Key: WDI = Whole *D. iburua*, WDE = Whole *D. exilis*, MCF = maize-based food, DIF = *D. iburua* food, DEF = *D. exilis* food, *RV (Recommended value) by FAO/WHO [1991].

32). The Na/K and Ca/P molar ratios ranged from 0.59 - 0.60 and 1.67 - 1.71 respectively. It was observed in this study that all formulated complementary food samples met the recommended values for Na/K (<1.0), but did not for the Ca/P (>2.0) (FAO/WHO, 1991, p. 144). This observation implies that the acha-based complementary foods may be essential for regulating the heart activities, but need to be complemented with micronutrient, particularly calcium for the building and maintaining of healthy bones. Nutritionally, the acha-based complementary foods contain appreciable amount of essential nutrients, hence, the food samples may be used as a substitute for the traditional complementary foods (Ogi, a corn gruel) which are low in vital nutrients (Anigo et al. 2010, pp. 65-2).

Anti–nutrient Compositions of Acha-based Complementary Foods

Antinutrient compositions (mg/100g) and phytate / (zinc & calcium) molar ratios (mol/kg) of acha (*D. exilis* and *D. iburua*) and formulated complementary foods are shown in **Table V**. The range values of phytate, oxalate and tannin in acha-based complementary foods were 1.04 - 1.10, 0.17 - 0.18 and 0.53 - 0.58 respectively, and these values were lower than values obtained for complementary foods reported by Emmanuel-Ikpeme, Ekpeyoung and Igile (2012, pp. 356-66). The range values of Ca/phytate, phytate/Zn and (Ca) (phytate) /Zn molar ratios were 0.01 - 0.02, 0.04 - 0.32 and 0.11 - 0.13 respectively, and these values were lower than the critical values reported by Oladimeji, Akindahunsi and Okafor (2000, pp. 136–37) and Davis and Warrington (1986, pp. 49–59) for Ca/Phy (6:1) and Phy/Zn (15:1) respectively. This finding showed that the antinutrients in acha-based complementary food may not interfere with the absorption and utlisation

Table IV. Minerals (mg/100g) and mineral ratios of acha (*D. exilis* and *D. iburua*) and formulated complementary foods (Minerales (mg/100g) y proporciones minerales de acha (*D. exilis* y *D. iburua*) y alimentos complementarios formulados).

Parameters	WDE	WDI	MCF	DEF	DIF	*RV
Macro-elements						
Calcium (Ca)	43.30±0.22[c]	42.36±0.21[c]	762.00±5.38[b]	769.29±4.61[ab]	774.29±3.87[a]	500.00
Phosphorus (P)	52.12±0.26[b]	54.61±0.27[b]	454.94±2.27[a]	457.13±2.74[a]	452.13±2.26[a]	456.00
Potassium (K)	45.09±0.22[c]	45.10±0.25[c]	515.61±2.57[b]	519.19±3.11[b]	530.11±2.65[a]	516.00
Sodium (Na)	37.06±0.18[d]	37.16±0.19[d]	302.01±1.51[c]	335.11±2.01[a]	319.58±1.59[b]	296.00
Magnesium (Mg)	42.09±0.21[c]	43.08±0.22[c]	79.17±0.39[a]	62.01±0.37[b]	78.05±0.39[a]	76.00
Micro-elements						
Iron	1.01±0.00[c]	1.08±0.01[c]	21.12±0.11[b]	22.03±0.13[a]	22.03±0.11[a]	16
Zinc	1.56±0.01[c]	1.73±0.01[b]	6.18±0.03[a]	6.11±0.04[a]	6.21±0.03[a]	3.2
Manganese	0.34±0.00[c]	0.34±0.00[c]	28.12±0.14[b]	30.56±0.18[a]	30.56±0.15[a]	32
Copper	0.06±0.00[c]	0.09±0.00[c]	189.39±0.94[b]	200.23±1.20[a]	188.81±0.91[b]	160
Mineral ratios						
[K/(Ca+Mg)]	0.53±0.00[c]	0.54±0.00[c]	73.85±0.36[a]	62.68±0.37[b]	62.68±0.31[b]	-
Na/K	0.82±0.00[a]	0.81±0.00[a]	0.59±0.00[c]	0.65±0.00[b]	0.60±0.00[c]	-
Ca/P	0.83±0.00[c]	0.78±0.00[d]	1.67±0.01[b]	1.68±0.01[b]	1.71±0.01[a]	-
Ca/Mg	1.03±0.00[d]	0.98±0.00[d]	10.41±0.05[c]	12.41±0.07[a]	11.38±0.05[b]	-

Means (±SEM) with different superscripts in the same row show significant difference at $P < 0.05$. Key: WDI = Whole *D. iburua*, WDE = Whole *D. exilis*, MCF = maize based food, DIF = *D. iburua* food, DEF = *D. exilis* food, *RV (Recommended value) by FAO/WHO [1991].

of protein and divalent metals in the body (Greiner & Konietzny 2006, pp.125-40; Habtamu 2014, pp. 45-4).

Nutritional Properties of Acha-based Complementary Foods in Wistar Rats

The growth performance of Wistar rats fed on acha-based complementary foods was comparable to those fed on commercial formula (CFF) and MCF (a maize-based complementary food) (**Figure 1**). This observation implied that acha-based complementary foods were of good nutritional qualities, and that the food samples may be suitable as infant foods.

The nutritional properties of acha-based complementary foods in Wistar rats are presented in **Table VI**. The biological values (BV) of acha-based complementary foods were 87.21 and 87.81% for DEF and DIF respectively, and these values were significantly (p≤0.05) higher than maize-based complementary food (MCF) (86.53%) and commercial formula (CFF) (84.32%). Protein efficiency ratios (PER) of the food samples were 3.12 and 2.94 for DEF and DIF respectively, and these values were significantly (p≤0.05) higher than that of MCF (2.52) and CFF (2.02). The BV and PER of these formulations met the FAO/WHO (1991, p. 144) recommended values of 70% and 2.7 respectively.

In comparing with other scientific studies, it was observed that the BV of the acha-based complementary foods were similar to those of earlier reports (Ijarotimi & Oluwalana 2013, pp.117-32; Ijarotimi & Olopade 2009, pp. 87-5; Laminu et al. 2014, pp. 536-42). The relative organ weights, that is, heart, lung, spleen, kidney and liver, of rats fed on the experimental foods were comparable to those rats fed on the commercial formula.

The production costs of acha-based complementary foods (DEF and DIF) ranged from N87.21 - 87.81 (Naira) (USD = 0.24), and were higher than maize-based complementary food (N23.00) (USD = 0.06), but lower than commercial formula (CFF) (N325.00) (USD = 0.9). This observation implies that these experimental food samples was not only adequate in nutrient to support growth and development in children, but affordable by the large resource poor mothers who cannot afford the

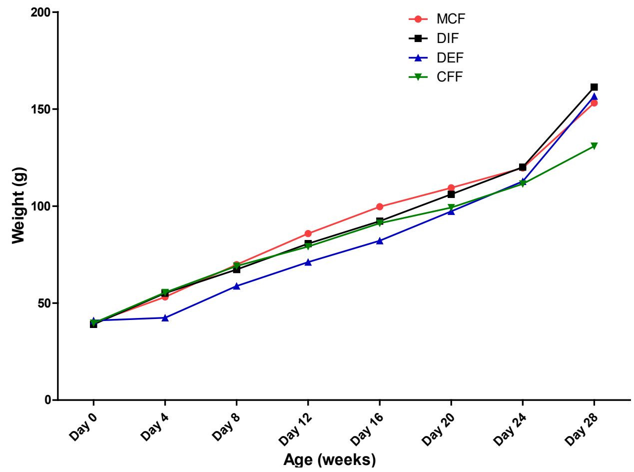

Figure 1. Nutritional status (Weight-For-Age) of rats fed *D. iburua*-based complementary and commercial infant food (MCF = maize-based food, DIF = *D. iburua* food, DEF = *D. exilis* food, CFF (commercial formula) (Estado nutricional (Peso para la Edad) de ratas alimentadas con alimentos complementarios a base de *D. iburua* y alimentos comerciales para bebés (MCF = alimentos a base de maíz, DIF = alimentos *D. iburua*, DEF = alimentos *D. exilis*, CFF (fórmula comercial)).

Table V. **Antinutrient compositions (mg/100g) and phytate /(zinc & calcium) molar ratios (mol/kg) of acha (*D. exilis* and *D. iburua*) and formulated complementary foods (**Composiciones antinutrientes (mg/100g) y ratios molares de fitato /(zinc y calcio) (mol/kg) de acha (*D. exilis* y *D. iburua*) y alimentos complementarios formulados**).**

Parameters	WDE	WDI	MCF	DEF	DIF
Anti-nutritional factors					
Phytate	42.24±0.21^{a}	42.24±0.11^{a}	1.08±0.01^{b}	1.10±0.00^{b}	1.04±0.01^{b}
oxalate	10.10±0.05^{a}	10.20±0.05^{a}	0.16±0.00^{b}	0.17±0.00^{b}	0.18±0.01^{b}
Tannin	11.02±0.06^{a}	11.05±0.05^{a}	0.55±0.00^{b}	0.53±0.01^{b}	0.58±0.00^{b}
Molar ratios					
Ca: Phytate	0.06±0.00^{a}	0.06±0.00^{a}	0.02±0.00^{b}	0.01±0.00^{c}	0.01±0.00^{c}
Phytate: Zn	2.68±0.00^{a}	2.42±0.00^{b}	0.31±0.01^{d}	0.04±0.00^{e}	0.32±0.01^{c}
[Ca][Phytate]/[Zn]	2.90±0.01^{a}	2.56±0.01^{b}	0.11±0.00^{c}	0.13±0.00^{c}	0.11±0.00^{c}

Means (±SEM) with different superscripts in the same row show significant difference at P < 0.05.
Key: WDI = Whole *D. iburua*, WDE = Whole *D. exilis*, MCF = maize-based food, DIF = *D. iburua* food, DEF = *D. exilis* food.

Table VI. **Nutritional Properties and impacts on Wister rats fed acha-based complementary foods (**Propiedades nutricionales e impactos en ratas Wister alimentadas con alimentos complementarios a base de acha**).**

Parameters	MCF	DEF	DIF	CFF
Average weight gain (g/day^{-1})	4.41±0.04^{b}	4.95±0.05^{a}	5.08±0.05^{a}	3.61±0.03^{c}
Average feed intakes (g/day^{-1})	10.31±0.10^{a}	10.38±0.11^{a}	10.45±0.10^{a}	10.39±0.13^{a}
Feed conversion ratio	2.54±0.03^{b}	2.10±0.02^{c}	2.17±0.02^{c}	3.22±0.03^{a}
Biological value	86.53±0.86^{ab}	87.21±1.90^{ab}	87.81±1.87^{a}	84.32±0.84^{b}
Food Efficiency	0.39±0.00^{c}	0.48±0.01^{a}	0.46±0.01^{b}	0.31±0.00^{d}
Protein Efficiency Ratio	2.52±0.02^{c}	3.12±0.03^{a}	2.94±0.02^{b}	2.02±0.02^{d}
*Cost of feed/100g weight gain (Naira)	23.00±0.23^{c}	37.00±0.37^{b}	41.00±0.41^{b}	325.00±3.25^{a}
Relative organ weights (g/kg^{-1} body weight)				
Heart	5.11±0.05^{b}	4.95±0.04^{b}	6.48±0.06^{a}	5.04±0.05^{b}
Lungs	10.40±0.10^{b}	10.55±0.11^{b}	13.42±0.13^{a}	10.43±0.10^{b}
Spleen	4.48±0.04^{c}	4.88±0.04^{b}	6.23±0.06^{a}	4.38±0.04^{c}
Kidneys	8.25±0.08^{b}	8.51±0.09^{b}	10.42±0.10^{a}	8.46±0.08^{b}
Liver	38.10±0.38^{b}	37.56±0.37^{b}	44.54±0.44^{a}	38.74±0.38^{b}

Means (±SEM) with different superscripts in the same row show significant difference at P < 0.05. Key: *Cost calculated on the basis of current market price. WDI = Whole *D. iburua*, WDE = Whole *D. exilis*, MCF = maize-based Food, DIF = *D. iburua* food, DEF = *D. exilis* food, CV%= coefficient of variation [%].

expensive proprietary complementary foods (Bruyeron et al. 2010, pp. S154-67; Muhimbula, Issa-Zacharia & Kinabo 2011, pp. 26 - 1).

Biochemical Variables of Wistar Rats Fed on Acha-based Complementary Foods

The biochemical variables of rats fed acha-based complementary foods and commercial formula are shown in **Table VII**. The total blood protein, serum albumen and blood cholesterol concentration of Wistar rats fed experimental foods ranged from 38.71 – 46.90 g/100g, 26.70 – 27.47 g/100g and 11.67 – 17.39 mg/100mL respectively, and these values were comparable to those of rats fed on MCF and CFF food samples, but higher than recommended values, except for cholesterol (MVM, 2012; Wikivet, 2013). This observation further confirmed the protein qualities of acha-based complementary foods.

The range values of haematological variables, that is, pack cell volume (PCV), red blood cells (RBC), white blood cells (WBC), heamoglobin concentration (Hbc) and lymphocytes of rats fed on acha -based complementary foods were 42.07 – 42.20%, 5.25 – 9.84 x106 mm3, 1.32 – 1.62 x103 mm3, 10.56 – 14.26 g/100mL and 60.4 – 64.8% respectively, and were within the recommended values, except for WBC (MVM, 2012; Wikivet, 2013). However, slight variation was observed between the haematological indices obtained in this present study compared to the report of Aikhuomobhogbe-Arueya and Osundahunsi (2014, pp.419-26). These variations could be attributed to the quality of diets used, concentration of proteins, breed of the rats and methods of blood analyses. The high values of PCV, RBC and Hb concentrations that were observed in acha-based complementary foods further confirmed the nutritional qualities of the formulated food products. Scientific studies have reported that Hb, PCV, MCHC and lymphocyte concentrations depend on the nutritional quality of dietary protein intake; and that diets containing poor protein may affect haemoglobin formation (Roberts et al. 2000, pp. 763-65; Oluwole et al. 2001, pp. 91–8) and immune status (Aikhuomobhogbe & Orheruata, 2006, pp. 743 - 48) as these indices

Table VII. Biochemical properties of acha-based complementary foods in Wistar rats (Propiedades bioquímicas de los alimentos complementarios a base de acha en ratas Wistar**).**

Parameters	MCF	DEF	DIF	CFF	*RV
Serum metabolites and cholesterol					
Total protein (g/100g)	35.46±0.35[c]	38.71±0.38[b]	46.9±0.46[a]	35.65±0.35[c]	5.60-7.60
Albumen (g/100g)	27.81±0.27[a]	27.47±0.25[ab]	26.7±0.26[b]	27.81±0.28[a]	3.80-4.80
Globulin (g/100g)	7.65±0.10[a]	7.24±0.07[b]	2.02±0.02[c]	7.84±0.08[a]	-
Albumen/globulin	3.64±0.03[a]	2.44±0.02[b]	1.32±0.01[c]	3.55±0.03[a]	-
ALT (µ/DL)	9.31±0.09[a]	9.12±0.10[a]	8.32±0.08[b]	9.31±0.01[a]	17.50-30.20
AST (µ/DL)	12.4±0.11[b]	12.17±0.13[b]	13.7±0.10[a]	12.3±0.12[b]	45.70-80.80
Cholesterol (mg/100ml)	18.68±0.18[a]	17.69±0.17[b]	11.39±0.11[c]	18.67±0.18[a]	40.0-130.0
Haematological variables					
ESR (%)	0.5±0.00[b]	0.5±0.00[b]	0.6±0.00[a]	0.60±0.00[a]	-
PCV (%)	32.20±3.10[c]	42.07±1.08[a]	42.20±2.23[a]	37.00±3.00[b]	37.6-50.60
RBC ($x10^6$ mm^3)	4.53±0.01[c]	9.84±0.03[a]	5.25±0.00[b]	4.61±0.01[c]	6.76-9.75
WBC ($x10^3$ mm^3)	1.33±0.03[b]	1.62±0.00[a]	1.32±0.00[b]	1.29±0.01[b]	6.60-12.60
Hbc (g/100 mL)	11.26±0.11[b]	14.26±0.06[a]	10.56±0.15[c]	10.62±0.14[c]	11.5-16.10
MCV (μm^3)	79.18±3.03[a]	51.58±2.71[b]	78.86±4.10[a]	78.6±4.03[a]	-
MCH (pg)	16.08±2.01[a]	15.12±1.05[b]	16.2±0.08[a]	15.91±0.72[a]	-
MCHC (%)	33.3±3.00[a]	33.32±2.03[a]	33.03±0.98[a]	33.3±0.34[a]	-
Lymphocytes (%)	63.6±4.01[a]	60.4±2.11[b]	64.8±3.04[a]	64.0±1.23[a]	4.78-9.12
Neutrophils (%)	26.6±0.43[a]	26.6±0.09[a]	26.5±0.51[a]	26.5±1.21[a]	1.77-3.38
Monocytes (%)	7.18±0.00[a]	6.6±0.04[b]	6.6±0.02[b]	6.6±0.00[b]	0.01-0.04
Eosinophil (%)	2.0 ±0.00[a]	2.0±0.00[a]	2.0±0.00[a]	2.0±0.00[a]	0.03-0.08

Means (±SEM) with different superscripts in the same row show significant difference at $P < 0.05$. *Rat Haematological reference ranges [51, 52]. MCF - maize-based food, DIF = *D. iburua* food, DIM= *D. iburua* meal, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), ESR=erythrocyte sedimentation rate, PCV= packed cell volume, RBC= red blood cells, WBC = white blood cell, Hbc= haemoglobin concentration MCV=mean cell volume, MCH=mean cell haemoglobin, MCH= mean cell haemoglobin concentration, lymph= lymphocytes, Neutr= neutrophils, Mono= monocytes, Eosino= eosinophil, CFF (commercial weaning foods).

are the major variables needed for the determination of anaemic condition of a growing child.

CONCLUSION

The present study demonstrated that the experimental complementary foods were characterized with essential nutrients, energy, ability to support growth and affordable. Hence, it could be concluded that the formulated infant foods could be valuable and excellent source for low-priced complementary foods, which could provide adequate nutrients for the prevention of protein-energy malnutrition in pre-school children.

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