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Research Article

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Nutritional Quality and Safety Assessment of Complementary Food Produced from *Acha* (*Digitaria exilis*) Flour and *Kariya* (*Hildergardia barteri*) Protein Concentrate Blends

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Abstract

Complementary food is the intermediate food given to infant alongside with breast milk. This study is aimed at producing complementary foods from blends of *acha* flour and *kariya* protein concentrate.

Acha flour was produced from cleaned and milled raw *acha*. Raw *kariya* was processed to flour and protein concentrate flour. These flours were blended at 80:20 (*acha: kariya*). The nutritional qualities of the products were assessed using animal feeding experiment. The protein quality parameters and safety of the blends to the experimental animal were monitored. Data obtained were analyzed using appropriate descriptive and inferential statistics.

The result showed that the feed intake ranged between 104.46 and 196.86 g/rat/28 days. There were significant differences ($p \geq 0.05$) in growth rates of the animals. Growth of the animals fed with *acha* blended with processed *kariya* ceased after the 13*th* day, while the other groups progressed with varied degrees of increase in weights. The Protein Efficiency Ratio (-1.55 to 1.85), Food Efficiency Ratio (-0.24 to 0.28), True Digestibility (54.26 to 62.89%) and Net Protein Retention (-4.04 to 1.00) of the experimental animals varied with the diets. The mortality rate of 100% was recorded for diets containing *acha* and processed *kariya* after the 13th day of the experiment.

Despite the high nutritional value and decrease in anti-nutrients contents of *kariya* after series of processing, the blends were still toxic to the experimental animals (all the animals fed with samples containing *kariya* died). The *kariya* containing diets may not be safe for infant consumption.

Keywords

Food intake, Growth rate, Mortality rate, Protein efficiency ratio, Toxic

Introduction

The period of complementary feeding has been defined as a transitional phase during which food other than mother's milk is expected to supply increasing proportion of a child's nutritional requirement [1]. Large numbers of infants and young children in developing countries suffer from the short and long-term health effects of poor breastfeeding and complementary feeding practices which in turn impact negatively on social and economic development [2]. In Africa, high prevalence of protein energy malnutrition among infants above six months old has been recorded [2, 3].

Many traditional complementary diets are mainly based on plants; cereals, or roots, and the large amounts of starches in these plant sources result in a thick, gelatinous porridge, which often has a low nutrient content [4]. In addition, mineral bioavailability is poor in many plant-based foods [5]. However, these traditional complementary foods could be improved by combining locally available plant based foods that complement each other in such a way that new patterns of amino acids are created [6]. Enrichment of cereal-based food with other protein source such as legumes, oilseeds etc, have received considerable attentions since investigations have revealed that cereals are deficient in lysine and tryptophan but have sufficient sulphur-containing amino acids which are limiting in legumes [7]. In view of these nutritional challenges, quite a number of studies have investigated ways of formulating quality complementary foods through a combination of available plant based foods to meet the nutritional demands of infants of weaning age [6-18].

Despite all these interventions, it is quite evident that the objective of these efforts which was to develop low-cost foods is still far from being met. These products are still not available to poor families and therefore had little impact on the prevalence of child malnutrition [19].

As a means of addressing this problem, the present study is focused on the utilization of locally available nutritious and lesser known food materials *acha* and *kariya* to develop high dense nutrient and low-cost complementary foods.

Acha (fonio), a tropical millet native to West Africa, one of the most nutritious of all grains [20]; rich in methionine and cystine, amino acids vital to human health and deficient in today's major cereals [21]; has the advantage to be minimally processed which limited the loss of the native nutritional value during milling [20]. The use of *acha* is mainly limited to traditional foods such as thick and thin porridges, steam cooked products (e.g. Couscous), and alcoholic and non-alcoholic beverages [21]. *Acha* can be used for complementary foods of low dietary bulk and high calorie density. Such complementary preparations with good acceptability and tolerance by children have been investigated [11, 13, 18, 22].

Kariya (*Hildegardia barteri)* primarily an ornamental tree in West Africa [23] whose flowers, which are usually borne on leafless branches, mature into one-seeded pods [24]. The mature pods drop completely when dry and are disposed as refuse in many places, only in few parts of West Africa the kernels are eaten raw or roasted like peanuts [25] or used as condiments in traditional food preparations. The essential amino acid pattern as reported by Inglett et al. [25] showed that the seeds are high in lysine; therefore the use of the meal as complement to cereal grain would be beneficial in improving the amino acid balance.

The use of a combination of *acha* and *kariya* concentrate has not been investigated as a possibility of producing a veritable complementary food for infants in the developing countries. Therefore, this study aimed at producing complementary diets from blends of *acha* and *kariya* protein concentrate and assessment of the proximate composition, physicochemical and functional properties of the products.

Materials and Methods

Materials

Acha grains were purchased from Abuja central market, Abuja. Dried *kariya* pods were gathered from ornamental *kariya* trees in Obafemi Awolowo University, Ile-Ife, Nigeria. All reagents used were of analytical grade and were obtained from Sigma Aldrich chemical company, USA.

Production of *acha* flour

The *acha* grains were weighed (1.00 kg), washed thoroughly to remove dirt's, oven dried at 60 ^{0}C for 12 hrs and milled into flours [18].

Production of raw and defatted fermented *kariya* flours

The *kariya* nuts extracted from the dried pods were sorted to remove extraneous materials such as stones and leaves. The kernels were obtained by shelling the nuts manually which were then cleaned to remove chaff, broken, shriveled and immature kernels. The cleaned raw *kariya* kernels were divided into two portions. One portion was oven dried for 12 hrs, milled and sieved through 200 µm sieve to produce the raw *kariya* flour. The second portion was soaked for 24 h, cooked at 100 ^{0}C for 30 min, drained and allowed to ferment (in a calabash lined with clean plantain leaves at ambient temperature) for a period of 4 days followed by oven-drying at 60 ^{0}C for 20 hrs. The dried fermented seeds were comminuted using mortar and pestle to obtain coarse flour. The coarse flour obtained was then ground using Marlex Excella grinder (Marlex Appliances PVT., Daman) and sieved through 200 µm sieve. The resulting flour was defatted using food grade hexane solvent in soxhlet extractor to obtain defatted fermented *kariya* flour. The defatted fermented flour was desolventized by drying in a fume hood and the dried fermented flour was finally ground in a Marlex Excella grinder (Marlex Appliances PVT., Daman) to obtain homogenous defatted fermented flour [26].

Production of protein concentrate

Kariya protein concentrate was produced according to the method described by Cheftel et al. [26] and modified by Gbadamosi et al. [27] as shown in Figure 1.

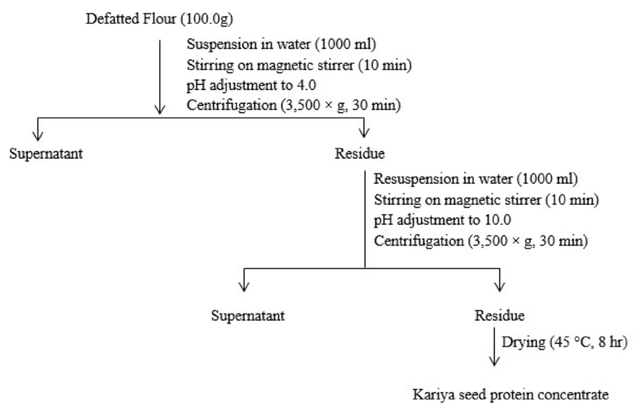

Figure 1: The production of *kariya* protein concentrate.
Source: Cheftel et al.[26] and modified by Gbadamosi et al.[27].

Formulation of the complementary food blends

The dietary blends (Table 1) were formulated with a target protein of 20% in accordance to the recommendation of the World Health Organization [28, 29].

Table 1: Formulation of complementary food blends.

Diet Sample	Formulation
ASD	100% Acha
ACD	80% Acha + 20% *kariya* Protein Concentrate
ARD	80% Acha + 20% Raw *kariya* Flour
ABD	Basal (non-protein diet) [4]
AED	Commercial Diet (Control)

Production of pre-gelatinized formulated diets

The dietary blends were mixed and pre gelatinized by cooking at 100 ^{0}C for 10 min. The pre-gelatinized blend was dried in an oven at 110 ^{0}C for 12 h and milled using attrition mill [30] as shown in Figure 2.

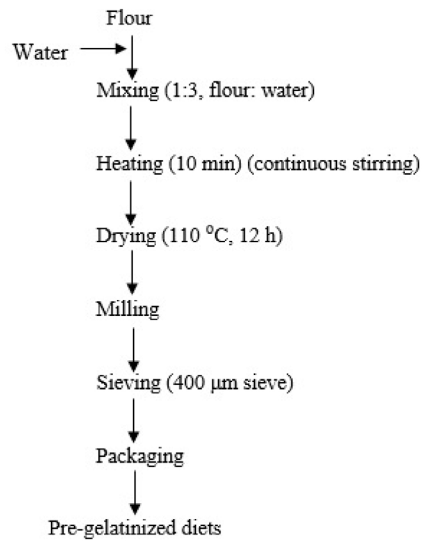

Figure 2: Flow Chart for the production of the pre-gelatinized formulated diets.
Source: Ikujenlola and Adurotoye [30].

Biological assessments

The method of Ikujenlola and Fashakin [4] was adopted. For this study, albino weanling rats of both sexes of varying weight were obtained from the Animal House, Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife. Animals were weighed and randomly distributed into individual metabolic cages and then acclimatized for 5 days. The animals were re-weighed and divided into groups. The average weight per group was approximately the same. A group of the animals (zero-day animal) served as control for the experimental groups and were sacrificed; the liver, kidney, and the plantaris muscle of the hind-leg were removed, weighed and frozen for chemical analysis. The remaining animals were fed the experimental diets and water *ad-libitum* over a period of twenty eight days. During this period, dietary intake and growth were monitored. The fecal discharge and urine were collected as from the fourteenth to the last day of the experiment and kept for nitrogen analysis. At the completion of the experiment, the animals were anesthetically sacrificed. Data collected during the experiment were used in determining protein quality parameters (Protein Efficiency Ratio (PER), Food Efficiency Ratio (FER), True Digestibility (TD), Net Protein Retention (NPR) and Protein Retention Efficiency (PRE). The study was carried out according to the rules of the Departmental ethics committee on animal studies/researches which is part of the University committee on animal researches.

$$PER = \frac{Weight\ gained}{Protein\ consumed}$$

$$FER = \frac{Weight\ gained}{Food\ consumed}$$

$$TD = \frac{Ni - (NF1 - NF2)}{Ni} \text{ X } 100$$

Ni = Nitrogen intake of animal fed with test diet, NF_1= Nitrogen excreted feces of animal fed test diet, NF_2= Nitrogen excreted from feces of animals fed protein free diet

$$NPR = \frac{Weight\ gain\ of\ the\ test\ group + Weight\ loss\ of\ protein\ free\ groups}{Weight\ of\ test\ protein\ consumed}$$

$$PRE = NPR \times 16$$

Statistical analysis

All determinations were made in triplicate and the data generated were subjected to one-way analysis of variance at 5% level of significance using SPSS 20.0 for Windows. Means were separated by Duncan's multiple range tests.

Results and Discussion

Food intake of the experimental animals during feeding experiment

The results showed that the mean food intake (Table 2) of the experimental animals fed on various formulated blends and the control varied from 104.46 g (basal diet) to 196.86 g (commercial diet). During the feeding experiment, it was observed that the rates and quantity of blend consumed varied not only between groups but also within the group. The protein intake of the animal followed a similar trend as the food intake, because the more the diet consumed, the more the protein (excluding basal). This agrees with the report of Vavidel and Pugalenthi [31] that placed the experimental animals (albino rats) on 20% protein.

Growth performance of the experimental animals

The growth performance of the experimental animals fed with the formulated complementary diets and control diet is presented in Figure 3. The mean weight gain (Table 2) ranged between -25.44 g (Basal) and 55.72 g (commercial diet). It was observed that the weight of the experimental animals decreased after 4 days into the feeding exercise; this could be as a result of adjustment to the test feed by the animal. Commercial diet supported the best weight gain/growth among all the complementary diets while the basal diet supported least weight gain. The increase in weight gained was largely influenced by the quality of the protein constituents of the diet. The protein content of the raw, processed *kariya* and diet are reported in another study underway. Protein is required among other things for good growth, healthy living, maintenance and production of cell and tissues of the body [7]. There were significant differences (p≤ 0.05) in the growth rates of the animals fed with the commercial diets and the formulated diets. The weight of animals fed on basal diet and ASD decreased as the feeding experiment progressed. This might be connected with the lack of protein in the basal diet but which is present in varying degrees in other samples. The basal diet and ASD did not support the growth; it was a negative growth rate (i.e. decline in growth rate), this was due to the fact that the basal diet lacks protein and ASD is deficient in some essential amino acids like lysine which is responsible for good growth and development. Low protein diets result in protein deficiency in weanling rats, which causes reduced growth, muscular wasting, emaciation and death if sufficiently severe [20]. All the animals that depend on the formulated diet for survival were found to become leaner and weaker each passing day of the experiment. Loss of weight were dramatic from 66.90 g and 66.92 g at day one to 41.46 g and 51.04 g at the twenty-eighth day for basal and ASD respectively.

On the other hand, the animals fed with other formulated diets i.e. ACD and ARD drastically reduced in weight from 66.78 g and 66.38 g at day one to 35.35 g and 37.50 g at 13th day. The two groups ceased to exist after the 13th day of the experiment i.e. all the animals fed with fortified *kariya* diets died. Although *kariya* flour and concentrate were high in protein but its inclusion did not support growth; the animals reduced in weight and eventually died. The death of the animals fed with *kariya* containing diets suggested that the diets possibly contained injurious substances apart from those analyzed under anti-nutritional factors. Also it suggested that the processing treatments to which the *kariya* seed was subjected to had no major reducing effects on the toxic substance(s) [32]. The values of the anti-nutritional factors are reported in another study underway. The mortality rate during the experiment is shown in Figure 4. The food intake and changes in the body weights may be influenced by the source of nitrogen and this was in accordance with the report of Obizoba [33] that the food intake and the changes in the body weights were influenced by the source of nitrogen and varietal differences in raw *kariya* and protein concentrate which were combined with *acha*.

The protein efficiency ratio (PER) and food efficiency ratio (FER)

The Protein Efficiency Ratio (PER) and Food Efficiency Ratio (FER) results are presented in Figure 5. The PER ranged between -1.55 (ASD) and 1.85 (commercial diet). PER for ACD (80% *acha* + 20% *kariya* protein concentrate) and ARD (80% *acha* + 20% raw *kariya*) could not be calculated because of the death of all the animals fed with the diets. From the results, it was observed that the PER was less than 2.5 which is regarded as the baseline for PER meant for weaning food. The PER gives indication of how well the protein has been effectively utilized by the animals. The lower PER signified that the diets did not support growth. ASD (100% *acha*) had low PER because it is deficient in some essential amino acids like lysine which might be responsible for the decline in the growth rate [7].

The FER ranged from -0.24 to 0.28. FER follows similar trend with the PER. The control diet had the highest FER (0.28) while the basal had the lowest FER (-0.24).

Table 2: Food intake of the experimental animals at the end of feeding experiment.

Dietary samples	Food Intake (g/rat/28 days)	Mean weight gained (g/rat/28 days)
Basal	0	0
ACD	0	0
ARD	0	0
ABD	104.46 ± 4.97	-25.44
AED	196.86 ± 8.00	-15.88
ASD	133.58 ± 3.48	55.72

Mean values in the same column followed by different superscripts are significantly different at p≥0.05

ARD: 80% *Acha* + 20% raw *kariya* flour; **ACD**: 80% *Acha* + 20% *kariya* protein concentrate; **ABD**: Basal; **AED**: Commercial Diet (Control); **ASD**: 100% *Acha*.

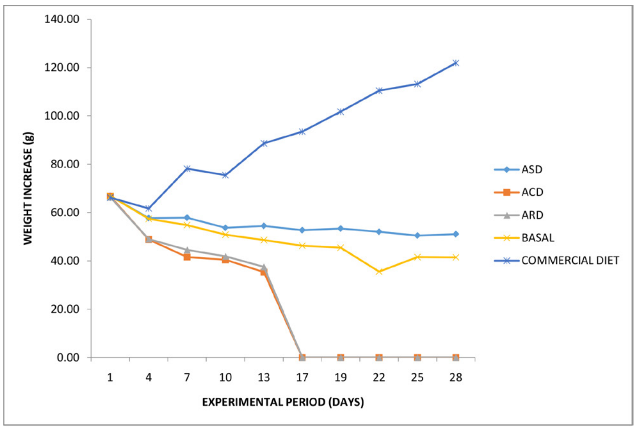

Figure 3: Growth rate of the experimental animals fed with the formulated diets and control. **ACD:** 80% *Acha* + 20% *kariya* Protein Concentrate; **ARD:** 80% *Acha* + 20% Raw *kariya*; **ASD:** 100% *Acha*.

True digestibility, net protein retention and protein retention efficiency

The true digestibility (TD) of the formulated diets is shown in Table 3 and it varied from 54.26% (commercial diet) to 62.89% (ASD). 100% *acha* had better digestibility than the commercial diets. Iwe [34] reported that legumes and oilseeds had reduced digestibility. This was attributed to the presence of anti-nutritional factors like tannins. It was also reported in the study that phenolic compounds like tannins exert influence by binding with various compounds including protein and making them less available to the animal because as dietary tannin content increase; the digestibility, energy and protein in the diet also decreases. Apart from the presence of anti-nutritional factor that reduces the protein digestibility, large intakes of dietary fibre, especially hemicelluloses increase the excretion of nitrogen in the feces, reducing the apparent protein digestibility of about 10% [29].

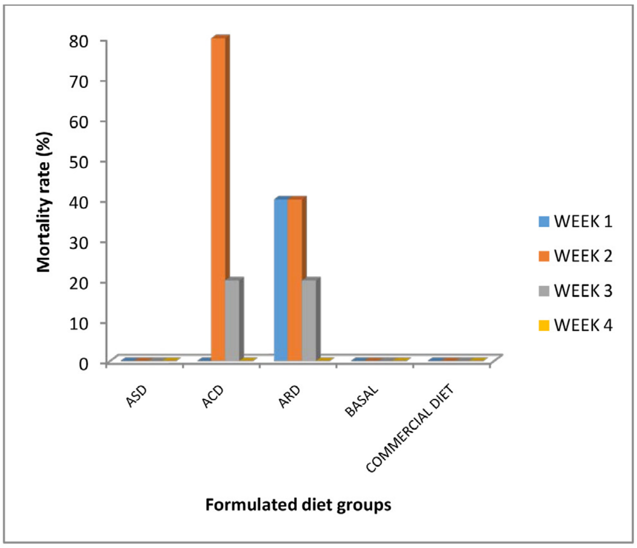

Figure 4: Mortality rate during the experimental period.
ACD: 80% *Acha* + 20% *kariya* Protein Concentrate; **ARD:** 80% *Acha* + 20% Raw *kariya*; **ASD:** 100% *Acha*.

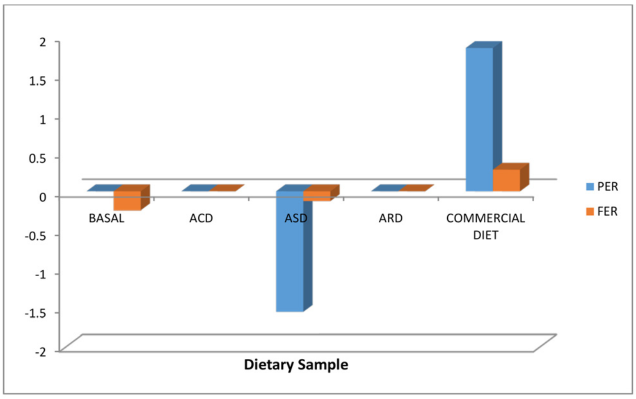

Figure 5: Protein Efficiency Ratio (PER) and Food Efficiency Ratio (FER) of the formulated diets.
ACD: 80% *Acha* + 20% *kariya* Protein Concentrate; **ARD:** 80% *Acha* + 20% Raw *kariya*; **ASD:** 100% *Acha*.

The NPR (Table 3) of the diet ranged from -4.04 (ASD) to 1.00 (commercial diet). The most favorable value was apparent in group fed with commercial diet whereas NPR is inferior in group receiving ASD. TD is a measure of the digestibility of food protein and the biological value of the amino acid mixture absorbed from food [35]. The PRE (Table 3) followed similar trend as observed in NPR values.

Weight of various tissues of the experimental animals

The mean weights of liver (Table 4) of the rats ranged from 2.10 g (basal diet) to 4.88 g (commercial diet). The sizes of the organs under consideration are related to the body weight of the experimental animals. The kidney size ranged from 0.53 g (basal diet) to 1.18 g (commercial diet). Generally, the tissues collected from the animals fed on basal diet were found to be very small and indeed much smaller than those of animals from other experimental groups which were due to protein deficiency in the diet. The weights of kidney and muscle tissues in the groups followed the same trend as that of the liver.

Table 3: Nutritional values of experimental diets compared with control diets.

Samples	TD	NPR	PRE
Basal	0	0	0
ACD	0	0	0
ARD	0	0	0
ASD	62.89	-4.04	-64.61
Commercial diet	54.26	1.00	16.06

ACD: 80% *Acha* + 20% *kariya* Protein Concentrate; **ARD**: 80% *Acha* + 20% Raw *kariya*; **ASD**: 100% *Acha*; **TD**: True digestibility; **NPR**: Net protein ratio; **PRE**: Protein efficiency ratio.

Total nitrogen in the tissues of the experimented animals

The total nitrogen in the tissues of the rats during the experimental period is shown in Figure 6. The mean total nitrogen of the tissues of the experimental animals followed the same trends as that of the weight of the tissues. There was increase in tissue (liver, kidney and plantaris muscle) nitrogen content in the group of animals fed the commercial diet compared to the other diets. The result observed might be as a result of the amount of the food consumed and subsequent nitrogen absorbed by the experimental animals.

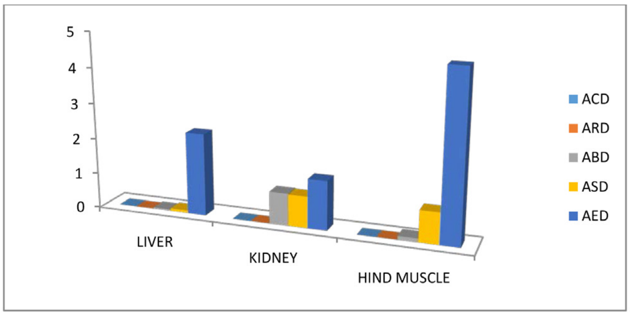

Figure 6: Total nitrogen in tissues of rats during the experimental period.
ABD: Basal; **ACD:** 80% *Acha* + 20% *kariya* Protein Concentrate; **AED:** Commercial Diet (Control); **ARD:** 80% *Acha* + 20% Raw *kariya*; **ASD:** 100% *Acha*.

Conclusion

This study concluded that there was a significant decline in growth and nutritional status of experimental animals fed with *acha-kariya* based complementary diets. The *acha–kariya* diets did not support healthy living in the animals. There was decline in their growth rate until they all died after the 13th day of the feeding exercise. The 100% *acha* diet gave lower values of PER, FER, NPR and PRE than the commercial diet. It showed that *acha* should be complemented with other protein rich foods. Moreover, the study concluded that despite the high nutritional value and decrease in anti-nutrients contents of *kariya* flour after series of processing, the blends were still lethal to the experimental animals; the diets containing *kariya* may not be safe for infant except thorough detoxification is ensured.

Table 4: Total weight of selected tissues of rats (g) during the experimental period.

Dietary samples	Liver	Kidney	Muscle (plantaris)
Stabilizing diet	3.25 ± 0.42	0.75 ± 0.11	0.82 ± 0.12
ABD	2.10 ± 0.13	0.53 ± 0.08	0.38 ± 0.18
AED	4.88 ± 0.25	1.18 ± 0.11	0.85 ± 0.48
ASD	3.11 ± 0.10	0.65 ± 0.12	0.51 ± 0.07

Mean values in the same column followed by different superscripts are significantly different at p≤ 0.05

ABD: Basal; **AED**: Commercial Diet (Control); **ASD**: 100% *Acha*.

Conflict of Interest

There is no conflict of interest. There is no impending conflict of interest as far as this study is concerned.

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