

Evaluation of Biochemical Parameters of Rats Fed with Corn Flour Supplemented with Cricket (*Acheta gossypii*) Proteins

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Abstract

African staple diets are mainly of protein-deficient corn. Dried yellow corn was fermented, dried, ground and supplemented with 5% and 10% of cricket (*Acheta gossypii*) flour. A standard diet of 5% and 10% soybean proteins-based corn flour was also prepared. The diets were fed to albino rats for four weeks. Standard methods for the serum levels of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), total proteins (TP), and albumin were determined in albino rats using 5% and 10% soybean supplemented corn diets as standard diets. The serum levels of ALP, AST, ALP, TP, and albumin in the rats fed with cricket supplemented diets were in the range of the normal healthy animals but were significantly different from the cornflour and soybean supplemented diets such that the ALP and AST values of the rats fed on the cornflour significantly decreased over the soybean supplemented and cricket supplemented experimental diets. The ALT activity, TP, and albumin levels of the rats fed the cornflour, and the soybean supplemented diets were significantly lower than those fed on the cricket protein supplemented diet ($p < 0.05$). Supplementing corn with crickets has shown improvement in the nutritional quality of corn, therefore, may be used to solve the malnutrition.

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INTRODUCTION

Hunger is on the rise in Nigeria and the underdeveloped countries. There is a massive problem of protein malnutrition and food shortages (Semba, 2016). The number of undernourished people has increased from around 804 million people in 2016 to around 821 million in 2017 and is worsening in developing nations of the world (Food and Agriculture Organization of the United Nations, 2019). The increase in the population of people had led to a higher demand for food. Furthermore, there is a dwindling economic crisis across the globe, especially in Africa and other developing countries (Kearney, 2010). Foods, if available, are not affordable. This has placed many people in a state where accessibility to regular food

becomes difficult and unaffordable. This creates a need for a cheaper and affordable food source (Tacoli, 2017; Kimani-Murage *et al.*, 2014).

Corn (*Zea mays*) is farmed as an everyday staple across the Savannah regions of Africa. Corn is cheap but deficient in proteins (Nuss & Tanumihardjo, 2011). This could be improved by supplementing cornflour with cricket (*Acheta gossypii*) proteins. Crickets are available, but underutilized (Stull *et al.*, 2018). Since corn is cheap and crickets are available, protein malnutrition, as well as food shortages, could be reduced by supplementing cornflour with cricket (Gunaratna *et al.*, 2019; Henchion *et al.*, 2017).

In certain parts of Africa including Nigeria, crickets, like other insect is ubiquitous in its various species both at home and in the wild (Ooninx *et al.*, 2015; Havlik *et al.*, 2014). Various species of crickets are reportedly consumed as snacks among the lower class in Africa. However, *Brachytrupes membranaceus*, *Gryllus bimaculatus* De Geer, *Acheta spp.*, and *Henicus whellani* are the most common species eaten (Musundire *et al.*, 2014). These all belong to the order orthoptera with high proteins (43.9-77.1 g/kg) and energy values (3319.3-5239.7 kJ/kg) as reported by Kelemu *et al.* (2015). Crickets can also be farmed with a turnover of just six weeks it could serve as a better protein alternative to others sources like beef that takes years and other expensive resources to be reared before conversion to meat (van Huis & Ooninx, 2017). More so, crickets have the more efficient conversion of phytomass to zoomass and the land required to farm crickets highly negligible per protein mass-produced when compared to the conventional sources as well producing less harmful gases that are capable of altering the climate (Rumpold & Schlüter, 2013).

In evaluating the nutritional quality of any food, the protein quality is the most important. Estimation of the protein quality involves the quality of the proteins as well as the digestibility (Wolfe *et al.*, 2016). Also, for new diets, safety and sustainable and non-toxic foods are of significant concern. Many corn-based diets are lacking in the nutrient, as mentioned earlier composition partially due to its low protein content (Sebastiani *et al.*, 2019). There is a need, for this reason, for the development of affordable diet with sufficient proteins that could support growth and development in children as well as in adults from available and acceptable raw resources with the use of easy processing methods which can be accessible and affordable to the poor and the average Nigerian (Chadare *et al.*, 2019).

Based on this background, this study aims to determine the biochemical parameters of test animals given a mixture of cornflour with cricket. As a test animal, male albino rats are used. The biochemical parameters observed were serum concentrations of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), total proteins (TP), and albumin.

MATERIALS AND METHODS

Materials

Assay Kits for ALP, AST, ALT, TP, and albumin determination, were products of Randox Laboratory Limited, County Atrim, United Kingdom. All the chemicals used were of general-purpose grade manufactured by the British Drug House (BDH) Limited, Poole, England and Sigma Aldrich Chemical Company Incorporation, Wisconsin, USA.

Tools

Tools used include the Beckman refrigerated centrifuge (TJ-6 model, USA), rotary evaporator (RE500 Yamato Scientific America Incorporation, USA), UV-Vis spectrophotometer (UV-1800 models, USA) analytical balance (Ohaus Adventurer model A223CN), water bath (Light Water, Surrey GU185TA, UK) medium-scale grinding engine (Henry West G160, 5.5 HP) moisture extraction oven (Gallenkamp, size 2, Brainweigh B, UK) and hot plate (Biotec, India).

Samples collection, identification, and treatment

The yellow corn samples were bought from Maikunkele Market, Bosso Local Government, Minna, Niger State, Nigeria and was identified in the Department of Plant Biology, Federal University of Technology, Minna. The corn kernels were cleaned steeped in a plastic container to ferment for 68 hours to allow for fermentation without sprouting. The fermented product was rinsed with clean

water and sun-dried after which they were wholly grinded severally to powder using an electric grinder. This was kept in a plastic container for further analyses. The roasted cricket flour sample was commercially obtained from Ankpa roadside market, Ankpa Local Government, Kogi State, Nigeria. Also, the cricket flour was identified at the Department of Plant Biology, Federal University of Technology, Minna. The roasted cricket was grinded into flour and stored in plastic containers for analyses. The soybean (*Glycine max*) sample was obtained from Bosso market, Bosso Local Government, Minna, Niger State, Nigeria. The samples were also identified in the Department of Animal Biology, Federal University of Technology, Minna. The dry soybean seed samples were washed, sun-dried and wholly grinded severally to powder using an electric grinder.

Formulation of cricket supplemented corn flour diets

The diets were formulated into 5% and 10% cricket supplements. The formulation was done based on the percentage of the cricket and corn flours respectively, to yield an increment of 5% and 10% cricket content in the experimental diets. The diets were kept in plastic containers for further analyses. The same procedure was repeated for soybean-based diets (Hanboonsong & Durst, 2014).

Determination of biochemical parameters

The methods for determining the biochemical parameters used were as described by Chauynarong *et al.* (2009) and Seralini *et al.* (2007). Twenty healthy male albino rats of an average initial weight of 68.42 g were fed with standard rat feed to acclimatize for 48 hours and grouped randomly into five groups. The experimental design was as shown in **Table I**.

Table I. Experimental design

Animal groups	Diet used as feed	Code
A	Free-corn flour supplement	0
B	5% cricket supplemented diet	C5
C	10% cricket supplemented diet	C10
D	5% soybeans supplemented diet	S5
E	10% soybeans supplemented diet	S10

Each group was housed and fed by the aid of a metal trough fitted to a corner of the housing cage, and at the same time, water was provided using an overhead nipple drinker for each cage. Each of the cages was cleaned every day. Feed and water were supplied ad libitum and feed intake were calculated in g. The weekly weight change was also recorded total feed intake, and total weight change of each rat was recorded from the corresponding weekly live weight change up to the end of the feeding period. After the 28 days feeding period, the animals were anaesthetized with chloroform. Each anaesthetized animal was sacrificed, and blood was collected. As much as 1 g blood collected carefully from the sacrificed animals with the aid of a pair of gloves and dissecting instruments, into 15 ml sucrose 0.25 N and then homogenized. The supernatant was carefully decanted into specimen bottles and kept below 4°C for assay.

Alkaline phosphatase assay

In a cuvette, 10 µl of the sample was mixed with 500 µl of Randox reagent (a solution containing *p*-NPP in full). The initial absorbance was read at 405 nm, and subsequently over three minutes. The mean absorbance per minute was used in the calculation:

$$ALP \text{ activity (IU/l)} = 2742 \times \Delta A \text{ 405 nm/min}$$

2742 = Extinction coefficient of Absorbance;

$\Delta A \text{ 405 nm/min}$ = change in absorbance per minute for the homogenate sample

Aspartate phosphatase assay

The sample (50 µl) was mixed with 500 µl of the AST reagent in a test tube, and the initial absorbance at 340 nm was read after a minute. The timer was started

simultaneously, and further readings of the absorbance were taken after 1, 2, and 3 minutes and calculations were made as below:

$$AST \text{ activity (nm/min)} = 1746 \times \Delta A_{340} \text{ nm/min}$$

$\Delta A_{340} \text{ nm/min}$ = change in absorbance per minute for the homogenate sample,
1746 = Extinction coefficient of 1 M Absorbance of NADH/NAD⁺

Alanine transaminase assay

The sample (50 μ l) and 500 μ l of the ALT reagent were mixed in a test tube, and the initial absorbance at 340 nm was read after a minute. The timer was started simultaneously, and further readings of the absorbance were taken after 1, 2, and 3 minutes and calculations were made:

$$ALT \text{ activity (nm/min)} = 1746 \times \Delta A_{340} \text{ nm/min}$$

$\Delta A_{340} \text{ nm/min}$ = change in absorbance per minute for the homogenate sample,
1746 = Extinction coefficient of Absorbance.

Total protein assay

In a cuvette, 10 μ l of blank (distilled water) was mixed with 1 ml of the Biuret reagent. It was left to incubate for ten minutes. Then the absorbance was read at 546 nm. The procedure was followed for the standard and the sample and repeated three times. The mean absorbance was used in the calculation:

$$C_{\text{sample}} = \frac{A_{\text{Sample}} \times C_{\text{Standard}}}{A_{\text{Standard}}}$$

A: absorbance (\hat{A})
C: concentration

Albumin assay

In a cuvette, 10 μ l of blank (distilled water) was pipetted and mixed with 1 ml of Randox reagent (R1). It was left to incubate for three minutes. Then, the absorbance was read at 628 nm. The procedure was followed for the standard and the sample and repeated three times. The mean absorbance was used in the calculation:

$$C_{\text{sample}} = \frac{A_{\text{Sample}} \times C_{\text{Standard}}}{A_{\text{Standard}}}$$

A: absorbance (\hat{A})
C: concentration

Statistical analysis

Data obtained were subjected to statistical analysis for the determination of mean and standard deviation values using Microsoft excel. Separate determinations for the significant differences ($p < 0.05$) between the mean values were analyzed using the Duncan's Multiple Range Test with Statistical Package for Social Statistics (SPSS) 15.0 (Ajibola *et al.*, 2016).

RESULTS AND DISCUSSION

Biochemical parameter test results from male albino rat blood samples given variations of the dietary supplement cricket and soybean are presented in **Table II**. The ALP activity values of the rats fed on the cornflour diet were significantly lower than the soybean and cricket supplemented diets. The ALP activity values between the rats fed with the cricket supplemented diets were not significantly ($p > 0.05$) different from each other. The ALP activity values in the rats fed the soybean supplemented diets were significantly different from each other and all other diets ($p < 0.05$). The AST activity values of the rats fed the cornflour, and the rats fed the soy supplemented diets were significantly higher than in the rats fed the 5 % and 10 % cricket supplemented diets ($p < 0.05$). The ALT activity of the rats fed the cornflour, and the soy supplemented diets were significantly ($p < 0.05$) lower than those fed with the cricket supplemented diets ($p < 0.05$). As with the ALT, a similar pattern was obtained with albumin, and total protein values in the rats fed the various diets.

Table II. Effects of experimental diets on biochemical parameters in male albino rats

Groups	Biochemical parameters				
	ALP (U/L)	AST (U/L)	ALT (U/L)	TP (g/dl)	Albumin (g/dl)
Corn flour	55.0 \pm 10.0 ^a	37.9 \pm 10.0 ^b	10.9 \pm 2.65 ^b	3.80 \pm 2.50 ^b	3.75 \pm 0.16 ^b
5% cricket	82.5 \pm 38.9 ^c	13.8 \pm 0.00 ^a	14.7 \pm 3.62 ^c	4.09 \pm 0.05 ^b	3.72 \pm 0.16 ^b
10% cricket	82.5 \pm 20.0 ^{bc}	18.9 \pm 12.1 ^a	15.2 \pm 0.27 ^c	5.04 \pm 1.33 ^c	4.09 \pm 0.16 ^b

5%	96.3 ±	61.2 ±	9.40 ±	2.60 ±	2.77 ±
soybean	13.8 ^c	10.8 ^c	1.30 ^a	1.37 ^a	0.83 ^a
10%	68.8 ±	58.8 ±	11.3 ±	2.79 ±	3.00 ±
soybean	19.5 ^b	2.34 ^c	3.00 ^b	2.90 ^a	0.60 ^a
References*	50.0 -	10 -	10 -	4.0 -	3.8 - 4.8
	128	45	35	6.5	

^{a,b,c} values with different superscript are significantly different from each other (p<0.05).

* (Kong *et al.*, 2016; van Huis *et al.*, 2013).

The results obtained showed a not too significant difference from the supplementation of both cricket and soybean at a concentration of 5% and 10%, except for ALP in soybean supplements. However, compared to references from previous studies by Kong *et al.* (2016) and van Huis *et al.* (2013), the average value indicated is still in the range of the results of previous studies. While in general, the use of cricket supplements showed an increase in the parameters of ALT, TP, and albumin compared to soybean. Conversely, on AST parameters, the results shown by the use of cricket are much lower than soybean.

Serum enzymes are biomarkers for the toxicity of some substances in animals. Some insects secrete toxic chemicals for self-defence. They might also harbour microorganism, which could be a source of toxicity when consumed as food (Schroegel & Watjen, 2019; Kim *et al.*, 2019). Although no known toxic chemical substance had been attributed to cricket (van Huis, 2013), there is a need to probe the chance of toxicity that may arise. The enzymatic activity of serum enzymes of the rats fed with the cricket supplemented diets agrees with the range of values reported by Kong *et al.* (2016) and van Huis *et al.* (2013), which are in the normal safe range. This suggests that subject to histochemical analysis of the underlying organs, and the diets are healthy.

The increased activity of AST in the rats fed with the soybean protein supplemented diet (5 and 10 %) could point to the fact that increase in supplementation with soybean protein could be hepatotoxic in rats. Although no clinical sign was noticed, continuous intake of the supplemented diets could lead to toxicity (Lee *et al.*, 2015).

This makes the cricket supplemented diets more superior to the soybean protein supplemented diets.

On the other hand, the low levels of the total serum proteins and albumin in the animals placed on the cornflour is a result of low protein level in cornflour (Table II). The low levels of serum proteins and albumin in the animals placed on the 5% and 10% soybean diet could be attributed to the possible presence of anti-nutritional factor in soybean (Gu *et al.*, 2010).

CONCLUSION

The biochemical parameters showed that there was no significant biochemical change in the characteristics of the biomarkers of liver toxicity for all the experimental diets except for ALP in the soybean supplemented diet. Thus, cricket can be used as a supplement for the cornflour diet as a substitute for soybean.

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