ASSESSMENT OF KIDNEY FUNCTIONS AND TRANSAMINASE ACTIVITIES IN WEANLING *WISTAR* RATS MAINTAINED ON DIFFERENT WEANING DIETS

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Abstract: This research was conducted to evaluate kidney functions and transaminase activities in *Wistar* rats fed on *Caelifera*-supplemented weaning diets made from locally available feed ingredients. Twenty-four (24) weanling *Wistar* rats, with mean weight of 39.27 ± 0.3 g were divided into four (4) groups of equal average weight and were randomly assigned to four experimental diets. Diet 4 (control) is Nestle wheat cerelac. Diet 1 contained *Digitariaexilis* (45%), *Caelifera* (12.5%), *Daucuscarota* (10%), *Glycine max*, (30%) and sucrose (2.5%) while diet 2 contained *Triticumaestivum* (45%), *Caelifera* (12.5%), carrot (10%), *Glycine max* (30%) and sucrose (2.5%). Diet 3 was made up of *Pennisatumtyphoides* (45%), *Caelifera* (2.5%), *Daucuscarota* (10%), *Glycine max* (30%) and sucrose (2.5%). The *Wistar* rats were fed on their respective diets and water *ad libitum* for six weeks. The serum concentrations of urea, uric acid, creatinine, aspartate and alanine transaminases were determined. The serum concentrations of urea, uric acid and creatinine were not significantly ($p>0.05$) affected by test diets. Serum ALT and AST were significantly ($p<0.05$) higher in *Wistar* rats maintained on diet 1. It is therefore concluded from the findings that the kidney functions may not be altered by the test diets.

Keywords: *Caelifera*, diet, function, kidney, supplemented, transaminases, weaning

Introduction

Malnutrition particularly protein energy malnutrition (PEM) among infants and children in most developing countries like Nigeria is a great source of concern to the government and all stakeholders (Andy *et al*., 2016). It leads to morbidity and mortality, impaired intellectual development and working capacity, and increased risk of adult disease (Kim *et al*., 2009). The major risk factors for malnutrition include unbalanced diet, psychological problems, digestive complaints and stomach conditions, lack of food, high food prices and lack of adequate breastfeeding (Khan *et al*., 2017).

The period during which solid foods or food other than milk are introduced into the infant`s food (weaning period) is increasingly being more critical owing to the increasing decline in the percentage of women who inadequately breast feed their children. Consequently, early formula feeding of the infant has always been resorted to. Commercial infant formulas are often believed to be nutritious, safe and widely accepted to infants (), however most of these commercial infant formula are usually not affordable to most low income households. The reports by researchers on the nutritive composition of legumes, cereals and vegetables in formulating traditional complementary weaning diets has no doubt shown great potential (Ladeji *et al*., 2000; Solomon, 2000; Umar *et al*., 2005).

Therefore, it would be of utmost advantage if weaning diets are formulated from readily available foodstuffs in communities. *Caelifera* (grasshopper) is an edible insect consumed in most parts of Northern Nigeria, Katsina State inclusive. It has been shown by researchers that grasshopper is rich in protein, contains trace and macro elements and is low in antinutritional factors (Kinyuru *et al*., 2011; Ghosh *et al*., 2016). Idoko *et al.* (2017) reported that though rats fed on the *Caelifera*-supplemented weaning diets formulated from some locally available food ingredients maintained steady but gradual growth, they were however found to be inferior to the commercial Nestle wheat cerelac in terms of achieving full growth potential and proper erythropoiesis. However, information on the role the *Caelifera*-supplemented weaning diets may play in some kidney functions and expression of transaminase activities remained scanty. This research therefore aimed at assessing some kidney functions and transaminase activities in *Wistar* rats maintained on *Caelifera*-supplemented weaning diets.

Materials and Methods

Materials

Acha grains (*Digitariaexilis*), millet (*Pennisatumtyphoides*), wheat (*Triticumaestivum*), soya beans (*Glycine max*), and grasshopper (*Caelifera*) were purchased from Katsina Central market. Carrots (*Daucuscarota*) and sugar (sucrose) were purchased from Dutsinma local market of Katsina state.

The weanling rats (39.3 g ±2.56) were purchased from Nigerian Institute for trypanosomiasis Research (NITR) Kaduna, Kaduna state, Nigeria. The commercial weaning food, wheat Cerelac is a product of Nestle Nigeria Plc. and was purchased from a supermarket in Dutsinma, Katsina.

Sample preparation

The samples were prepared in accordance with the method of Idoko *et al.* (2017). In this method, the wheat, millet, soybean and acha were cleaned to remove dirt, rot and unwanted substances. They were then washed and air dried for two days. The soybeans were soaked in water for twelve hours. Thereafter the beans were hulled by rubbing in between the palms and washing several times with water. The soybeans were then strained of water followed by air drying for two days. The dried soybeans samples were then roasted on a hot plate at 55^0C for 30 min. The carrots were thoroughly washed with clean water sliced into pieces and spread on a flat surface to allow easy drying. It was air-dried for three days. The dry grasshopper was sorted for dirt, other insects and unwanted substances. All the food substances were pulverized separately and stored in a clean plastic container. The pulverized grasshopper was sieved using 75 micron aperture to remove large particles.

Feed formulation

The pulverized cereals (millet, wheat and acha) were separately mixed with other ingredients to formulate three experimental diets (Idoko *et al*., 2017).

Diet 4 - Nestle Cerelac (control)

Diet 1- Acha: soya beans: *Caelifera*: carrot: sucrose (45:30:12.5:10:2.5%w/w)

Diet 2- Wheat: soya beans: *Caelifera:* carrot: sucrose (45:30:12.5:10:2.5%w/w)

Diet 3-Millet: soya beans: *Caelifera*: carrot: sucrose (45:30:12.5:10:2.5%w/w)

The formulated diets were then subjected to proximate analysis.

Assigning of experimental animals

The experimental rats were randomly distributed into four (4) groups of six (6) rats each, housed in standard laboratory compartment cage and fed with normal rats feed for 2 days so as to get stabilized and become acclimatized to the environment. The rats were starved for 1 day before the commencement of the feeding trial. The rats were given their respective experimental diets and water *ad libitum* for six weeks.

Sample collection

At the end of the feeding period, rats were weighed and anesthetized with chloroform. Blood was obtained through the jugular vein of the rats and collected in a plain sample container for analysis. The blood was allowed to clot for 40 min before centrifuging at 3000 rpm for 15 min. The supernatant (serum) was carefully transferred into a clean, dry and labeled sample container and stored until required (Akanji and Ngaha, 1989).

Determination of uric acid in serum

The method described by Fossati *et al.* (1980) was used for the determination of uric acid concentration in the serum. This is based on the principle that uricase converts uric acid to allantoin and hydrogen peroxide. The hydrogen peroxide formed further reacts with a phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colouredquinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of uric acid present in the sample.

Determination of creatinine in serum

Serum creatinine was determined using the standard assay kits following the method of Bartels and Bohmer (1972). This method is based on the reaction of creatinine in alkaline solution with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Determination of aspartate transaminase and alanine transaminase activity

The method described by Reitman and Frankel (1957) was used to determine the AST. The method is based on the use of 2, 4-DNPH to produce a coloured phenyl hydrazine of oxaloacetate in the aspartate – α-ketoglutaate transamination reaction catalyzed by the AST.

L – Aspartate + α- ketoglutaate $\xrightarrow{AST}$ Oxaloacetate + L glutamate

The activity of the enzyme is estimated by measuring the amount of oxaloacetate – phenyl hydrazine complex produced calorimetrically.

The procedure was the same for the alanine transaminase.

Statistical analysis

Results were expressed as means ± SEM. The statistical package program (SPSS 16.0 version) was used to assess statistical significance where $p < 0.05$ was set as the level of significance.

Results and Discussion

Proximate composition of the formulated diets

The proximate compositions of the test diets as reported in the previous publication by Idoko *et al.* (2017) is presented in Table 1. The test diets had significantly ($p<0.05$) lower ash and fibre, but higher crude protein ($p<0.05$) contents than the control.

Table 1: Proximate composition of the experimental diets

Nutrient (%)	Diet 1	Diet	Diet 3	Diet 4
Ash content	0.63±0.04^{a}	0.29±0.01^{b}	0.49±0.03^{c}	3.25±0.01^{d}
Crude Protein	17.43±0.00^{a}	19.28±0.00^{b}	19.25±0.00^{b}	15.00±0.58^{c}
Crude Fat	9.85±0.23^{a}	10.05±0.44^{a}	10.66±0.37^{a}	10.00±0.58^{a}
Crude Fiber	0.85±0.01^{a}	0.93±0.15^{a}	0.91±0.01^{a}	2.00±0.58^{b}
Total Carbohydrate	68.60±0.04^{a}	65.57±1.28^{b}	65.41±0.57^{b}	66.50±0.06^{ab}

Results are means of 3 determinations ± SEM .Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different

Serum concentration of some kidney functions of rats fed Caelifera-supplemented diets.

Compared with the control, serum concentrations of urea, uric acid and creatinine were not significantly ($p>0.05$) affected (Table 2). Uric acid is the final oxidation product of purine metabolism and is renally excreted (Johnson *et al*., 2011). Therefore, elevated serum uric acid levels are seen in patients with reduced glomerular filtration rate (GFR). While hyperuricemia may or may not predispose a patient to developing *de novo* renal disease, studies have indicated that the development of hyperuricemia leads to progression of existing renal disease and an increase in mortality (Weiner *et al*., 2008).

Kidneys urea is filtered out of blood by glomerulli and is partially being reabsorbed with water (Corbett, 2008). The most frequently determined clinical indices for estimating renal function depends upon concentration of urea in the serum. It is useful in differential diagnosis of acute renal failure and pre-renal condition (Mitchell, 2006).

Creatinine originates from the creatine/phosphocreatine pathway. Creatine is synthesized in the kidneys and the liver (Wyss *et al*., 2000) and stored mainly in striated muscle cells (Kushmerick *et al*., 1992; Sant'Ana *et al*., 1996), where it is phosphorylated to phosphocreatine by creatine kinase. In turn, phosphocreatine is used to phosphorylate ADP into ATP when energy demand is high. Both creatine and phosphocreatine spontaneously degrade to creatinine. Besides endogenous creatinine production, dietary intake of cooked meat and fish may contribute to the creatinine pool and affect serum creatinine levels (Baxmann *et al*., 2008). Since these metabolic wastes (urea, uric acid and creatinine) are removed by the kidney, their concentrations increase in blood during renal diseases (Jaspreet *et al*., 2000). Therefore, the formulated weaning diets may have no harmful effects on the kidney as could be inferred from the non significant variations in the evaluated metabolites. Similar results were obtained by Solomon (2005). It could be inferred that the *Caelifera*-supplemented diets may produce no harmful effects on the kidney.

Table 2: **Serum concentrations of uric acid, urea and creatinine of rats fed with *Caelifera*-supplemented weaning diets**

Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Uric Acid	2.56 ± 0.24^{a}	3.03 ± 0.33^{a}	2.80 ± 0.53^{a}	2.90 ± 0.35^{a}
UREA	5.94 ± 1.52^{a}	6.31 ±1.35^{a}	7.80 ±0.34^{a}	5.39 ± 0.35^{a}
Creatinine	59.47±21.85^{a}	39.64 ± 7.85^{a}	79.29±29.67^{a}	97.31 ± 8.26^{a}

Results are means of 3 determinations ± SEM .Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different

Table 3: Serum activities of ALT and ASTof rats fed with *Caelifera*-supplemented weaning diets

Parameter	Diet 1	Diet 2	Diet 3	Diet 4 (Control)
ALT (IU/L)	11.50±0.38^{a}	8.47±0.57^{b}	7.57±0.72^{b}	7.00±0.72^{b}
AST(IU/L)	15.00±0.26^{a}	13.43±0.23^{ab}	14.13±1.23^{ab}	12.53±0.55^{b}

Results are means of 3 determinations ± SEM. Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different

Activities of serum alanine transaminase (ALT) and aspartate transaminase (AST) of rats fed with Caelifera-supplemented diet

The serum concentrations of transaminase enzymes in rats fed *Caelifera*- supplemented diet are shown in Table 3. The rats maintained on diet 1 had significantly ($p>0.05$) higher concentration of the transaminases when compared with the concentration in rat fed the control diet. The serum activities of the transaminases (AST and ALT) are markers for the functions and integrity of the heart and liver (Adeniyi *et al.,* 2010) and aid in the diagnosis, monitoring and treatment of liver diseases because they reflect the inflammatory activity of the liver (Omar *et al*., 2018). The serum levels of these liver enzymes are reported to decrease in kidney disease patients (Omar *et al*., 2018). The possible mechanisms for this reduction may include reduction in pyridoxal‑5‑phosphate which is a coenzyme of aminotransferase, presence of ultraviolet absorbing materials and high levels of uremic toxins. Other possibilities include decreased synthesis and inhibition of release of AST and ALT from hepatocytes. It may even be due to accelerated clearance from serum (Fabrizi *et al*., 2001). The result is consistent with the non-significant variations in the serum concentrations of urea, uric acid and creatinine which also imply that the *Caelifera*- supplemented diets may not have harmful effect on the kidney.

However the observed significant increase of serum AST in rats fed *Caelifera*-supplemented weaning diet based on acha (Diet 1) as compared to the control is unclear and calls for further investigation on the combination of acha *Caelifera* in the formulation of weaning diet.

Conclusion

In conclusion, inclusion of *Caelefera* in weaning diets formulated from locally available ingredients such as acha, wheat and millet could improve the protein quality of the weaning diet and may not affect the kidney functions.

Conflict of Interest

Authors declare that there is no conflict of interest.

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