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Extruded breakfast meal from malted finger millet (*Eleusine coracana*) and watermelon (*Citrullus lanatus*) seed flour: in-vivo nutritional qualities study

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Abstract

Background: The study aimed at evaluating the in-vivo nutritional qualities of extruded breakfast meal produced from flour blends of malted finger millet and watermelon seed.

Results: The proximate compositions of the flour blends revealed that there was progressive increase in protein (12.83–15.14) %, with increase in the watermelon substitution. The protein quality evaluation of the extrudate showed that the protein efficiency ratio ranged from 0.64 to 89.75, while the biological values were between (87.82–89.75)%. The relative organs weight of rats fed with extruded breakfast meal showed that, the weights of the kidney and liver of rats fed with extruded breakfast meal were significantly lower compared with rats fed with goldenmorn. The hematological indices showed that the packed cell volume and the red blood cell counts of rats fed with the formulated diets were significantly lower compared with those fed with goldenmorn but significantly higher than rats fed with basal. Meanwhile, the values of the white blood cells count for the formulated diet shows no significant difference compared with rats fed with goldenmorn.

Conclusions: Evidently, the growth performance of the rats fed with the extruded breakfast meal revealed that the formulated diets promote growth status of the animals with relatively low effect on organs of experimental rat used in this study. Hence, formulated diet may serve as alternative to expensive commercial breakfast meal.

Keywords: Extruded breakfast meal, Nutritional evaluation, Biochemical indices

Background

Food processing techniques are used to enhance nutritional quality, improve the digestibility and bioavailability of food nutrients with reducing anti-nutrients (Oladiran and Emmambux 2020). These food processing techniques includes decortications, milling, soaking, cooking, germination, fermentation, malting, popping and extrusion (Bolade 2018). Germination is a biochemical process which involves transition of a seed from dormant state to vital active state and this process could enhance protein content, mineral bioavailability and dietary fibre (Chauhan 2017).

Extrusion cooking has been described as an important technique for modification and manufacturing of a wide variety of traditional and novel food products (Altaf et al. 2020). Expanded snack foods, ready to eat cereals and dry pet foods are manufactured from cereals (finger millet) and starches by high temperature short time extrusion cooking (Olapade and Adetuyi 2007).

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Finger millet contains protein (5–8%), fat (1–2%), and carbohydrates (65–75%) (Chethan and Malleshi 2007; Nakarani et al. 2020). It has the highest calcium content among cereals (344 mg/100 g). The total dietary fibre (22%) of finger millet grains were reported to be relatively higher than that of wheat (12.6%), rice (4.6%), maize and sorghum (12.8%) (Siwela, et al. 2010; Ambre et al. 2020). Finger millet is the best source of micronutrients such as iron, phosphorus, zinc and potassium and were reported to be higher than that of rice and equal to that of wheat (Nakarani et al. 2020; Ambre et al. 2020). However, finger millet is limiting in lysine which is found abundance in fruit such as watermelon (Anitha et al. 2020).

Watermelon (*Citrullus lanatus*) is a typical fruit from the family of *Cucurbitaceae* grown in the warmer part of the world whose seeds are underutilized. The seeds are rich source of dietary fibre (> 5%), high in protein (35%), fat (50%), magnesium, calcium, potassium, iron, phosphorus, zinc with some excellent functional properties which have been found to be effective in baking (Maoto et al. 2019; Rekha and Rose 2016; Wan-Shafiin et al. 2020). They are rich sources of B vitamins, as well as phytochemicals and on consumption they promote strong bones, teeth, and hemoglobin formation (Braide et al. 2012; Wan-Shafiin et al. 2020).

The food industry is primarily driven by consumer health trends. A present day dietary concern is the consumption of expensive sugar-rich commercial stable foods, and which have been found to be associated with an increase in the incidence of diabetes and related health problems, including coronary heart disease (CHD) (Boobier et al. 2006; Ifesan 2020). Hence, in the present study, finger millet—A rich source of carbohydrate was fortified with watermelon seed flour. The extruded breakfast meal produced from finger millet and watermelon seed flour were subjected to nutritional evaluation studies using experimental animals in order to determine the nutritive value of the extruded products.

Methods

Sources of food materials

Finger millets were obtained from Ganawuri market, Jos, Nigeria; while watermelon fruits, ginger, common salt and granulated sugar were all purchased from Ozoro market, Delta, Nigeria. Extrusion was carried out at Food Processing Laboratory, Federal University of Agriculture, Abeokuta (FUNAAB) using locally fabricated food extruder (manufactured by NASOD Engineering Ltd., Ogun, Nigeria). All reagents used were of analytical grade.

Processing of flour samples and food formulation

Malted finger millet flour

Malted finger millet flour was processed into flour using the method described by Owheruo et al. (2019). Finger millet grains were cleaned, picked manually and winnowed to remove all forms of foreign particles and defects. The finger millet was soaked in water for 6 h. After soaking, the finger millet was spread thinly on a moist jute sack, sprinkled intermittently with water to facilitate sprouting, malted for 72 h and dried in hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 65 °C for 15 h. Dried malted finger millets were milled (Laboratory blender, Model KM 901 D, Kenwood Electronic, Hertfordshire, UK) and sieved through 200 mm mesh sieve (British Standard) to obtain malted finger millet flour and packaged in airtight polyethylene bag until further use.

Watermelon seed flour

Watermelon fruits were washed with potable water and cut manually into eight equal longitudinally parts using a stainless steel table knife. The seeds were manually extracted, washed, drained and dried in hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 65 °C for 10 h. Dried watermelon seeds were mill (Laboratory blender, Model KM 901 D, Kenwood Electronic, Hertfordshire, UK) and sieved through 200 mm mesh sieve (British Standard) to obtain watermelon seed flour and kept in airtight polyethylene bag until further use.

Formulation of malted finger millet-watermelon seed flour blends

The flour blends were formulated using Response Surface Methodology (RSM) Design-Expert version 10.0.0 (Stat-Ease, Inc., USA) to generate the following samples MFMWM90: Malted finger millet:Watermelon seeds (90.00:10.00%); MFMWM86: Malted finger millet:Watermelon seeds (86.25:13.75%); MFMWM82: Malted finger millet:Watermelon seeds (82.50:17.50%); MFMWM78: Malted finger millet:Watermelon seeds (78.75:21.25%); MFMWM75: Malted finger millet:Watermelon seeds (75.00:25.00%); and Goldenmorn (a commercial breakfast meal) was used as control as shown on Table 1.

Extrusion of malted finger millet-watermelon seed flour blends

Extrusion of malted finger millet-watermelon seed flour blends was carried out on a single screw extruder

Table 1 Formulation of flour blends

Flour composition (%)			Other ingredient (%) of total flour composite			Extruded meal
Run	FM	WS	Ginger	Sugar	Salt	
1	90.00	10.00	5.00	3.00	0.50	MFMWM90
2	86.25	13.75	5.00	3.00	0.50	MFMWM86
3	82.50	17.50	5.00	3.00	0.50	MFMWM82
4	78.75	21.25	5.00	3.00	0.50	MFMWM78
5	75.00	25.00	5.00	3.00	0.50	MFMWM75

FM finger millet flour, *WS* watermelon flour seed

(manufactured by NASOD Engineering Ltd., Ogun, Nigeria) with configuration (304:18.5 L/D ratio, 18 mm screw diameter, 1.74 KW power, 304 mm barrel length, 0.8 cm die length and extruder speed 125 rpm). The extrusion cooking process occurred at a barrel temperature of 120 °C and 17.5%, and extrudate were cut in to small shapes, cooled at room temperature for 30 min and were kept in airtight polyethylene bag until further use.

Proximate composition determination

The proximate compositions (moisture, crude protein, fat, total ash) of the extruded malted finger millet-watermelon seed flour were determined according to Association of Official Analytical Chemist (AOAC 2012). Carbohydrate was obtained by difference; while the energy value was calculated using the Atwater factor method $[(9 \times \text{crude fat}) + (4 \times \text{crude protein}) + (4 \times \text{carbohydrate})]$ as described by Kayode et al. (2020).

Nutritional quality evaluation

The experiments on animals were conducted in accordance with the laws and regulations as regards animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC 1993).

Experimental animals

Thirty-five Albino Wistar rats (*Rattus novergicus)* of both sexes with average weight of 25 g were obtained from the Animal House Unit, Department of Biochemistry, Federal University of Technology, Akure, Nigeria. The rats were divided into seven groups ($n=5$) and the rats were housed individually in metabolic cages in a climate-controlled environment with free access to feed and water for 7 days' acclimatization. After these, rats in each groups were fed on formulated extruded breakfast meal, control and basal diet (animal chow) with water ad libitum for 28 days. Records were kept on the food intakes, weight and length gained by the rats. Faeces and urine voided for the past seven days of the experimental periods were collected. The faeces were oven dried at 60 °C, while urine was preserved in 10 mL of 10% sulphuric acid to eliminate microbial activities and to prevent nitrogen losses by evaporation of ammonia, and the samples were stored in a deep freezer (− 20 °C) prior to nitrogen determination. The feed efficiency, protein efficiency ratio, biological value (BV) and feed conversion ratio were calculated (Oluwajuyitan et al. 2020).

Anthropometric measurements of the rats

Height and weight of the animals were measured at 4 day intervals for 28 days using standard techniques. All measurement was carried out in the morning between 9 a.m. and 12 noon. Weight was determined using a digital weighing scale (Salter, SL20348, London, UK) calibrated to the nearest 0.1 kg. Length was measured using a meter board constructed and calibrated to the nearest 0.1 cm. The animal was stretched along the meter board with the nostril touching the zero mark and the measurement was taken at the tip of the tail stretched along the meter board to the nearest 0.1 cm. The weight and length of the animals were related using weight-for-length and length-for-age indices to determine nutritional status.

Hematological and biochemical indices determination

Collection of blood samples

On 28 day of the experimental period, all the rats were starved for about 3 h and weighed. Each rat was anaesthetized with chloroform before been euthanized by cervical dislocation. Blood was collected into Bijour bottles containing a speck of dried tetracetic ethylenediamine acid powder and a portion of the blood was also collected without anticoagulant for serum biochemical parameters. After the experiment, the animals' carcass was hygienically buried below the soil level as detailed by the study ethical protocol committee.

Hematological indices determination

Hematological indices were determined as described by Oluwajuyitan and Ijarotimi (2019). Packed cell volume (PCV) was estimated by centrifuging about 75 Fl of each blood sample in heparinized capillary tubes in a hematocrit microcentrifuge for 5 min. Total red blood cell count (RBC) was determined using normal saline as the diluting fluid. Hemoglobin (Hb) was estimated using the cyanomethemoglobin method and corpuscular hemoglobin concentration (MCHC) mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) were calculated.

Biochemical indices determination

The biochemical parameters were analysed using methods described by Jasper et al. (2012). The blood sample was first centrifuged at 1500×*g* for 10 min at ambient temperature. The serum was then separated and used for liver function assessment employing measurements of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline Phosphate (ALP). Renal function was evaluated using serum concentrations of urea and creatinine. These tests were performed using disposable kits obtained from Labtest Diagnostica S.A. (Lagoa Santa, Minas Gerais, Brazil).

Statistical analysis

Data were subjected to analysis of variance using SPSS (IBM version. 20.0, SPSS Inc., Quarry Bay, Hong Kong) and presented as means (± SEM). Comparisons between different groups was done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $p < 0.05$ were considered as statistically significant.

Results

Proximate composition of extruded breakfast meal

The moisture and protein content of the extruded breakfast meal from malted finger millet-watermelon seed flour blends ranged from 6.48 g/100 g in MFMWM86 to 7.44 g/100 g in MFMWM78 and 12.83 g/100 g in MFMWM90 to 15.14 g/100 g in MFMWM75 respectively as shown on Table 2. The moisture contents of the developed extruded breakfast meals were significantly higher ($p < 0.05$) than 5.16 g/100 g obtained in reference samples (goldenmorn) whereas the extruded products were higher in protein content except for the sample with 10% watermelon seed flour than the value obtained in goldenmorn. Ash content ranged from 1.40 g/100 g (MFMWM90) to 4.56 g/100 g in MFMWM86. Fat content of samples ranged from 10.48 g/100 g in MFMWM82 to 12.53 g/100 g in MFMWM78 while, the energy value ranged from 400.60 kcal/100 g in MFMWM86 to 411.07 kcal/100 g in MFMWM90. The fat content and the energy value of developed extruded samples were significantly higher than (6.60 g/100 g and 373.00 kcal/100 g) obtained in goldenmorn.

Nutritional quality of rats fed with extruded breakfast meal

The feeding experiment showed that the mean weight gain (Table 3) of the experimental animals fed with the extruded breakfast meal from malted finger millet-watermelon seed flour blends significantly lower compared with animals fed with goldenmorn. Among the extrudates, rats fed with sample MFMWM75 had the highest weight gain (34.00 g). The mean food intake of the animals fed with sample MFMWM90 was significantly higher than those fed with the goldenmorn and other experimental samples. Feed efficiency ratio, nitrogen retention, biological value and net protein utilization shows that animal fed with developed extruded breakfast meal were significantly lower compared with animal fed

Table 2 Proximate composition (g/100 g) and energy value (Kcal/100 g) of malted finger millet-watermelon seed flour blends

Parameters	Goldenmorn	MFMWM90	MFMWM86	MFMWM82	MFMWM78	MFMWM75	Basal
Moisture	5.16 ± 0.02^{c}	7.39 ± 0.33^{a}	6.48 ± 0.23^{b}	6.57 ± 0.04^{b}	7.44 ± 0.29^{a}	7.02 ± 0.01^{ab}	7.47 ± 0.37^{a}
Total ash	2.13 ± 0.01^{d}	1.40 ± 0.27^{e}	4.56 ± 0.01^{a}	2.64 ± 0.01^{c}	3.44 ± 0.03^{b}	2.57 ± 0.02^{c}	1.33 ± 0.12^{e}
Crude protein	13.00 ± 0.05^{e}	12.83 ± 0.01^{f}	13.43 ± 0.26^{d}	13.83 ± 0.01^{c}	14.45 ± 0.01^{b}	15.14 ± 0.01^{a}	10.30 ± 0.01^{g}
Crude fat	6.60 ± 0.03^{f}	11.07 ± 0.01^{d}	11.36 ± 0.01^{b}	10.48 ± 0.01^{e}	12.53 ± 0.01^{a}	11.17 ± 0.01^{c}	10.44 ± 0.12^{e}
Crude fibre	7.20 ± 0.06^{a}	2.27 ± 0.01^{f}	3.02 ± 0.01^{d}	2.92 ± 0.01^{e}	3.33 ± 0.01^{b}	3.15 ± 0.01^{c}	2.13 ± 0.01^{g}
Carbohydrate	65.50 ± 0.50^{b}	65.03 ± 0.25^{b}	61.16 ± 0.48^{d}	63.56 ± 0.03^{c}	59.17 ± 0.09^{e}	60.95 ± 0.01^{d}	68.33 ± 0.51^{a}
Energy	373.00 ± 0.29^{c}	411.07 ± 11.34^{a}	400.60 ± 8.14^{ab}	403.88 ± 9.91^{ab}	407.25 ± 10.42^{ab}	404.89 ± 9.22^{ab}	408.48 ± 10.48^{ab}

Means (± SEM) with different alphabetical superscripts in the same row are significantly different at $P < 0.05$

Key: Goldenmorn (commercial control breakfast meal); Basal (animal chow); MFMWM90: Malted finger millet:Watermelon seeds (90.00:10.00%); MFMWM86: Malted finger millet:Watermelon seeds (86.25:13.75%); MFMWM82: Malted finger millet:Watermelon seeds (82.50:17.50%); MFMWM78: Malted finger millet:Watermelon seeds (78.75:21.25%) and MFMWM75: Malted finger millet:Watermelon seeds (75.00:25.00%)

Table 3 Nutritional qualities of rat fed with malted finger millet-watermelon seed extruded breakfast meal

Parameters	Goldenmorn	MFMWM90	MFMWM86	MFMWM82	MFMWM78	MFMWM75	Basal
Weight gained (g)	52.00^{a}	7.00^{e}	5.00^{f}	11.00^{d}	27.17^{c}	34.00^{b}	4.50^{g}
Food intake (g)	588.00^{b}	251.19^{g}	330.85^{f}	365.35^{e}	424.61^{d}	487.15^{c}	593.69^{a}
Food efficiency ratio	0.11^{a}	0.01^{d}	0.01^{d}	0.03^{c}	0.06^{b}	0.10^{a}	0.01^{d}
Nitrogen retention (%)	16.29^{a}	13.33^{b}	9.96^{d}	8.65^{e}	12.51^{c}	10.60^{d}	3.67^{f}
Biological value (%)	93.03^{a}	89.75^{b}	87.91^{c}	88.35^{b}	87.82^{c}	88.54^{b}	47.15^{d}
Net protein utilization (%)	90.80^{a}	88.75^{b}	85.65^{c}	84.51^{d}	85.92^{c}	85.86^{c}	45.58^{e}
Protein efficiency ratio	1.74^{a}	0.49^{d}	0.33^{e}	0.64^{c}	1.62^{b}	1.79^{a}	0.11^{f}
Protein retention	8.98^{a}	1.48^{d}	0.76^{f}	1.30^{e}	4.73^{b}	4.44^{c}	0.02^{g}
Relative organ weight (g)							
Kidney	2.86^{b}	0.26^{c}	0.34^{a}	0.20^{e}	0.23^{d}	0.26^{c}	0.08^{f}
Liver	2.45^{b}	2.63^{a}	1.89^{d}	1.48^{e}	1.30^{f}	2.16^{c}	0.87^{g}
Heart	0.35^{a}	0.26^{b}	0.24^{c}	0.20^{d}	0.26^{b}	0.23^{c}	0.07^{e}

Means with different alphabetical superscripts in the same row are significantly different at $P < 0.05$

Key: Goldenmorn (commercial control breakfast meal); Basal (animal chow); MFMWM90: Malted finger millet:Watermelon seeds (90.00:10.00%); MFMWM86: Malted finger millet:Watermelon seeds (86.25:13.75%); MFMWM82: Malted finger millet:Watermelon seeds (82.50:17.50%); MFMWM78: Malted finger millet:Watermelon seeds (78.75:21.25%) and MFMWM75: Malted finger millet:Watermelon seeds (75.00:25.00%)

with goldenmorn. However, sample MFWM90 biological value (89.75%) was significantly higher compared with other developed extruded breakfast meal.

The weight of some vital organs of animals fed with experimental diets are shown in Table 3. The relative weight of organs (kidney, liver and heart) of the experimental rats, fed on extruded breakfast meal significantly lower compared with animal fed with goldenmorn.

Growth performance of rats fed with extruded breakfast meal

The growth performance in terms of weight-for-age (WFA) (underweight, a measure of combination of chronic and acute malnutrition) and length-for-age (LFA) (stunting, a measure of past nutritional status) indices of the rats fed with extruded breakfast meal and control diets (Figs. 1, 2) showed that the growth rate of the rats fed with the control diet (goldenmorn) were significantly ($p > 0.05$) higher compared to the developed extruded samples. However, the rats fed with MFMWMF75 had the highest growth performance followed by MFMWMF78 and MFMWMF82 among the formulated extruded breakfast meal.

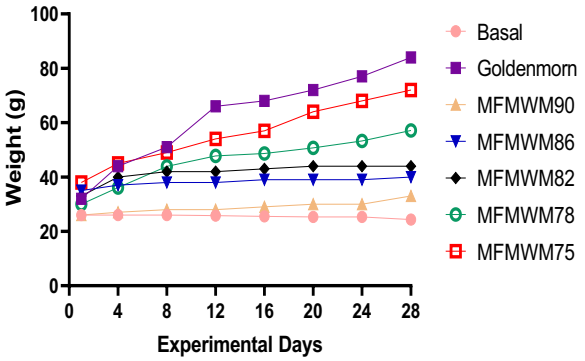

Fig. 1 Weight-for-days of the experimental animal. Key: Basal (animal chow); Goldenmorn (commercial control breakfast meal); MFMWM90: Malted finger millet:Watermelon seeds (90.00:10.00%); MFMWM86: Malted finger millet:Watermelon seeds (86.25:13.75%); MFMWM82: Malted finger millet:Watermelon seeds (82.50:17.50%); MFMWM78: Malted finger millet:Watermelon seeds (78.75:21.25%) and MFMWM75: Malted finger millet:Watermelon seeds (75.00:25.00%)

Haematological and biochemical indices of rats fed with extruded breakfast meal

The haematological property of the extruded breakfast meal (Table 4) shows that the packed cell volume (PCV) and red blood cells (RBC) obtained from the rats fed with the formulated diets were significantly lower compared with animal fed goldenmorn but significantly higher than animals fed with basal. Meanwhile, the values of the white blood cells (WBC) for the formulated diet shows no significant difference compared with animals fed with goldenmorn. It was also observed that rats fed with formulated extruded breakfast meal RBC and WBC were slightly lower than the recommended normal range for animal with those fed with basal and goldenmorn. However, the mean concentration hemoglobin (MCH) in animals fed with MFMWM90 and MFMWM75 were significantly higher compared with animals fed with goldenmorn.

The albumin (5.14–11.24 mg/dl), globulin (7.78–26.34 mg/dl), and total protein (13.10–31.48 mg/dl) of experimental rats fed with formulated extruded breakfast meal were found to be significantly ($p > 0.05$) higher

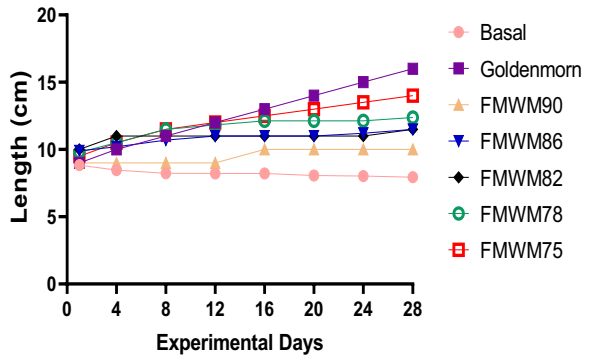

Fig. 2 Length-for-days of the experimental animal. Key: Basal (animal chow); Goldenmorn (commercial control breakfast meal); MFMWM90: Malted finger millet:Watermelon seeds (90.00:10.00%); MFMWM86: Malted finger millet:Watermelon seeds (86.25:13.75%); MFMWM82: Malted finger millet:Watermelon seeds (82.50:17.50%); MFMWM78: Malted finger millet:Watermelon seeds (78.75:21.25%) and MFMWM75: Malted finger millet:Watermelon seeds (75.00:25.00%)

than rats fed with goldenmorn (3.00; 12.10 and 15.10) mg/dl and basal (1.00; 3.03 and 4.03) mg/dl (Table 4) respectively. Similarly, the values obtained in rat fed with formulated extrudates and goldernmorn were comparatively higher than normal range values for serum albumin (3.80–5.00 g/dL), and protein (6.00–8.00 g/dL) respectively. Variation could be attributed to the quality of protein in each experimental breakfast meal sample. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) are all enzyme markers used to determine the functionality of the liver. The AST values of the formulated extrudates ranged from 23.04 U/L in MFMWM90 to 79.82 U/L in MFMWM78, and were significantly ($p > 0.05$) higher than goldenmorn (21.00 U/L). For ALT, the values were from 11.78 U/L in MFMWM90 to 29.46 U/L in MFMWM75 and MFMWM78. The ALP values ranged from 83.64 U/L in MFMWM90 to 116.86 U/L in MFMWM75.

Discussion

High protein values observed in the extruded sample may be attributed to the inclusion of watermelon seed flour owing to the fact that watermelon seeds are rich in protein (Kausar et al. 2020; Behere et al. 2020). The high protein value observed in the present study is in line with

Table 4 Hematological and biochemical parameters of rat fed with malted finger millet-watermelon seed extruded breakfast meal

Parameters	Basal	Goldenmorn	MFMWM90	MFMWM86	MFMWM82	MFMWM78	MFMWM75	NR*
PCV%	18.00 ± 0.03^{g}	46.00 ± 0.30^{a}	31.00 ± 0.07^{e}	19.00 ± 0.04^{f}	33.00 ± 0.03^{d}	36.00 ± 0.06^{c}	43.00 ± 0.06^{b}	37.6–50.6
Hb (g/dl)	$6.00. \pm 0.02^{e}$	12.10 ± 0.05^{a}	7.00 ± 0.05^{d}	6.30 ± 0.05^{e}	7.70 ± 0.01^{d}	8.70 ± 0.02^{c}	11.00 ± 0.02^{b}	11.5–16.1
WBC ($\times 10^3 mm^{-3}$)	4.03 ± 0.03^{b}	5.03 ± 0.04^{a}	4.00 ± 0.03^{b}	4.00 ± 0.03^{b}	3.00 ± 0.03^{c}	5.00 ± 0.03^{a}	5.00 ± 0.03^{a}	6.6–12.6
RBC ($\times 10^3 mm^{-3}$)	2.00 ± 0.01^{f}	4.00 ± 0.07^{a}	2.35 ± 0.20^{d}	2.15 ± 0.14^{e}	2.55 ± 0.15^{d}	2.90 ± 0.10^{c}	3.70 ± 0.10^{b}	6.76–9.75
MCHC (g/dL)	33.30 ± 0.02^{c}	33.60 ± 0.09^{a}	33.30 ± 0.05^{c}	33.10 ± 0.08^{d}	33.40 ± 0.06^{b}	33.40 ± 0.07^{b}	33.30 ± 0.09^{c}	28.2–34.1
MCH (pg)	30.00 ± 0.05^{c}	30.20 ± 0.10^{b}	33.30 ± 0.08^{a}	29.30 ± 0.17^{d}	30.20 ± 0.11^{b}	30.00 ± 0.08^{c}	33.30 ± 0.10^{a}	16.0–33.1
MCV (fl)	70.00 ± 0.04^{a}	70.00 ± 0.38^{a}	69.30 ± 0.17^{b}	68.30 ± 0.31^{c}	70.10 ± 0.10^{a}	69.60 ± 0.17^{b}	69.10 ± 0.14^{b}	50.0–77.8
Neutrophils (%)	36.00 ± 0.03^{e}	21.00 ± 0.54^{g}	50.00 ± 0.57^{a}	39.00 ± 0.14^{d}	41.00 ± 0.33^{c}	42.00 ± 0.33^{b}	30.00 ± 0.00^{f}	5.3–38.1
Lymphocytes (%)	58.00 ± 0.04^{c}	76.00 ± 0.19^{a}	44.00 ± 0.05^{f}	57.00 ± 0.13^{d}	53.00 ± 0.57^{e}	53.00 ± 0.03^{e}	67.00 ± 1.15^{b}	56.7–93.1
Monocytes (%)	3.00 ± 0.00^{b}	3.00 ± 0.00^{b}	3.00 ± 0.00^{b}	0.00 ± 0.00^{d}	2.00 ± 0.00^{c}	5.00 ± 0.00^{a}	3.00 ± 1.00^{b}	0.00–7.7
Eosinophils (%)	3.00 ± 0.00^{b}	0.00 ± 0.00^{c}	3.00 ± 0.00^{b}	4.00 ± 0.00^{a}	3.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.0–3.4
Basophils (%)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.0–0.4
Albumin (mg/dl)	1.00 ± 0.03^{g}	3.00 ± 0.04^{f}	5.31 ± 0.93^{d}	6.95 ± 0.85^{c}	8.53 ± 0.77^{b}	11.24 ± 0.35^{a}	5.14 ± 0.05^{e}	3.80–5.00
Globulin (mg/dl)	$3.03. \pm 0.02^{g}$	12.10 ± 0.05^{d}	7.78 ± 0.62^{f}	10.16 ± 0.82^{e}	12.48 ± 0.98^{c}	16.44 ± 0.62^{b}	26.34 ± 0.74^{a}	-
Total Protein (mg/dl)	4.03 ± 0.03^{g}	15.10 ± 0.09^{e}	13.10 ± 0.92^{f}	17.11 ± 0.27^{d}	21.02 ± 0.72^{c}	27.68 ± 0.07^{b}	31.48 ± 0.24^{a}	6.00–8.00
Kidney function indices								
Urea (mg/dl)	1.00 ± 0.03^{d}	2.90 ± 0.10^{a}	2.91 ± 0.62^{a}	0.81 ± 0.61^{e}	1.75 ± 0.17^{b}	1.63 ± 0.53^{c}	2.91 ± 0.62^{a}	7.00–20.00
Creatinine (mg/dl)	0.90 ± 0.04^{a}	0.40 ± 0.08^{e}	0.65 ± 0.05^{c}	0.79 ± 0.06^{b}	0.67 ± 0.04^{c}	0.51 ± 0.07^{d}	0.59 ± 0.06^{d}	0.20–0.80
Liver function indices								
AST (U/L)	96.00 ± 0.03^{a}	21.00 ± 0.54^{f}	23.04 ± 0.48^{e}	58.69 ± 0.65^{d}	66.52 ± 0.74^{c}	79.82 ± 0.61^{b}	79.56 ± 0.22^{b}	45.70–80.80
ALT (U/L)	18.00 ± 0.04^{c}	22.00 ± 0.19^{b}	11.78 ± 0.14^{d}	22.03 ± 0.14^{b}	21.78 ± 0.71^{b}	29.46 ± 0.82^{a}	29.46 ± 0.29^{a}	17.50–30.20
ALP (U/L)	73.00 ± 0.00^{f}	83.00 ± 0.00^{e}	83.64 ± 0.23^{e}	87.40 ± 0.09^{d}	90.76 ± 0.43^{c}	110.96 ± 0.23^{b}	116.36 ± 0.34^{a}	56.80–128.00

Means with different alphabetical superscripts in the same row are significantly different at $P < 0.05$

Key: Basal (animal chow); Goldenmorn (commercial control breakfast meal); MFMWM90: Malted finger millet:Watermelon seeds (90.00:10.00%); MFMWM86: Malted finger millet:Watermelon seeds (86.25:13.75%); MFMWM82: Malted finger millet:Watermelon seeds (82.50:17.50%); MFMWM78: Malted finger millet:Watermelon seeds (78.75:21.25%) and MFMWM75: Malted finger millet:Watermelon seeds (75.00:25.00%)

*NR: Giannini et al. (1999) and Diana (2007)

the report of Kausar et al. (2020) where an increase in protein was recorded by inclusion of watermelon seed flour to cookies. Ash is an indication of mineral content of food and it has been reported that watermelon seed has high ash content (Ojinnaka et al. 2018; Falade et al. 2020). Crude fibre is known to aid the digestive system of human (Jin et al. 2020; Li et al. 2020). Energy is a function of the protein, fat and carbohydrate composition of any food product which could be the reason for variation in the energy values of the products (Ojinnaka et al. 2018). High fat content recorded in the present study must have been responsible for high energy value. This is beneficial as it will provide sufficient calories to carry out daily activities (Hasan et al. 2020; Simanjuntak et al. 2020).

It was also observed that the weight gains of animals placed on experimental diet increased with increasing watermelon seed flour. However, the rats fed with reference sample recorded higher weight gained (52.00 g) compared to the rats fed with the formulated diets. The weight gain was probably influenced by the quality of the protein in the diets. This is in agreement with the findings of Oluwajuyitan et al. (2020) that high quality protein diet promotes weight gain in animals. A similar trend was reported on experimental animals by Mhlomi et al. (2019). Biological value evaluates the competence of protein to support growth through nitrogen retention in the body (Oluwajuyitan and Ijarotimi 2019). It is the assessment of the absorbed protein from food that becomes part of the body (Grzeszczuk et al. 2020). This result indicates that the extrudate may promote growth and maintenance of the body. Foods with high feed efficiency ratio tends to add to weight gain while low feed efficiency ratio are prone to be used as energy rather than stored as body weight (Laminu et al. 2014). The protein efficiency ratio of the extruded diets (0.33–1.79) were significantly ($p > 0.05$) higher than the value (0.08–0.32) reported by Olapade and Aworh (2012) in extruded complementary foods from blends of fonio and cowpea. However, these values were lower than (2.80–4.88) reported in the complementary foods produced from fermented popcorn, African locust bean and bambara groundnut (Ijarotimi and Keshinro 2012). The finding from this work agreed with other researches that were reported on the nutritional qualities of foods formulated from combinations of two or more plant-based food materials (Okpala and Okoli 2011; Ijarotimi and Keshinro 2012; Oluwajuyitan and Ijarotimi 2019).

The extruded samples supported the growth and development of the organs (kidney, liver and heart) of the animals which may be due to increase in the tissue nitrogen of the experimental animals must especially in MFMWM86 and goldenmorn. The liver is one of the major important organs in the body that metabolize amino acids and it acts as the body's chemical factory meanwhile, the kidney regulates toxic substances. Therefore, it can be said that the weight gain in experimental animal organs could be attributed to the quality of formulated diets fed on experimental animal. The result obtained from this study is in relation to weight gain observed in experimental rats' organ reported by Ibironke et al. (2012) and Ijarotimi and Keshinro (2012).

Nutritionally, from this present study, it could be deduced that these extruded breakfast food samples, particularly MFMWMF75, may serve as a cheap meal suitable to promote growth and reducing malnutrition among the young children. Recently, more attention has been given to research on the increase of protein-energy malnutrition (PEM) in developing countries due to low nutritional qualities of traditional foods (Ikujenlola and Adurotoye 2014; Oluwajuyitan and Ijarotimi 2019). Hence, a low cost food that is high in protein and energy–density such as MFMWMF75 may be a preferred substitute to replace expensive imported foods and low quality local foods (Ijarotimi and Keshinro 2012; Oluwajuyitan and Ijarotimi 2019).

Packed cell volume also known as haematocrit (Ht or Hct) is the percentage of red blood cells in the whole blood (Purves et al. 2003). According to Isaac et al. (2013) packed cell volume is involved in the transportation of oxygen and absorbed nutrients, hence increased packed cell volume shows a better transportation and thus results in an increased primary and secondary polycythemia. It was observed from the present study that the developed samples show high percentage of packed cell volume and were within the recommended value of 37.60–50.60% for healthy rat (Diana 2007). It was observed that values obtained were within the safety limits recommended by Diana (2007). The MCHC, MCH, and MCV values are major indicators of subject assessment tendency to anaemia and a low level is an indication of anaemia (Aster 2004). The values obtained for MCHC, MCH, and MCV in this study showed that animal fed with the diets are not at risk of anaemia.

The creatinine and urea values of rats fed with extrudate, goldenmorn and basal were observed to be within the normal range reported by Giannini et al. (1999), which implies that the formulated diets had no negative side effect on the kidney functionality. Healthy kidneys remove creatinine and urea nitrogen from blood, but the level of creatinine and urea in blood rises with kidney failure and malfunction, that is, the higher the creatinine and urea value the less effective the kidney function (Rusul and Haider 2014).

However, the AST, ALT, and ALP values for experimental food samples were within the normal range values (45.70–80.50; 17.50–30.20 and 56.80–128.00 U/L)

respectively (Giannini et al. 1999; Diana 2007). This observation implies that the extruded samples may be suitable for consumption, and that consumption of these food samples may not damage liver cells. Scientific studies have reported that high concentration of AST or ALT in the blood is an indication of liver malfunction and damage (Al-Mamary et al. 2002; Aniagu et al. 2005; Aliyu et al. 2007).

Conclusions

Results from this study revealed that the extruded breakfast meal produced from blends of malted finger millet and watermelon seed flour possessed high crude protein, promotes growth with relatively low effect on organs of experimental rat used in this study. The implication of this research therefore is that a careful selection of indigenous plant protein sources could be of nutritional benefit in terms of consumer weight gain and nitrogen utilization that may be comparable to commercial market sample.

Abbreviations

CHD: Coronary heart disease; FUNAAB: Federal University of Agriculture, Abeokuta; RSM: Response Surface Methodology; AOAC: Association of Official Analytical Chemist; CCAC: Canadian Council on Animal Care Guidelines and Protocol Review; PCV: Packed cell volume; RBC: Red blood cell count; Hb: Hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphate; WFA: Weight-for-age; LFA: Length-for-age (LFA); MFMWM90: Malted finger millet:Watermelon seeds (90.00:10.00%); MFMWM86: Malted finger millet:Watermelon seeds (86.25:13.75%); MFMWM82: Malted finger millet:Watermelon seeds (82.50:17.50%); MFMWM78: Malted finger millet:Watermelon seeds (78.75:21.25%); MFMWM75: Malted finger millet:Watermelon seeds (75.00:25.00%).

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Authors' contributions

OJO carried out the laboratory analysis and wrote the first draft of the manuscript while OTD carried out the statistical analysis and proof read the first draft. IBTO and BMK supervised the research work and corrected the final draft of the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

Data are available on request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethical Committee for Laboratory Animals of School of Agriculture and Agricultural Technology, Akure, Nigeria (FUTA/SAAT/2019/036).

Consent for publication

Not Applicable.

Competing interests

No competing interest within authors.

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