GROWTH PERFORMANCE AND HAEMATOLOGICAL CHARACTERISTICS OF WEANLING WISTAR RATS FED *CAELIFERA* SUPPLEMENTED DIETS

Idoko A. S., Balkisu A., and Zaharaddeen A. S.[*]

Department of Biochemistry, Federal University, Dutsin-Ma, Dutsin-Ma, Katsina State, Nigeria.
(Author to whom correspondence should be addressed; E-Mail: zayuba@fudtsinma.edu.ng
Phone +2348039498069

ABSTRACT

This research was conducted to determine the growth performance and haematological status of rats fed with *Caelifera*-supplemented weaning diets. Twenty four (24) weanling albino rats with mean weight of 39.27g ± 0.3g were divided into four (4) groups of equal average weight and randomly assigned to four experimental diets. Diet C (control) was Nestle wheat Cerelac. Diet A contained *Rheum australe* (45%), *Caelifera* (12.5%) *Daucus carota* (10%), *Glycine max* (30%), and sucrose (2.5%) while diets W and M respectively contained *Triticum aestivum* and P*ennisetum glaucum* instead of *Rheum austral.* The rats were fed *ad libitum* for six weeks. Proximate compositions of the diets, growth and haematological parameters were determined using standard methods. The results revealed that ash content of Diet A (0.630 ± 0.04), Diet W (0.287±0.01), and Diet M (0.490 ± 0.03) were significantly ($p < 0.05$) lower than that of the control (3.250 ± 0.01). The protein contents of these diets were however significantly ($p < 0.05$) higher compared to the control. Although there was no significant ($p > 0.05$) variations in the average weekly feed intake, the rats fed test diets had significantly ($p < 0.05$) higher feed conversion ratio and lower average weekly weight gain. The rats fed with the test diets had significantly ($p < 0.05$) lower PCV, Hb and MCHC concentrations. It could be drawn from the findings that the diets, even when supplemented with *Caelifera* may not be as good as Nestle Cerelac. It is therefore recommended that the test diets be fortified with minerals in order to raise their nutritional status.

INTRODUCTION

Malnutrition which is said to contribute to nearly half of all child deaths each year (Robert, 2013) has been described as a condition that occurs when people consistently do not consume or absorb the right amounts and types of food as well as essential nutrients(FMHN, 2016). This is due to increased susceptibility to contracting diseases in malnourished people. According to Global Nutrition Report (2015), 159 million children are stunted, 50 million are wasted and 41 million are overweight. Similar report indicated more than 90% of the world's stunted children live in Africa and Asia, and concluded that the prevalence of stunting and underweight among children under five years of age worldwide had insufficiently decreased since 1990, leaving millions of children at great risk (WHO, 2012). Stunting, according to UNICEF (2013) is the main indicator of childhood malnutrition and leads to poor physical growth and brain development, preventing them from thriving and living up to their full potential. Adequate nutrition during the weaning period is essential to avert malnutrition and its consequences. Weaning is the process of expanding the diet to include foods and drinks other than breast milk or infant formula. In case of other mammals like mice and albino rats, it is the moment when the pups are transferred out of the mothers' cage.

Adequate complementary (weaning) foods should contain the daily nutritional requirements beside the human milk (Nair *et al*., 2016). Traditional complementary (weaning) foods in Nigeria consist mainly of single mono-grains prepared from any of the wheat, millet, maize and acha fermented into gruels referred to as pap. These gruels have been reported to be of poor nutritional values (Modu *et al*., 2010). The commercial complementary (weaning) foods are exorbitant and cannot be afforded by most people in rural areas. In Nigeria, about 40% of the population live below poverty line and cannot afford commercial complementary (weaning) foods for their infants or good quality animal source of protein (Adebayo-Oyetoro, 2012). The high cost of nutritionally adequate commercial complementary (weaning) foods is a major challenge facing baby weaning mothers. The purpose of this research therefore, was to formulate *Caelifera*-supplemented complementary (weaning) foods from locally available materials such as wheat (*Triticum aestivum*), acha (*Rheum australe*), soybeans (*Glycine max*), farra (*caelifera*), and millet (P*ennisetum glaucum*); to study the growth performance characteristics and determine the haematological indices of weanling Wistar rats fed the formulated foods.

MATERIALS AND METHODS

The research was conducted in Dutsin-Ma LGA of Katsina State, Nigeria. Dutsin-Ma lies on latitude 12°26'N and longitude 07°29'E. The climate of Katsina State (Dutsin-Ma inclusive) is the tropical wet and dry type with average annual rainfall of about 700 mm. The mean annual temperature ranges from 29 °C – 31 °C [9] (Abaje *et al*., 2015).

Materials

Raw materials; wheat (*Triticum aestivum*), acha (*Rheum australe*), soybeans (*Glycine max*), grasshopper (*Caelifera*), and millet (P*ennisetum glaucum*) were purchased from Katsina central market. Sucrose and carrot (*Daucus carota*) were purchased from Dutsinma local market of katsina state, Nigeria. 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 state.

Feed Formulation

The processed feed ingredients were mixed in the following ratio to formulate three different experimental diets;

Diet A : Acha – soybean – *Caelifera* (grasshopper) – carrot - sucrose (45:30:12.5:10:2.5)
Diet B : Wheat - soybean – *Caelifera* (grasshopper) – carrot - sucrose (45:30:12.5:10:2.5)
Diet C : Millet – soybean – *Caelifera* (grasshopper)– carrot - sucrose (45:30:12.5:10:2.5)
Diet D : Nestle wheat Cerelac

Proximate Analysis of the Experimental Diet

The proximate (crude protein, lipid, ash and fibre) compositions were determined in accordance with the method of AOAC (1995), while carbohydrate was determined by subtracting the sum of percentage of crude protein, crude fat, crude fiber and ash from 100.

Experimental Design

The rats were placed in a cage and fed with normal rat feed for 2 days, after which the rats were starved for 1 day before commencement of the feeding trial. The rats were divided into four groups consisting of six rats each. The four (4) experimental diets were then randomly assigned to the groups. The groups were offered their respective experimental diets and water *ad libitum* for six weeks. The recommendation of ARRP (2007) for keeping experimental animals was complied with throughout the experimental period.

Determination of Feed Intake and Growth Response

To determine feed intake, the quantity of feed left over was subtracted from the quantity of feed served. The rats were weighed at the commencement of the experiment and thereafter on weekly basis to determine change in weight. The weekly feed conversion ratio was calculated by **dividing the total feed consumed by total weight** gain.

Preparation of Feed Materials

The wheat, millet, soybeans, and acha grains were cleaned to remove dirt, rot and other unwanted substances. The wheat, millet and acha were then washed, and air dried for two days. The soybeans were soaked in water for 12 hours. Thereafter, the beans were hulled by rubbing in-between the palms and washing several times with more water. Thereafter, the soyabeans were strained of water and air-dried for two days. The dried beans sample was then roasted at 55°C on a hot plate for 30 minutes. 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 3 days. The dry grasshopper was sorted for dirt, other insects, and unwanted substances. All the food materials were separately pulverised and stored in clean plastic container. Furthermore, the pulverised grasshopper was sieved using 75 micron aperture to remove larger particles.

Blood Sample Collection and Evaluation of Haematological Parameters

At the end of the 42- day period of feeding trial, the animals were weighed and anaesthetized in a jar containing cotton wool soaked in chloroform. Blood was obtained through the jugular veins into tubes containing EDTA as anticoagulant (Akanji and Ngaha, 1989). The animal sacrifice was preceded by 12-hour fasting. The analysis of the haematological parameters was done using an automated haematology analyser, SYSMEX KX21- JAPAN.

Statistical Analysis

The data were analyzed using SPSS version 16.0. The analysis of variance (ANOVA) was performed to determine significant differences among the means using Duncan Multiple Range Test where p value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The result of the proximate composition of the test diets indicates that 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). The significantly higher protein contents of the test diets may be as a result of the *Caelifera* supplements in the diets, although the test and control diets all fall within the recommended amount for infant diet. The

recommended Adequate Intake (AI) of protein for infants from birth to six months of age is 10 g (1.43 g/kg bodyweight) per day, and for infants aged seven to 12 months is 14 g (1.60 g/kg bodyweight) per day (NHMRC, 2006). Therefore, any difference in growth performance and haematological indices may not likely be due to the difference in protein content. The significantly lower ash content of the test diet may be due to low mineral contents of the respective composite feed materials. This difference may cause consequential difference in the evaluated parameter since minerals play important roles in metabolism. They are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life (Soetal *et al.*, 2010). The need for minerals becomes even more pronounced in infants and young animals because they need adequate micronutrients to maintain normal growth and development (Rush, 2000). Furthermore, the presence of minerals and trace elements at optimum levels is essential for the production and maintenance of many enzymes involved in the anabolic and catabolic processes (Tamari, 2016). Therefore, maintaining infants solely on the test diets may cause problems associated with mineral deficiency.

There was no significant difference between the average feed consumption (p > 0.05) by the test groups and the control group. The feed conversion ratios of the test groups were significantly (p < 0.05) higher than that of control group. Compared to the control, the test groups had significantly lower (p < 0.05) average weight gain (Table 2). The non-significant difference in feed consumption among the groups is interesting and indicates that the formulated diets have good taste and texture. Farran *et al.* (2005) had reported that taste and texture of finished feeds influence intake and satiety in animals. Feed intake could also be affected by freshness, appearance, mould, spoilage, moisture, temperature and odour. Feed conversion ratio (FCR) measures animal's efficiency in converting consumed feed into increases in weight gain.

The significantly higher FCR in rats maintained on the test diets is indicative of higher quality of the commercial diet (cerelac) and this could be due to low mineral contents of the test diets as indicated by the low ash contents of the test diets. For instance, trace elements like iron are essential components of metabolic enzymes (Soetan *et al.*, 2010). Many of the proteins of the electron-transport chain contain iron-sulphur clusters involved in the electron transport chain and ultimately ATP synthesis (Kamil *et al.*, 2006). Chromium's presence

facilitates the entry of glucose into the cell by facilitating the interaction of insulin with its receptor on the cell surface (Krejpcio, 2001). It has also been shown that low levels of magnesium, manganese, chromium and zinc may cause difficulties in glucose metabolism. As a result of its prominent roles in anabolic and energy metabolism, a Zinc deficiency contributes to growth failure and susceptibility to infections (Ekweagwul *et al.*, 2008). The significantly lower weight gain of rats placed on the test diets is understandable because when metabolism is affected, it would translate into inefficiency in converting feed into weight gain. The control group had remarkably higher weekly average weight gain which agrees with remarkably lower FCR in the group. It follows from the finding that long term solely consumption of the test diets may prevent attainment of full growth potential in Wistar rats.

The packed cell volume (PCV), haemoglobin concentration (Hb Conc.) and mean corpuscular concentration (MCH) values in the test groups were significantly lower (P<0.05) than the values in the control group (Table 3). The platelet and white blood cell values did not significantly vary (p > 0.05) among the groups. The significantly lower PCV and haemoglobin in rats maintained on the test diets could also be attributed to the lower ash (mineral) content of the test diets. Low iron, for instance, could cause decreased synthesis of haemoglobin which in turn decreases the oxygen-carrying capacity of red blood. Also, zinc is a required cofactor for an enzyme that synthesizes the haeme portion of haemoglobin and severely deficient zinc diets can result in anaemia. In addition, more severe copper deficiency can cause anaemia from the lack of iron mobilization in the body for red blood cell synthesis (Klevay, 2006). Long term solely consumption of the test diets may lead to complications associated with decreased red blood cell indices. The levels of white blood cells and platelets were not affected indicating that the test diets could maintain the potentials to fight disease and prevent blood loss due to bleeding in the experimental rats. White blood cells are mainly meant to fight infection while platelets prevents over bleeding from cuts.

The cost of formulating weaning diets from local feed ingredients was remarkably cheaper than the commercial one (Table 4). The formulation of the diets from local ingredients remarkably reduced the cost of the weaning diets, making them far more affordable than the commercial ones. This is understandable since the local materials from which the diets were formulated were cheap.

Table 1. Proximate composition of the experimental diets

Parameters	Diet A	Diet W	Diet M	Diet C
Ash (%)	0.630 ± 0.04^a	0.287 ± 0.01^b	0.490 ± 0.03^c	3.250 ± 0.01^d
Protein (%)	17.43 ± 0.00^b	19.28 ± 0.00^a	19.25 ± 0.00^a	15.00 ± 0.57^c
Lipid (%)	9.85 ± 0.03^a	10.04 ± 0.43^a	10.65 ± 0.37^a	10.00 ± 0.58^a
Fibre (%)	0.85 ± 0.01^a	0.92 ± 0.01^a	0.91 ± 0.01^a	2.00 ± 0.58^b
Carbohydrate (%)	68.59 ± 0.37^b	65.56 ± 1.28^a	65.40 ± 0.57^a	66.50 ± 0.57^{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.

Table 2. Average feed consumption, feed conversion ratio, and the average weight gain of the experimental animals

Parameters	Group A	Group W	Group M	Group C
FC	53.43 ± 2.81	55.16 ± 3.72	55.02 ± 4.70	59.75 ± 4.76
FCR	6.66 ± 1.10^b	6.06 ± 1.33^b	5.27 ± 0.75^{ab}	2.94 ± 0.46^a
AWG	9.02 ± 1.43^a	10.97 ± 1.81^a	11.30 ± 1.63^a	22.49 ± 3.65^b

Results are means of 5 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.
FC: Feed consumed
FCR: Feed consumption ratio
AWG: Average weight gain

Table 3. Haematological parameters of the experimental animals

S/N	Parameters	Group A	Group W	Group M	Group C (Control)
1	PCV (%)	39.67 ± 2.19^a	39.33 ± 1.45^a	39.67 ± 0.88^a	45.67 ± 1.76^b
2	HB (G/DL)	13.23 ± 0.74^a	13.10 ± 0.49^a	13.27 ± 0.31^a	15.20 ± 0.59^b
3	WBC (X10^9/L)	7.03 ± 0.55^a	6.70 ± 0.55^a	8.10 ± 0.42^a	6.93 ± 0.74^a
4	PLATELETS (X10^9)	299.33 ± 2.33	358.33 ± 14.62	380.33 ± 36.70	326.67 ± 48.46
5	MCH (G)	26.47 ± 1.48^a	26.20 ± 0.97^a	26.53 ± 0.64^a	30.40 ± 1.17^b
6	MCHC (G/DL)	33.33 ± 0.07	33.27 ± 0.03	33.47 ± 0.12	33.27 ± 0.03

Results are means of 5 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.PCV: Packed cell volume; Hb: Haemoglobin concentration; WBC: White Blood Cell count; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration. Group A: Rats

Table 4. The cost of diet

Diet	Cost of average weekly feed consumption in Naira
A	45.18 ± 0.38^a
W	31.17 ± 0.01^b
M	26.07 ± 0.01^c
C	119.50 ± 0.12^d

CONCLUSION

In conclusion, this research has revealed that the formulated diets from the locally available food ingredients, even when supplemented with *Caelifera* would still be nutritionally inferior to the commercially available wheat Cerelac.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abaje I.B., Ogoh A.O., Amos B.B., and Abashiya, M. (2015). Climate Change, Flood Disaster Assessment and Human Security in Katsina State, Nigeria. *American Journal of Human Ecology 4 (4):47-56*

Adebayo-Oyetoro O.A., Olatidoye O.P., Ogundipe, O.O., Akande, E.A., and Isaiah C.J. (2012). Production and Quality Evaluation of Complementary Food Formulated from Fermented Sorghum, Walnut and Ginger. *Journal of Applied Biosciences. 54: 3901-3910*

Akanji M.A. and Ngaha E.O. (1989). Effect of repeated administrations of berenil on urinary excretion with corresponding tissue pattern in rats. *Pharmacology and Toxicology*, 64: 272-275

Animal Research Review Panel (ARRP). (2007). Guideline 20: Guidelines for the Housing of Rats in Scientific Institutions. Accessed 20 July 2013. Available: ww.animalethics.org.au.

Association of Official Analytical Chemists (AOAC). (1995). Official methods of analysis. Washington D.C.

Ekweagwul E., Agwu, A.E., and Madukwe, E. (2008). The role of micronutrients in child health: A review of the literature. *African Journal of Biotechnology, 7:3804-3810*

Farran M.T., Halaby W.S., Barbour G.W., Uwayjan M.G., Sleiman F.T., and Ashkarian V.M. (2005). Effects of feeding ervil (Vicia ervilia) seeds soaked in water or acetic acid on performance and internal organ size of broilers and production and egg quality of laying hens. Poultry Science, 84 (11): 1723-1728.

Federal Ministry of Health, Nigeria. Malnutrition. (2016). Nigeria's Silent Crisis. Accessed 15 March 2016. Available: www.prb.org/pdf15/nigeria-malnutrition-factsheet.pdf.

Global Nutrition Report. Levels and trends in child malnutrition UNICEF – WHO – World Bank Group joint child malnutrition estimates. 2015. Accessed 10 December 2016. Available: https://www.unicef.org/media/files/JME_2015_edition_Sept_2015.pdf.

Kamil B., Sylwia M., Marcin K. (2006). Iron-sulfur cluster proteins: electron transfer and beyond. *Acta Biochemican Polonica, 53: 685–691*

Klevay L.M. Myelodysplasia myeloneuropathy, and copper deficiency. (2006). Mayo Clinic Proceedings, 81: 132-132.

Krejpcio Z. (2001). Essentiality of Chromium for Human Nutrition and Health. *Polish Journal of Environmental Studies, 10: 399-404.*

Modu S, Laminu H.H, Abba S.F. (2010). Evaluation of the nutritional value of a composite meal prepared from Pearl millet and cowpea. Bayero Journal Pure and Applied Science, 31:164-168.

National Health and Medical Research Council. Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes. 2006. Accessed 20 July 2013. Available: www.foodstandards.gov.au/.../monitoringnutrients/.../nutrientreferenceva

Nair M.K.C, Piyush G. and Alok G. Nutrition. Accessed 20 December 2016. Available: bpni.org/Article/nutrition-chapter-IAP-Textbook.

Robert E.B. (2013). Maternal and Child Undernutrition and Overweight in Low-Income and Middle-Income Countries. Lancet. Accessed 20 October 2016. Available: www.prb.org/pdf15/nigeria-malnutrition-factsheet.pdf

Rush D. (2000). Nutrition and Maternal mortality in the developing world. *American Journal of Clinical Nutrition*, 72: 2125-2405.

Soetan K.O., Olaiya C.O., Oyewole O.E. (2010). The importance of mineral elements for humans, domestic animals and plants. *African Journal of Food Science, 4(5): 200-222.*

Tamari G. M. Metabolic Role of Minerals: Analytical and Clinical Considerations. Accessed 21 November 2016.
Available:
www.anamol.com/index2.php?option=com_content&do_pdf=1&id=98

United Nations Children Fund. Improving Child Nutrition. The Achievable Imperative for Global Progress. 2013. Accessed June 2016. Available: www.unicef.org/publications/index_68661.html

United Nations Children's Fund, World Health Organization, The World Bank. Child Malnutrition Estimates. 2012. Accessed 10 June 2016. Available: www.who.int/nutgrowthdb/cover.pdf