Agrosearch, 21(1&2), 1-17, (2022) https://dx.doi.org/10.4314/agrosh.v21i1-2.1

EFFECTS OF SUBSTITUTING MELON SEED PEEL MEAL FOR YELLOW MAIZE MEAL ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES IN *Clarias gariepinus* FINGERLINGS

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ABSTRACT

A feeding trial was conducted to assess the effects of substituting melon (*Citrullus lanatus*) seed peel meal (MSPM) for yellow maize meal on the haematological and serum biochemical indices of *Clarias gariepinus* fingerlings for 56 days. Fifteen fish each were stocked in eighteen plastic bowls (50-litre capacity) and fed six iso-nitrogenous diets at graded MSPM levels of 0%, 20%, 40%, 60%, 80% and 100% twice daily at 5% of their body weight. Selected haematological and serum biochemical parameters were determined by standard procedures and data analyzed using analysis of variance at $p = 0.05$. The results showed that the fish fed with diet 6 had the highest PCV (45.00%), Hb (15.03 gm/100mL), RBC (4.81 x 10^{12}/mL), Platelets (34.73 x 10^{9}/mL), MCH (31.32 µg/mL), total protein (74.00%) and albumin (40.00%). The fish fed with diet 4 had the highest WBC (12.68×10^{9}/mL) and globulin (35.00%) while those fed diet 3 had the least WBC (9.36×10^{9}/mL) and globulin (33.00%). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increased and varied ($p < 0.05$) between 10.00 - 30.67 mg/dL and 11.00 –17.05 mg/dL respectively. Blood glucose significantly ($p < 0.05$) increased in fish fed with diets 3 to 6. This study revealed that melon seed peel meal can replace yellow maize meal in the diet of *C. gariepinus* without adverse effects on its haematological and serum biochemical indices.

Keywords: *Clarias gariepinus*, Blood glucose, Haemoglobin, Melon seed peel, Unconventional ingredients

INTRODUCTION

Clarias gariepinus is an important cultivable fish in Africa (particularly in Nigeria) but its extensive culture (as it is generally applicable to most cultured fish species) is becoming unattractive due to the expensive nature of most conventional fish feedstuffs. Despite the vast aquatic resources, oil seeds, cereals and legumes which Nigeria is endowed with, most fish feed manufacturers in the country still rely on imported feed ingredients and fish feeds from other countries. This importation of fish meal and other fish ingredients makes fish farming expensive (Aduku and Bolorunduro, 2016).

Despite the considerable success of aquaculture, the rising cost and scarcity of desirable feeds have constituted serious constraints against the successful operations of intensive aquaculture enterprises in Nigeria. The expensive nature of most conventional feedstuffs is a major challenge faced by the majority of local fish farmers (Abowei and Ekubo, 2011). Ogunlade (2007) reported that high demand for the conventional feed ingredients by other sectors and also for human consumption has contributed to expensive and competitive nature of these conventional feed ingredients. Unconventional feedstuffs and agricultural by-products can serve as substitutes for expensive conventional feedstuffs in fish diet preparation. The paradigm shift is intended to minimize feed production cost without compromising feed quality (Houlihan *et al.,* 2001).

Maize as one of the main sources of metabolisable energy in most formulated catfish diets is readily digestible by fish (Olurin *et al*., 2006). However, maize is extensively used for human consumption in Nigeria and its inadequate production, increasing cost and scarcity have necessitated the need to consider other hitherto under-utilized energy-rich alternatives (FAO, 2005). A few studies involving replacement of maize by unconventional ingredients as alternative energy sources in fish diets included the use of *Moringa oleifera* leaves (Ayoola *et al*., 2013), *Chrysophyllum albidum* (Jimoh *et al*., 2014) and melon seed peel (Iheanacho *et al*., 2018). Consequently, the use of melon seed peel as an unconventional fish feed ingredient may contribute to profitable fish farming.

Melon (*Citrullus lanatus*) is among the most popular African indigenous vegetable crops produced in Nigeria on a large scale. Melon seeds have been reported to contain mineral nutrients (magnesium, sodium, potassium, zinc, calcium, iron and phosphorus), oils and substantial amounts of protein ranging from 18 - 28% (Obi *et al.,* 2011) while melon seed peel is common and usually discarded as an agricultural waste that constitutes environmental hazards. Moreover, Orire and Ricketts (2013) viewed melon seed peel meal as a good dietary energy source in the diet of *O. niloticus* and recommended that it could be utilized effectively as a feed ingredient for fish and livestock. According to Ogbe and George (2012), melon seed peel has a gross energy

content value of 1440.11 Kcal/Kg which, however, is lower than that of maize (3390 Kcal/Kg) reported by Tuleun *et al.* (2005). Chemical analysis of melon seed peel has shown that it contains the following nutrients: 10.02 - 16.19% crude protein; 2.42 - 8.9% moisture; 14.91 - 20.09% crude lipid; 8.12 - 15.56% crude fibre; 6.35 - 12.09% total ash and 26.89 - 39.30% nitrogen-free extract (Ogbe and George, 2012; Ogu and Orjiakor, 2017; Akiode *et al*., 2018; Omovwohwovie and Omoruwou, 2019).

Nutrient requirements of fish species vary and therefore necessitate fish farmers to identify the nutritional requirements of their preferred species in order to prepare nutritionally balanced diets that will ensure optimal fish growth and general wellbeing. Many of the unconventional plant-based ingredients which are expected to supplement conventional ingredients are not readily digested due to their inherent anti-nutritional factors and therefore contribute to stunted growth and low profitability of fish farming. For instance, melon seed peel has been reported to contain anti-nutrients such as saponins (0.02 - 1.47%), hydrogen cyanide (0.06%), alkaloids (0.26%), flavonoids (0.38%), oxalates (0.71%), trypsin inhibitors (2.01%), phytates (2.05%) and tannins (15.15%) (Ogbe and George, 2012; Akiode *et al*., 2018). Consequently, attempts have been made to remove or significantly reduce the amounts of these anti-nutrients in melon seed peel by means of processing methods such as boiling, fermentation, sun-drying, oven-drying, soaking and enzyme treatment (Fagbohun *et al*., 2011; Ogbe and George, 2012; Ogu and Orjiakor, 2017; Iheanacho *et al*., 2018).

Meanwhile, haematological and biochemical indices are important parameters for evaluating physiological and pathological changes in fish (Erhunmwunse and Ainerua, 2013). Haematological study also provides reliable information on health status, metabolic disorders and chronic stress status before and after clinical examination of specimens (Bahmani *et al*., 2001). Their applications are valuable in fish biology especially in the assessment of fish health and stress-related responses (Dacie and Lewis, 2011). Adeyemo (2005) recommended ichthyo-haematology as a useful tool in the assessment of fish condition and evaluation of the effects of toxic substances. Therefore, this study assessed the effects of the utilization of melon seed peel as an alternative dietary energy source on the haematological and serum biochemical parameters in *C. gariepinus* fingerlings

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MATERIALS AND METHODS

Processing of Melon Seed Peels and Preparation of Experimental Diets

Six (6) kilograms of melon seed peels were collected from the melon shelling and milling factory at Bodija Market, Ibadan, Oyo State where they were heaped as waste. The peels were winnowed and dirt was sieved out using a hand sieve. The peels were dried at 50°C for 48h in an electric oven (Fan Azma Gostar, BM 55 Model), ground into a powdery form using a grinding machine and stored in an airtight container. Six diets were prepared (at 40% crude protein) from various ingredients by means of Pearson's square method as shown in Table 1. Oven-dried melon seed peel meal (MSPM) was substituted for yellow maize meal at graded levels of 0% (0.00 g = diet 1), 20% (4.46 g = diet 2), 40% (8.93 g = diet 3), 60% (13.39 g = diet 4), 80% (17.86 g = diet 5) and 100% (22.32 g = diet 6) as presented in Table 1. The proximate composition of melon seed peel meal (MSPM) as determined in this study showed the following: 14.65% crude protein; 4.27% crude lipid; 6.05% crude fibre; 8.56% ash; 7.83% moisture and 58.64% nitrogen-free extract. Each diet was prepared by thoroughly mixing the dry ingredients using a mixer (Pars Electric Company, Tehran, Iran) after which palm oil and warm water were added to homogenize the dry mixture into a paste. Each diet paste was extruded through a pelleting machine (Hobart A-2007 Model, UK) to obtain 2-mm pellets which were sundried for three days on clean concrete slabs, cooled to room temperature and kept in separate airtight containers prior to use.

Experimental design and fish feeding trial

Three hundred and twenty (320) *C. gariepinus* fingerlings (mean weight = 7.40 ± 0.08 g) were purchased from Ibukunoluwa Fish Farm in Okitipupa and transported in an open 50-litre water-filled plastic container to the fish nutrition laboratory in the Department of Fisheries and Aquaculture Technology, Olusegun Agagu University of Science and Technology, Okitipupa. The fish were acclimatized in an open 2 m x 2 m x 1 m plastic tank for 7 days during which they were fed with the control diet. Fifteen (15) similar-sized *C. gariepinus* fingerlings were weighed and randomly distributed into each of the eighteen-plastic bowls. Each bowl had a water-holding capacity of 50 litres and was filled with 35 litres of clean water. Six dietary treatments (arranged as three replicates per treatment) were randomly allotted to the experimental bowls making eighteen (18) experimental units. Fish were fed twice daily (07.00 and 17.00 hours) at 5% of their body weight for 56 days. Fish mortality was monitored daily while fish weight was determined weekly and diet quantity adjusted based on new weight gain. Water in the bowls was changed every two days to avoid fouling resulting from faeces and uneaten feed. Water temperature, dissolved oxygen and pH in the bowls were monitored and measured weekly using mercury-in-

glass thermometer and water testing kits. Eight (8) grams of each diet sample as well as six (6) fish per treatment sacrificed at the commencement and end of the feeding trial were proximately analyzed by means of standard methods (AOAC, 2011).

Table 1: Gross ingredient composition (g/100g diet) of experimental diets

Dietary ingredients	DIET 1 0% MSPM (Control)	DIET 2 20% MSPM	DIET 3 40% MSPM	DIET 4 60% MSPM	DIET 5 80% MSPM	DIET 6 100% MSPM
Melon seed peel meal	-------	4.46	8.93	13.39	17.86	22.32
Yellow maize	22.32	17.86	13.39	8.93	4.46	-------
Groundnut cake	23.00	23.00	23.00	23.00	23.00	23.00
Fishmeal	24.00	24.00	24.00	24.00	24.00	24.00
Soybean meal	20.68	20.68	20.68	20.68	20.68	20.68
Wheat offal	3.00	3.00	3.00	3.00	3.00	3.00
Salt	2.00	2.00	2.00	2.00	2.00	2.00
Palm oil	2.00	2.00	2.00	2.00	2.00	2.00
Cassava starch	2.00	2.00	2.00	2.00	2.00	2.00
Vit./mineral premix*	1.00	1.00	1.00	1.00	1.00	1.00
Total (g)	100.00	100.00	100.00	100.00	100.00	100.00
Gross energy value (Kcal/kg)	2762.52	2631.25	2601.45	2623.82	2640.61	2619.73

MSPM = melon seed peel meal

*Each kilogram of vitamin/mineral premix contained:

Vitamin A: 10,000 IU; Vitamin B1: 400 mg; Vitamin B2: 40 mg; Vitamin B6: 20 mg; Vitamin B12: 0.04 mg; Vitamin D3: 2,000 IU; Vitamin E: 100 mg; Vitamin K: 20 mg; Biotin: 0.2 mg; Choline chloride: 1,200 mg; Folic acid: 10 mg; Inositol: 200 mg; Niacin, 200 mg; Pantothenic calcium: 100 mg. $MgS0_4.2H_20$: 127.5 mg; KCl: 50 mg; NaCl: 60 mg; $CaHPO_4.2H_2O$: 727.8 mg; $FeSO_4.7H_2O$: 25 mg; $ZnSO_4.7H_2O$ 5.5 mg; $CuSO_4.5H_2O$ 0.785 mg; $MnSO_4.4H_2O$: 2.54 mg; $CuSO_4.4H_2O$: 0.478 mg; Ca $(IO_3)_2.6H_2O$: 0.295 mg; $CrCl_3.6H_2O$: 0.128 mg.

Maker: Pars Kilka Company, Miroud, Mazandaran Province, Iran

Fish blood sample collection and determination of haematological parameters

Six samples of live fish from each treatment were randomly selected at the beginning and end of the feeding trial. Both pre- and post-treatment fish were anaesthetized with 150 mg/L of tricane methane sulphonate as previously described by Wagner *et al.* (1997). A small cut was made in the caudal peduncle with a sharp dissecting blade as described by Stoskopf (1992). Six (6) mL of blood sample was gently drawn from the caudal peduncle artery of each fish by means of sterile

disposable 2 mL plastic syringes and needles and mixed with an anti-coagulant EDTA (ethylene diamine tetra-acetic acid) within EDTA bottles. The blood samples collected were analyzed at the Haematology Laboratory of Ondo State Specialist Hospital, Okitipupa to determine Packed Cell Volume (PCV) or Haematocrit (Hct), Haemoglobin (Hb) concentration, White Blood Cell (WBC) counts and Red Blood Cell (RBC) counts using the methods described by Shah and Altindag (2004) and Dacie and Lewis (2011) as reported by Adesina *et al.* (2017). The derived haematological indices were calculated as follows:

Packed Cell Volume (PCV) = $\frac{100 \times (\text{Volume of blood} - \text{volume of plasma})}{\text{Volume of blood}}$

Blood volume = $\frac{\text{Volume of plasma} \times 100}{100 - \text{PCV}}$

Mean corpuscular volume (MCV) (µg/mL) = $\frac{PCV}{RBC} \times 100$

Mean corpuscular haemoglobin (MCH) = $\frac{Hb}{RBC} \times 100$

Mean corpuscular haemoglobin concentration (MCHC) (g/100mL) = $\frac{Hb\ per\ 100\ mL\ of\ blood}{Haematocrit\ (\%)} \times 100.$

Serum biochemical assay

Serum biochemical indices were calculated by means of standard methods. Total protein, albumin and globulin were estimated colorimetrically according to Peter *et al*. (1982), blood glucose determined colorimetrically using a spectrophotometric method according to Trinder (1969), selected serum enzymes [Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] determined by means of standard enzymatic methods as described by Bush (1991) while Blood Urea Nitrogen (BUN) was measured according to Patton and Crouch (1977) as reported by Adesina *et al*. (2017).

Statistical analysis

Statistical analyses in this study were conducted using Statistical Package for Social Science (SPSS Version 22.0, SPSS Inc., Chicago, IL). Data obtained from each parameter were subjected to one-way analysis of variance (ANOVA) and the Tukey's Multiple Comparison Test applied to determine the differences between mean values at $P < 0.05$. Data were presented as mean values ± standard deviation.

RESULTS AND DISCUSSION

Proximate composition of experimental diets

The proximate composition of the experimental diets varied significantly ($p < 0.05$) and possibly suggested that the substitution levels of melon seed peel meal affected the proximate composition of the diets (Table 2). The crude protein content was highest (41.40%) in Diet 1 (control) and lowest (39.40%) in Diet 2, although not significantly different ($p > 0.05$) across the diets. Despite the variation, the amounts of crude protein in the diets still met the protein requirements of *C. gariepinus* fingerlings. According to Adegbesan *et al*. (2018), ideal growth rate and feed conversion efficiency in *C. gariepinus* could be achieved with a diet containing 38 - 42% crude protein. The values obtained in this study were also consistent with 43.97 – 44.28% reported by Iheanacho *et al*. (2018) for melon seed peel meal-based diets fed to *Oreochromis niloticus* juveniles. The crude lipid content which was highest (6.50%) in Diets 1 and 5 and lowest (5.50%) in Diet 4 however exceeded 4.15 – 4.37% reported by Iheanacho *et al*. (2018) in similar melon seed peel meal-based diets. The crude fibre content was lowest (4.80%) in Diet 1 and highest (5.90%) in Diets 4 and 6. The crude fibre values recorded during the study were approximately twice 2.57 – 2.71% recorded by Iheanacho *et al*. (2018) for similar melon seed peel meal-based diets. Values of the ash content ranged from 7.80% in Diet 4 to 11.20% in Diet 6 and were similar to 10.73 – 11.35% obtained in melon seed peel meal-based diets reported by Iheanacho *et al*. (2018) while the moisture content values (7.90 - 12.30%) however surpassed 5.37 – 6.08% reported by them. The NFE values (24.60 – 31.45%) in the diets were comparable with 31.56 – 32.86% documented by Iheanacho *et al*. (2018) and therefore confirmed the dietary potential of melon seed peel meal as an unconventional energy feedstuff.

Table 2: Proximate composition of experimental diets

Proximate indices (%)	Diet 1 0% MSPM (Control)	Diet 2 20% MSPM	Diet 3 40% MSPM	Diet 4 60% MSPM	Diet 5 80% MSPM	Diet 6 100% MSPM
Dry matter	90.90±0.27^{b}	91.80±0.52^{a}	92.10±0.01^{a}	89.70±0.41^{b}	91.60±0.32^{a}	87.70±0.07^{c}
Moisture	9.10±0.56^{c}	8.20±0.27^{d}	7.90±0.11^{d}	10.30±0.24^{b}	8.40±0.35^{d}	12.30±0.16^{a}
Crude protein	41.40±0.01^{a}	39.40±0.21^{a}	39.90±0.31^{a}	39.80±0.01^{a}	40.15±0.13^{a}	40.30±0.51^{a}
Crude lipid	6.50±0.11^{a}	6.10±0.12^{a}	5.90±0.04^{a}	5.50±0.13^{b}	6.50±0.11^{a}	5.70±0.31^{b}
Crude fibre	4.80±0.22^{b}	5.40±0.41^{a}	5.60±0.15^{a}	5.90±0.32^{a}	5.60±0.22^{a}	5.90±0.32^{a}
Ash	10.50±0.34^{a}	10.90±0.32^{a}	9.60±0.15^{b}	7.80±0.72^{c}	7.90±0.34^{c}	11.20±0.16^{a}
Nitrogen-free extract	27.70±0.24^{b}	30.00±0.11^{a}	31.10±0.32^{a}	30.70±0.41^{a}	31.45±0.05^{a}	24.60±0.13^{c}

Mean values with different superscripts along the same row are significantly different ($p < 0.05$).
MSPM = melon seed peel meal

Haematological indices of *C. gariepinus* fingerlings fed melon seed peel meal-supplemented diets

Table 3 shows that values of tested haematological indices in *C. gariepinus* fingerlings fed with melon seed peel meal-supplemented diets exhibited significant ($p < 0.05$) variations among the dietary treatments compared to the control treatment and the initial values, thus suggesting that the treatments applied affected blood indices. Packed cell volume (PCV) increased from initial 39.00% to final values ranging between 42.00% (in the fish fed with Diet 1) and 45.00% (in those fed with Diet 6). These values agreed with 20 - 50% reported by Pietse *et al*. (1981) and Etim *et al*. (1999) who stated that fish haematocrit values rarely exceed 50%. Piotr *et al*. (2014) stated that increased PCV can result from increased number of RBCs, erythrocyte swelling or reduced volume of water in the circulating blood. By contrast, these values exceeded 16.67 – 32.00% reported by Iheanacho *et al*. (2018) for *O. niloticus* juveniles fed with melon seed peel meal-based diets. Haemoglobin (Hb) content increased from initial 13.0 gm/100mL to final values ranging between 14.04 gm/100mL (in the fish fed with Diet 1) and 15.03 gm/100mL which were comparatively higher than 5.37 – 10.33 gm/100mL reported by Iheanacho *et al*. (2018) for *O. niloticus* juveniles fed with melon seed peel meal-based diets as well as 7.06 – 8.33 gm/100mL observed in *C. gariepinus* juveniles fed with *M. oleifera* leaf meal-based diets (Ayoola *et al*., 2013). These high Hb values corroborated those of Dienye and Olumuji (2014) who stated that haemoglobin concentration is usually higher in fishes capable of aerial respiration, hence the present high haemoglobin values can be associated with both the diets as well as the air-breathing characteristic and high anaerobic metabolic capacity of *C. gariepinus*. Initial red blood cell (RBC) count was 4.90×10^{12}/mL while the final values ranged between 4.33×10^{12}/mL and 4.81×10^{12}/mL which were higher than 1.99 – 2.48×10^{12}/mL found in *C. gariepinus* juveniles fed with *M. oleifera* leaf meal-based diets (Ayoola *et al*., 2013). RBC count above 1.00×10^{6} mm^{-3} is considered high and indicative of high oxygen-carrying capacity of the blood which is characteristic of fishes capable of aerial respiration and high metabolic activity (Jimoh *et al*., 2012). By contrast, the present RBC count was lower compared to 5.33 – 7.17×10^{12}/mL documented by Iheanacho *et al*. (2018) for *O. niloticus* juveniles fed with melon seed peel meal-based diets.

Initial white blood cell (WBC) count was 10.70×10^{9}/mL while the fish fed with Diet 4 had the highest count (12.68×10^{9}/mL) and those fed with Diet 3 had the least (9.36×10^{9}/mL). These WBC counts were similar to 8.33 – 12.53×10^{9}/mL reported by Adesina (2017) for *C. gariepinus* juveniles fed with mechanically extracted sunflower seed meal-based diets but were higher than 2.88 – 11.22×10^{9}/mL and 2.57 – 8.10×10^{9}/mL found in *Sarotherodon melanotheron* (Ayoola *et*

al., 2014) and *O. niloticus* juveniles fed with melon seed peel meal-based diets (Iheanacho *et al*., 2018) respectively. Kori-Siakpere *et al.* (2009) associated high WBC count with a release of more cells to maintain homeostasis while low WBC count represents a common stress response. White blood cells are known to perform significant functions in the immune system and responses of living organisms. The Low WBC count in the fish fed with Diets 1 and 3 could be linked to a reduction in the number of lymphocytes. Alkahem (1994) attributed reduced WBC counts in *O. niloticus* to the effects of toxicants and a stress response resulting from increased pituitary-interrenal activity. Platelets (PLT) which rose from 29.10×10^9/mL to 29.73×10^9/mL (in the fish fed with Diet 3) and 34.73×10^9/mL (in those fed with Diet 6) markedly surpassed 1.73 – 3.50×10^9/mL observed in *O. niloticus* juveniles fed with melon seed peel meal-based diets (Iheanacho *et al*., 2018) as well as 10.00 – 14.00×10^9/mL found in *C. gariepinus* juveniles (Adesina, 2017). They also exceeded 19.25 x 10^3/µL, 17.33 x 10^3/µL, 16.67 x 10^3/µL and 10. 67 x 10^3/µL recorded for *Clarias anguillaris*, *Clariabranchus*, *Heteroclarias* and *Heterobranchus bidorsalis* respectively (Diyaware *et al*., 2013).

Mean corpuscular volume (MCV) rose from 79.60 µg/mL to 93.62 µg/mL (in the fish fed with Diet 5) and 99.72 µg/mL (in those fed with Diet 2) which exceeded 34.67 – 37.00 µg/mL and 35.00 – 38.67 µg/mL observed in *C. gariepinus* juveniles (Adesina, 2017; Adesina *et al*., 2017). MCV indicates RBCs' size and reflects a normal/abnormal cell division during red blood cell production. Increased MCV may be due to RBC swelling leading to macrocytic anaemia. Mean corpuscular haemoglobin (MCH) was initially 26.50 µg/mL while the final values ranged between 11.00 µg/mL (in the fish fed with Diet 4) and 12.75 µg/mL (in those fed with Diet 6) which were similar to 11.00 – 12.00 µg/mL observed in *C. gariepinus* juveniles (Adesina, 2017; Adesina *et al*., 2017). However, the values were lower when compared with 24.24 µg/mL reported by Omitoyin (2006) for *C. gariepinus* juveniles. According to Diyaware *et al*. (2013), higher MCH signifies an ideal volume of haemoglobin which indicates effective oxygen transportation in the bloodstream for fish wellbeing. Mean corpuscular haemoglobin concentration (MCHC) values (33.00 – 33.45 gm/100mL) were uniform ($p > 0.05$) and agreed with 32.00 – 33.00 gm/100mL earlier reported for *C. gariepinus* juveniles (Adesina, 2017; Adesina *et al*., 2017). MCHC is a quantitative measurement of mean amount of haemoglobin per erythrocyte in biological organisms (Moses, 2007).

Serum biochemical parameters and percentage survival of *C. gariepinus* fingerlings fed with melon seed peel meal-supplemented diets

Table 4 shows serum biochemical parameters of *C. gariepinus* fingerlings fed with melon seed peel meal-supplemented diets. Total protein was initially 72.00 g/100mL while the final values ranged between 70.24 g/100mL recorded in the fish fed with Diet 2 to 74.00 g/100mL in those fed with Diet 6 and were higher than 40.19 g/100mL, 2.30 – 3.60 g/100mL and 2.60 – 4.17 g/100mL respectively reported by Ayoola (2011), Adesina *et al*. (2017) and Adesina (2017) for *C. gariepinus* juveniles. These increased blood protein levels possibly suggested that MSPM inclusion in the diets somehow enhanced protein synthesis which, according to Yadav *et al.* (2003), determines the quantity of protein. Similarly, albumin content was initially 38.00 g/100mL while the final values ranged between 37.21 g/100mL in the fish fed with Diet 2 to 40.00 g/100mL in the fish fed with Diet 6. Globulin content was initially 34.00 g/100mL while the final values ranged between 33.00 g/100mL in the fish fed with Diets 1 and 3 to 35.00 g/100mL in the fish fed with Diet 4. Albumin and globulin contents exhibited a similar trend of increased values as total blood protein. Alanine aminotransferase (ALT) level rose from 13.00 mg/dL to values ranging between 10.00 mg/dL (in the fish fed with Diet 4) to 30.67 mg/dL (in the fish fed with Diet 1) while aspartate aminotransferase (AST) level increased from 11.00 mg/dL to final values between 11.00 mg/dL (in the fish fed with Diet 1) and 17.05 mg/dL (in the fish fed with Diet 3). However, these values were lower than 25.67 – 46.67 mg/dL (ALT) and 21.33 – 72.00 mg/dL (AST) respectively reported by Adesina (2017) and Adesina *et al*. (2017) for *C. gariepinus* juveniles. The observed higher ALT and AST values suggest that the blood serum enzymes effectively utilized amino acids for metabolic purposes and these corroborated the observation of Adesina (2008) on *O. niloticus* fingerlings and juveniles. Transferases are important enzymes used for monitoring the health status of fish and have been reported to escape into the bloodstream from damaged liver cells (Racicot *et al*., 1975). Improved AST and ALT levels in the blood serum of fish are usually linked with dying or damaged liver cells while a decrease could suggest leakage of these enzymes into the serum (Ozovehe, 2013). The slightly lower ALT values recorded in fish fed diets 3 to 6 probably confirmed similar findings by Mousa and Khattab (2003) who associated reduced AST and ALT values with the inhibition of AST and ALT activities in the liver of catfish after intoxication with dietary ochratoxin. Abdel Tawwab *et al*. (2001) also observed a similar reduction in liver AST and ALT enzyme levels of Nile tilapia after exposure to mercury and associated them with liver necrosis and damage to the liver cells caused by the toxicants. The decrease in the activities of these enzymes could be attributed to their inhibition or a reduction in

the rate of their synthesis in the liver. Aminotransferase levels in fish also rise as a response to stress (Tiwari and Singh, 2004).

Initial glucose concentration of 4.90 mg/dl marginally reduced to values between 4.30 mg/dL (in the fish fed with Diet 1) to 4.84 mg/dL (in the fish fed with Diet 3) while it was significantly ($p < 0.05$) higher in the fish fed with Diets 3 to 6. However, the present values fell much below 47.00 – 81.33 mg/dL and 51.33 – 84.00 mg/dL respectively reported by Adesina (2017) and Adesina *et al*. (2017) for *C. gariepinus* juveniles. The generally low glucose levels could be due to the presence of residual anti-nutrients/anti-metabolites in the diets and this observation harmonized with that of Mahajan and Dheer (1983) who linked low or reduced glucose levels to severity of stress factor in the diets. The variations in the values of blood indices between the present study and previous reports could be due to differences in species and size or age that greatly influence the values of blood indices (Docan *et al*., 2010) while other factors may include genetic features, culture conditions, ingredient combinations and dietary treatments. The considerable improvement in the blood parameters studied reflected high acceptability of the MSPM-based diets by fish which could be ascribed to good handling, proper feed processing and suitability of oven-dried MSPM in the diet of *C. gariepinus*.

Table 3: Haematological indices of *C. gariepinus* fingerlings fed graded levels of processed melon seed peel meal-based diets

Haematological indices	Initial values	Experimental Dietary Inclusion Levels					
		Diet 1 0%MSPM (control)	Diet 2 20% MSPM	Diet 3 40%MSPM	Diet 4 60%MSPM	Diet 5 80% MSPM	Diet 6 100% MSPM
PCV (%)	39.00±0.58^{d}	42.00±0.18^{c}	43.00±0.10^{b}	44.00±0.28^{a}	43.00±0.30^{b}	44.00±0.13^{a}	45.00±0.23^{a}
Hb (gm/100mL)	13.00±0.17^{b}	14.04±0.06^{ab}	14.30±0.20^{ab}	14.72±0.26^{a}	14.32±0.10^{ab}	14.74±0.16^{a}	15.03±0.35^{a}
RBCs (x10^{12}/mL)	4.90±0.02^{a}	4.33±0.01^{b}	4.42±0.11^{b}	4.62±0.21^{a}	4.54±0.07^{a}	4.72±0.21^{a}	4.81±0.06^{a}
WBCs (x10^{9}/mL)	10.70±0.01^{b}	9.68±0.41^{c}	12.45±0.06^{a}	9.36±0.02^{c}	12.68±0.13^{a}	12.49±0.07^{a}	11.23±0.12^{b}
Platelets (x10^{9}/mL)	29.10±0.05^{e}	34.50±0.10^{a}	33.06±0.20^{b}	29.73±0.02^{d}	31.63±0.40^{c}	33.82±0.07^{b}	34.73±0.32^{a}
MCV (µg/mL)	79.60±0.06^{e}	97.70±0.28^{b}	99.72±0.02^{a}	95.72±0.18^{c}	95.60±0.58^{c}	93.62±0.13^{d}	98.83±0.12^{a}
MCH (µg/mL)	26.50±0.07^{a}	11.67±0.58^{b}	12.00±0.20^{b}	12.00±0.02^{b}	11.00±0.10^{b}	12.53±0.63^{b}	12.75±0.80^{b}
MCHC (gm/100mL)	33.30±0.10^{a}	33.00±0.06^{a}	33.00±0.30^{a}	33.00±0.64^{a}	33.00±0.01^{a}	33.45±0.15^{a}	33.31±0.28^{a}
Lymphocytes (%)	34.00±0.25^{a}	30.00±1.15^{b}	28.03±0.38^{c}	27.00±0.20^{c}	26.32±0.35^{d}	26.22±0.38^{d}	29.62±0.10^{b}
Neutrophils (%)	64.00±1.07^{d}	68.00±0.42^{c}	70.00±0.68^{b}	72.22±0.38^{a}	71.00±0.12^{a}	72.00±0.42^{a}	70.24±0.18^{b}
Monocytes (%)	2.00±0.37^{a}	1.00±0.16^{b}	2.00±0.05^{a}	1.00±0.26^{b}	2.00±0.07^{a}	1.00±0.10^{b}	1.00±0.12^{b}

Means with different superscripts along the same row are significantly different ($p < 0.05$).

MSPM = melon seed peel meal, PCV: packed cell volume; Hb: Haemoglobin content; RBC: red blood cells; WBC: white blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration

Table 4: Serum biochemical and survival indices of *C. gariepinus* fingerlings fed melon seed peel meal-based diets

Biochemical parameters	Initial values	Dietary Inclusion Levels					
		Diet1 0%MSPM (control)	Diet 2 20 %MSPM	Diet 3 40 %MSPM	Diet 4 60 %MSPM	Diet 5 80 %MSPM	Diet 6 100 %MSPM
Total protein (g/100mL)	72.00±0.16^{b}	71.00±0.02^{c}	70.24±0.01^{c}	72.33±0.11^{b}	73.20±0.23^{a}	72.00±0.04^{b}	74.00±0.15^{a}
Albumin (g/100mL)	38.00±0.14^{b}	38.00±0.06^{b}	37.21±0.01^{c}	39.00±0.02^{a}	38.23±0.41^{b}	39.02±0.12^{a}	40.00±0.26^{a}
Globulin (g/100mL)	34.00±0.06^{a}	33.00±0.12^{b}	33.01±0.10^{b}	33.00±0.12^{b}	35.00±0.15^{a}	33.23±0.06^{b}	34.60±0.04^{a}
ALT (mg/dL)	13.00±0.08^{c}	30.67±0.18^{a}	25.67±0.38^{b}	12.02±0.31^{c}	10.00±0.15^{d}	10.72±0.01^{d}	12.45±0.18^{c}
AST (mg/dL)	11.00±0.53^{d}	11.00±0.01^{d}	13.32±0.16^{c}	17.05±0.45^{a}	11.54±0.05^{d}	14.23±0.38^{c}	15.00±0.57^{b}
BUN (mg/dL)	0.02±0.01^{c}	0.03±0.01^{c}	1.00±0.03^{a}	0.06±0.02^{b}	1.11±0.03^{a}	0.02±0.01^{c}	0.02±0.01^{c}
Glucose (mg/dL)	4.90±0.72^{a}	4.30±0.73^{b}	4.32±0.32^{b}	4.84±0.15^{a}	4.70±0.86^{a}	4.65±0.62^{a}	4.74±0.25^{a}
Percentage survival (%)		86.67±0.31^{a}	86.67±0.24^{a}	82.22±0.41^{b}	84.44±1.23^{ab}	86.67±0.25^{a}	86.67±1.03^{a}

Mean values with different superscripts along the same row are significantly different ($p<0.05$).

MSPM = melon seed peel meal; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen

CONCLUSION AND RECOMMENDATIONS

The results from this study showed that oven-dried melon seed peel meal could replace yellow maize meal in the diet of *C. gariepinus* since the values of the blood parameters obtained were within acceptable limits for cultured fish and no traces of infection (such as anaemia) were observed in the cultured fish. It also provides helpful base-line information on the haematology and serum biochemistry of *C. gariepinus* that could be referred to in assessing biochemical changes in the physiology of this species. Improvement in the values of blood parameters, such as Packed Cell Volume, Haemoglobin concentration, Platelets, Mean Corpuscular Volume, Total Blood Protein and White Blood Cells, recorded in the study indicated positive contribution of oven-dried melon seed peel meal nutrient content in blood formation and improving immunity in the fish. Therefore, this study recommends substitution of oven-dried melon seed peel meal for yellow maize meal in the diet of *C. gariepinus* without obvious harmful effects on its physiology and health status as revealed in the haematological and biochemical parameters studied. Further processing methods and enzyme treatment are also recommended to expand the scope of utilization of melon seed peel in fish diets.

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