

Assessment of *Ricinus communis* (Castor Oil) Seed as Potential Protein Source in Weanling Diet Formulations

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Abstract: Protein-rich weanling food products, particularly those from animal sources, are not very accessible and affordable in several developing nations in Africa. Most women resort primarily to the use of only carbohydrate-based food such as cornstarch, to wean their toddlers from breast-feeding. This has led to several children getting affected by protein energy malnutrition and other associated conditions. The need to develop plant-based protein-rich weanling diets is therefore essential to ensure a balance in nutrient intake of toddlers. Growth and maintenance studies using *Ricinus communis* seed-diet formulations were investigated in wistar rats, to evaluate its potential as weanling diet. The diets were formulated using AIN 93G methods for laboratory rats during their growth, pregnancy and lactating periods. Thirty weanling male albino rats (40-60g) were utilized in this study, they were divided into five groups of six rats each based on sample treatment respectively. The groups includes; (CAS) casein diet, (BRC) boiled *R. communis* diet, (FRC) fermented *R. communis* diet, (FRC+C) fermented *R. communis* plus charcoal diet, and (NFD) nitrogen free diet. The rats were housed in individual metabolic cages equipped to separate faeces and urine. The growth study lasted for 21 days followed by a 7 days maintenance period. The proximate composition showed that *R. communis* seeds is high in protein (33.25%) and fat (37.70%). Boiling and fermentation treatments decreased the protein (23.13 and 20.37%) and fat (24.5 and 21.0%) contents, respectively. Sample-based diets were least consumed by the rats, which showed retarded growth as well, compared to CAS control and NFD diet groups. The nitrogen balanced studies showed that over 80% of the nitrogen consumed from the test diet were absorbed and retained, suggesting high utilization of the seed nitrogen.

Keywords: Proximate Composition, *Ricinus communis*, Protein Quality, Weanling Diet

1. Introduction

Plant materials, like the legumes, oil seeds and cereals form an important source of calories and protein for developing countries. The growth and maintenance needs of both children and adult can be achieved through eating of diet that are balanced especially with protein [1]. Protein is very essential in biochemical, biophysical and physiological processes [2].

It has been ascertained that the presence of some anti-nutrients, low palatability, digestibility and processing techniques reduces the utilization of these plant nutrients [3]. Different detoxification/processing techniques have been

employed in order to optimize the utilization of these plant foods. The efficacy of the adopted processing technique depends on the nature and properties of the antinutrients present [4].

Many processing methods have been shown to improve the nutritive value of plant foods for human nutrition [5]. Moderate heat treatment has been reported to improve the digestibility of plant proteins and inactivate several enzymes such as proteases, lipases, lipoxygenases, amylases, and other oxidative hydrolytic enzymes without developing toxic derivatives [5].

The castor oil plant *R. communis*, belongs to the plant family Euphorbiaceae. The plant has been cultivated for centuries in some countries for the oil produced by its seed.

Among the vegetable oils, castor oil is distinctive because of its high level of ricinoleic acid (over 85%) [6].

Castor oil has been in use for centuries for its antimicrobial and anti-bacteria properties. In Nigeria, It is mainly processed by fermentation to eliminate the ricin toxin. The fermented product commonly known as “*ogiri*” is utilized as food condiment in preparation of cocoyam based soup in eastern part of Nigeria.

This research is an attempt to explore the potentials of an under-utilized plant food as a source of protein.

As laboratory animals were intended for use in this study, approval was obtained from the ethics committee on the use and care of experimental animals of the Faculty of Biological Sciences, University of Nigeria Nsukka.

2. Material and Method

2.1. Sample Preparation

Boiled sample (BRC). The seeds were par-boiled for 30min in a metal pot on an electric cooker. After 30 minutes, the water was drained and the seeds were wrapped in fresh

plantain leaf and boiled properly in water in a ratio of 1:3 (W/V). The seed was boiled until the water was completely absorbed. The boiled sample was dried in an electric oven at 50°C, ground and stored in afreezer bag at 4°C.

Fermented sample (FRC). The seed were boiled using same procedure as BRC. However after boiling, it was covered in dried pot, allowed to ferment for four days and dried at 50°C, ground and stored in freezer bag at 4°C.

2.2. Diet Formulation

The diets were formulated using AIN 93G method for laboratory rats during their growth, pregnancy and lactating periods [7]. Casein, boiled *R. communis* (BRC), fermented *R. communis* (FRC) were sources of protein.

The dried ingredients for each diet were weighed into a bowl and mixed manually. An appropriate amount of oil was added and mixed thoroughly with the other ingredients, to a smooth texture by hand, water was added in small quantity at a time until a homogenous mixture was obtained and pelleted manually.

Table 1. Diet composition (g/kg diet).

Diet Ingredient	CAS	BRC	FRC	, FRC+C	NFD
CAS	114.30	-	-	-	-
BRC	-	439.06	-	-	-
FRC	-	-	490.99	490.99	-
Corn starch	206.74	206.74	165.20	165.20	557.99
Dextrinised corn starch	132.00	132.00	132.00	132.00	132.00
Sucrose	116.64	51.69	41.30	41.30	139.50
Fibre	50.00	50.00	50.00	50.00	50.00
Soy bean oil	70.00	70.00	70.00	70.00	70.00
Mineral mix	35.00	35.00	35.00	35.00	35.00
Vitamin mix	10.00	10.00	10.00	10.00	10.00
L-cystine	3.00	3.00	3.00	3.00	3.00
Choline bitartrate	2.50	2.50	2.50	2.50	2.50
TBH	0.014	0.014	0.014	0.014	0.014
Charcoal	-	-	-	16.00	-

CAS – Casein, TBH - Tetrabutyl hydroquinone, AIN - American Institute of Nutrition

2.3. Proximate analysis

Percentage concentrations of protein, lipid, crude fibre, moisture and ash of unprocessed (URC), boiled (BRC), fermented (FRC) *R. communis* were determined using the standard method of the Association of Official Analytical Chemist AOAC methods [8]. The crude protein content was calculated by multiplying the organic nitrogen content by a factor of 6.25 [9], while total carbohydrate content was calculated by subtracting the sum of other fraction from 100.

2.4. Experimental Design

Thirty weanling male albino rats (40-60g) were supplied by the Veterinary Department of the University of Nigeria Nsukka. The rats were divided into five groups of six rats based on sample treatment respectively. The groups included; casein diet (CAS) boiled *R. communis* (BRC) diet, fermented

(FRC) diet, fermented *Ricinus communis* plus charcoal (FRC+C) diet, and nitrogen free (NFD) diet groups. The rats were housed in individual metabolic cages equipped to separate faeces and urine. After a six-day acclimation period, the rats were weighed prior to access to their respective diets. Fifteen (15g) of casein and test diets were fed to the rats daily for a 21 day growth period. Daily food intakes were recorded and weight gain was measured on weekly intervals. The rats in NFD group were fed normal rat chow during the growth studies and NFD diet for maintenance studies. On day 21 and 28 after weight measurement, carmine red was added to each diet to mark the beginning and end of nitrogen balance, respectively. The black and red coloured faeces, collected during the growth and maintenance studies, respectively, were dried at room temperature for 48h, weighed, blended and stored at -10 °C until analysed.. The total urine collected (days 23 – 29) was treated with 0.09N HCL to avoid

microflora growth. The respective faecal and urinary samples were pooled together and stored at -10 °C, until analyzed. The rats were sacrificed under ether anesthesia on day 29, and relevant internal organs harvested and weighed.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS® software package (version 16). Data was assessed for normality and homogeneity using the Shapiro-Wilk test. Following a positive normality test, a one-way analysis of variance was performed to separate the means with significant differences determined at 95% confidence interval. Results were expressed as means of six determinations \pm standard deviations.

3. Results and Discussion

3.1. Proximate Composition

The results of proximate composition of *R. communis* seeds (Table 2) showed that the URC crude protein, fat and crude fibre contents were significantly higher ($p < 0.05$) when compared with the BRC and FRC samples. However, processing by boiling and fermentation increased the carbohydrate contents by more than 100% ($p < 0.05$) in relation to the unprocessed samples. The decrease in the protein content of *R. communis* following processing (boiling and fermentation) might be due to denaturing and subsequent loss of its non-protein nitrogen (NPN) in the water. Heating

by boiling is known to denature protein. Fermentation also creates a viable environment for microbial proteolytic enzymes to initiate the breakdown of proteins into component free amino acids. The increased free amino acids released leach into the water used in the process, which is eventually discarded. The crude protein composition of *R. communis* (32.25%), when compared with other conventional edible oil seed such as breadfruit (18.27%) [10] and bambara groundnut (23.41%) [11], is much higher. This high protein content of *Ricinus communis* seed positions it as a potential source for protein in diet formulations. The high fat content of *R. communis* (URC) is expected as it is an oil seed that stores fat as source of energy. The reduction of the fat content by fermentation and boiling can be attributed to loss in cooking and fermentation water following enzymatic hydrolysis by microorganisms as well as heat denaturation. The increased activity of lipolytic enzymes is generally known to increase the quality of fats in food as more free fatty acids (short and medium-chain) are released. The availability of fatty acids lead to potentially improved flavour and palatability of products [12]. Additionally, the increase in carbohydrate content of fermented and boiled samples might be attributed to increased amylolytic enzyme activity and gelatinization by cooking. Boiling carbohydrate-rich food caused gelatinization of the carbohydrate granules to increase the surface thus, exposing them to enzyme attack. The crude fibre ($3.25 \pm 0.70\%$) and ash content ($2.78 \pm 0.33\%$), were also higher in the URC while the moisture content showed the highest value in the BRC ($4.49 \pm 0.50\%$).

Table 2. Proximate composition of *R. communis* seeds (%).

Measured parameters	URC ^a	BRC ^b	FRC ^c
Moisture	3.45 ± 0.50^b	4.49 ± 0.50^{ac}	3.29 ± 0.30^b
Ash	2.78 ± 0.33^c	2.25 ± 0.27	2.00 ± 0.49^a
Fat	37.70 ± 0.80^{bc}	24.50 ± 0.50^{ac}	21.01 ± 1.00^{ab}
Crude protein	32.25 ± 0.30^{bc}	23.13 ± 0.20^{ac}	20.37 ± 0.60^{ab}
Crude fibre	3.25 ± 0.70^{bc}	1.65 ± 0.30^a	0.85 ± 0.20^a
Carbohydrate	20.54 ± 0.60^{bc}	43.98 ± 0.20^{ac}	52.47 ± 0.30^{ab}

Values are means from six rats \pm SD. Data on the same column with different superscript letters from the group are significantly different ($p < 0.05$).

3.2. Growth Study

The results of the growth study (Table 3) indicates that all the growth parameters measured (feed intake, weight gain, N intake and protein efficiency ratio-PER) were higher in the group of rats fed casein diet (control) and significantly different ($p < 0.05$) from those fed with the test diets (FRC+C, BRC and FRC). Although the FRC+C diet was consumed more than the BRC and FRC diets, the differences were observed to be non-significant. Moreover, all the rats in the test diet groups experienced a loss of weight because of low diet intake.

Feed intake is influenced by palatability, source of N and essential amino acid (EAA) profiles [13, 14]. The higher feed intake, resulting in higher N intake in rats fed CAS diet can be attributed to a combination of these factors as casein has desirable essential amino acid profile. However, the lower feed intake by rats fed with the test diets suggests that boiling

or fermentation did not produce acceptable flavour or improve the palatability of the diet to the taste of the test animals [15]. The low feed intake consequently, resulted in weight loss (negative weight values) in rats fed test diets. The protein efficiency ratio (PER) measures the effectiveness of a protein in promoting growth. It is the simplest method for evaluating protein quality, as it requires only an accurate measurement of dietary intake and weight gained by growing rats after 28 days of consumption of standard quantity of protein [16]. The higher PER of rats fed CAS diet is a reflection of the feed intake and the weight gained which were influenced by the desirable EAA pattern of casein. The lower PER values for rats fed test diets than those fed CAS diet might be due to the low feed intake and weight gain. The higher weight and PER of FRC group of rats than those fed BRC and FRC+C might be as a result of their high feed intake.

Table 3. Growth of rats fed with different *Ricinus communis* seed based diets (g).

Measured Parameter	CAS ^a	BRC ^b	FRC ^c	FRC + C ^d
Feed intake (g)	236.50±14.30 ^{bcd}	107.30±14.20 ^a	106.50±15.60 ^a	114.50±8.30 ^a
Weight gain (g)	54.00±10.70 ^{bcd}	-12.00±3.90 ^a	-5.50±1.50 ^a	-11.30±4.10 ^a
N intake (g)	19.90±1.20 ^{bcd}	4.90±0.60 ^{ad}	4.80±0.70 ^{ad}	6.40±0.50 ^{abc}
PER (cal)	2.70±0.40 ^{bcd}	-2.60±0.80 ^a	-1.20±0.50 ^a	-1.80±0.30 ^a

Values are means from six rats± standard deviation. Data on the same column with different superscript letters from the group are significantly different (p<0.05).

3.3. Nitrogen Balance Assessment

During the 7-day nitrogen balance study, the results (Table 4) for the feed intake and weight gain followed similar trend as those of the growth study. The rats fed CAS diet had higher feed intake and gained more weight than those in the groups fed with test diets (BRC, FRC and FRC+C). However, the difference in feed intake was not significantly different (p>0.05) except when compared with those fed BRC diets. Although the rats in the NFD group had the highest feed intake, they experienced a weight loss, which differed significantly (p<0.05) from those of the rats fed the test and CAS diets. This weight loss is expected, as their feed was deficient in N source (protein) which is essential for maintenance of body tissues.

The results of the protein quality indices (BV, NPU, and TD) estimated in this study revealed high values for both the CAS control group and the test diet groups. These values ranged from 85 – 99% (BV), 81 – 97% (NPU), and 89 – 98% (TD). In all, the CAS diet group had the highest values. The biological value of a protein is a measure of how efficiently food protein absorbed from the gastrointestinal tract is utilised in synthesis of body tissue [16]. The similarity of biological value of rats fed CAS diet and those fed test diets suggest that the test diets provided a good pattern of EAA

that can potentially be utilized for synthesis of tissue protein. Whitney and Rolfe reported that a protein with a BV of 70% or more can support growth and tissue maintenance as long as energy intake is adequate [17]. Thus, the high BV of test diets suggests that protein from these diets can support body growth and tissue maintenance. NPU is a measure of both digestibility and BV of the amino acid mixture absorbed from food [13]. The high net protein utilization value in rats fed the test diets, which are similar to those of CAS diet group, indicates that the test diets could provide adequate protein for tissue maintenance. Ene-Obong and Obizoba maintained that the net protein utilization is more of a practical magnitude than BV in protein quality evaluation [13]. This is because digestibility is an important and integrated part of the nutritive value of a dietary protein source. True digestibility, gives information on N intake absorbed by the body. Whitney and Rolfe observed that most protein is worthless to the body, if they are not digested and absorbed [17]. Overall, the high NPU values obtained for the test diets suggest that they contain desirable patterns of EAA. Also, fermentation produced better N balance indices than boiling because of extensive hydrolysis of proteins that release more free amino acids due to increased microflora enzyme activity- a commonly observed phenomenon.

Table 4. Nitrogen balance of rats fed different *Ricinus communis* Seed based diets.

Measured Parameters	CAS ^a	BRC ^b	FRC ^c	FRC+C ^d	NFD ^e
Feed intake (g)	36.25± 10.00 ^{bc}	16.25±2.80 ^{abc}	21.75±3.40 ^c	19.25±1.70 ^c	83.50±23.70 ^{abcd}
Weight gain (g)	2.75±0.30 ^c	-1.00±0.30 ^c	0.75±0.10 ^c	-1.25±0.20 ^c	-14.50±2.00 ^{abcd}
Faecal N (g)	0.27±0.03 ^c	0.30±0.03 ^c	0.29±0.01 ^c	0.31±0.01 ^c	0.22±0.02 ^{abcd}
Urinary N (g)	0.04±0.023 ^{ce}	0.09±0.02 ^{ce}	0.15±0.02 ^{abde}	0.09±0.01 ^{ce}	0.01±0.00 ^{abcd}
Consumed N (g)	3.05±0.80 ^{bcd}	0.74±0.01 ^a	0.99±0.10 ^a	1.10±0.06 ^a	
Absorbed N (g)	2.99±0.84 ^{bcd}	0.68±0.08 ^a	0.92±0.15 ^a	0.99±0.09 ^a	
Retained N (g)	2.96±0.87 ^{bcd}	0.59±0.08 ^a	0.78±0.14 ^a	0.92±0.09 ^a	
BV (%)	99.00±1.00 ^{bc}	89.00±9.00 ^a	85.00±6.00 ^a	93.00±2.00	
NPU (%)	97.00±3.00 ^{bcd}	81.00±8.00 ^a	84.00±6.00 ^a	85.00±2.00 ^a	
TD (%)	98.10±1.50 ^{bcd}	89.75±3.30 ^a	93.00±3.90 ^a	91.88±0.90 ^a	

Values are means from six rats± standard deviation. Data on the same column with different superscript letters from the group are significantly different (p<0.05)

3.4. Organ Weight Assessment

The weight of major organs (liver, heart, kidney, brain and lungs) assessed in this study showed that the CAS diet group had significantly higher (p < 0.05) organ weights when compared to the test diet groups. Also, the rats fed fermented

diets had higher lungs and brain weight which was significantly different from those fed boiled diets. Obizoba and Nnam observed that organ weights were influenced by body weight, food and N intake [18].

Table 5. Organ weight of rats fed various test diets and casein (g).

Organ weights	CAS ^a	BRC ^b	FRC ^c	FRC+C ^d
Liver (g)	5.25±0.95 ^{bcd}	3.00±0.81 ^a	3.50±0.58 ^a	3.25±0.50 ^a
Kidney (g)	1.11±0.04 ^{bcd}	0.71±0.14 ^a	0.7±0.11 ^a	0.73±0.05 ^a
Lungs (g)	0.78±0.16 ^{bcd}	0.38±0.03 ^{ac}	0.55±0.15 ^{ab}	0.45±0.04 ^a
Brain (g)	1.29±0.11 ^{bc}	0.93±0.03 ^a	1.06±0.14 ^a	1.15±0.20
Heart (g)	0.53±0.05 ^{bcd}	0.23±0.03 ^{ad}	0.23±0.03 ^a	0.23±0.03 ^{ab}

Values are means from six rats± standard deviation. Data on the same column with different superscript letters from the group are significantly different (p<0.05).

4. Conclusion

R. communis seed is rich in protein and processing particularly by fermentation, enabled release of desirable amino acids that showed good protein quality indices. However, the diets formulated using the processed samples were poorly consumed as observed in the low feed intake values among rats fed test diets. This strongly suggests that further study needs to be done to develop better or additional treatments that will enhance the flavour of the diets and improve palatability. However, during maintenance period, the high BV, NPU and TD values indicates that the seeds N derived from the little feed consumed, were well utilized in rats for tissue maintenance. More than 80% of N consumed from test diets was absorbed and retained. The processing methods used in this work (boiling and fermentation) are beneficial and are of nutritional importance to populations that prefer natural enhancement of nutrients to enrichment.

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