NUTRIENT UTILIZATION AND HEMATOLOGICAL INDICES OF HYBRID CATFISH (*Heteroclarias*) FED VARIEGATED GRASSHOPPER MEAL (*Zonocerus variegatus*) LINN MEAL

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ABSTRACT

This study was carried out to evaluate the nutrient utilization, digestibility and haematological indices of hybrid catfish fingerlings fed *Zonocerus variegatus* meal (ZVM) for 70 days. Six isonitrogenous diets were formulated in which ZVM was included in the diet at different inclusion levels - D1 (0%), D2 (10%), D3 (20%), D4 (30%), D5 (40%) and D6 (50%). Each treatment had three replicates, eighteen net hapa (0.5 × 0.5 × 1m) were suspended in three outdoor concrete tanks (8m×5m×1.5m). The concrete tanks were filled to 5/6 of its volume (40m^3) with filtered and dechlorinated tap water, 20 fish with the initial average weight of 2.17±0.14 were stocked in each hapa. Water temperature and other water quality parameters were monitored daily. The result showed that Fish fed D4 had the highest value in all nutrient utilization parameters recorded and was significant ($P<0.05$) except for protein efficiency (PE) value which was not different with D2. However D6 had the lowest total feed intake (TFI), Protein retention (PR) but there was no significant difference ($P>0.05$) between D6 and D5. Fish fed D4 had the highest apparent digestibility co-efficient of crude protein and was significantly different from others, except for D3. D6 had the lowest significant value but was not significantly different from D5. D3 had the highest apparent digestibility co-efficient of crude lipid value but was not significantly different from D1, D2, and D4. However they were significant higher ($p<0.05$) than D5 and D6. The result of the hematological indices showed that there was increase in all the values of all the hematological indices measured as compared to the initial. From this study it could be concluded that 30% inclusion of ZVM in the diet of hybrid catfish could improve their nutrient utilization without any negative impact on their hematological indices

Keywords: Nutrient utilization; Hematological indices; apparent digestibility co-efficient

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INTRODUCTION

Formulation of fish feeds containing high levels of conventional and non-conventional alternative protein source has become a major focus in aquaculture nutrition research. This has become imperative because of the ever-increasing cost and uncertain availability of fishmeal (Bake *et al.* 2013).The partial or complete replacement of fishmeal (FM) with alternative plant or animal protein ingredients generally results in an imbalance of amino acids, which can affect fish nutrient utilization efficiency and digestibility of the diet, resulting in higher N loading and eventual pollution of the culture medium and outbreak of disease to the culture fish itself (Rumsey, 1993; Sajiura, 1998; Bake *et. al* 2013).

Blood is a major index of physiological, pathological and nutritional status of an organism. Any change in the constituent component of blood sample when compared to the normal values could be used to interpret the metabolic state of the animal and the influence of treatment given to the animal (Babatunde *et al.,* 1992). Haematological study in fish nutrition is gaining more attention by fisheries researchers. This is because of its importance in monitoring the physiological condition and health status of the cultured fish (Hrubec *et al.,* 2000), which serves mainly for diagnostic purpose hence can be used to appraise the suitability of feeds, feed mixture pellets as well as to examine the effect of stress situation (Svobodova *et al.,* 1991). Changes in haematology of fish in response to stressing agents are indicators of the distress stage of fish, giving vital information to control any unfavourable condition that may affect the health status of the fish (Bello-Olusoji *et al.,* 2006). In culture fisheries, these studies are usually associated with the feed input. The red blood cells count (RBC), haematocrit (PCV) and haemoglobin (Hb) concentration vary with diet and strain as well as temperature, season of the year and nutritional status of the fish (Barnhart, 1969). Hence, optimal inclusion of any alternative ingredient in fish feed without any negative effect on the fish growth, nutrient utilization and culture medium is highly desirable to fish nutritionists. Furthermore, haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (Oyawoye and Ogunkunle, 1998).

The variegated grasshopper *Zonocerus variegatus* (Linn.) (Orthoptera: Pyrgomorphidae) is a large grasshopper with a high dry season population in the southwest and northern parts of Nigeria. It is recognized easily by the multicoloured markings on its body and the disagreeable odour. It is a polyphagus insect that feeds on and defoliates a large number of farm crops (Alegbeleye *et al.*2011). Their population is high enough to constitute a threat to plantations, especially cassava and maize (Thresh *et al.* 1994). On the positive side, they provide animal dietary protein supplements for rural populations in the southwest and northern parts of Nigeria, hence, alleviating animal protein scarcity in this zone (Ojewole *et al.* 2005).

Over the last three decades, Clariid species has been considered to hold great potential for fish farming in Africa and Nigeria in particular and of recent trend is the culture of its intergenetic and intraspecific hybrids. The hybrids of *Heterobranchus* species and *Clarias* species exhibit a high growth rate, resistance to handling and stress and well appreciated in a wide number of African countries (Eyo and Ezechie 2004). The main objective of intensive aquaculture is to obtain the maximum increase in weight (biomass) of fish/ unit area of volume/unit with a specific level of management practice; this require the knowledge of fish growth, carrying capacity, nutrition, yield and

water quality parameters. In fish farming, sufficient consumption of feed is essential for maximum yield and profitability. Because of the importance of these hybrid species, it is critical that feeds are both economically and environmentally sustainable. Good nutrition in animal production systems is essential to economically produce a healthy, high quality product in shortest time possible.

Although ZVM are in abundance especially in the tropics and not much work has been done on its utilization as an ingredient in the diet of hybrid catfish fingerlings and its subsequent impact on the haematological indices of the fish. It is in the light of this that this study was carried out with the main objective to assess feed utilization, nutrient digestibility and haematological indices of hybrid catfish fed variegated grasshopper meal (VGM) diets in concrete tank.

MATERIALS AND METHODS

The feeding trial experiment was carried out at the Old Research School of Agriculture and Agricultural Technology Farm, Federal University of Technology Minna, Nigeria Bosso campus. The adult variegated grasshopper were collected from the environment of Federal University of Technology Minna Nigeria, Bosso campus, they were weighed fresh, oven dried at 80°C for 24 hours and soaked in water at 120°C for 10mins to remove the waste and destroy harmful microorganism in the grasshopper. The grasshopper were sun-dried for 8 hours, milled with the aid of grinding machine. The fish meal used for the experimental diet was purchased from Sauki fish farm Km 16 Minna –Zungeru road Niger state. Soybean and ground nut cake was obtained from the Kure market Minna Niger state. The Soybean was then toasted using a frying pan and allowed to cool before milling with the aid of grinding machine. All the ingredients used were separately milled and fortified with vitamin and mineral premix, each experimental diet was thoroughly mixed starting from the less quantity ingredient in a plastic bowl and later mixed with water. The moist mixed ingredients were made into dough and pelleted with manual pelleting machine at the laboratory unit of the Department of Water Resources, Aquaculture and Fisheries Technology, with 2mm die. The pellets were sun dried and preserved in polythene bags.

Based on the nutritional requirements of African catfish fingerlings (NRC 1993), six isonitrogenous and isolipidic diets were formulated at 40 % protein and 9.5 % lipids, containing 10-50% ZVM at different levels of inclusion designated as D1 (0% inclusion), D2 (10% inclusion), D3 (20% inclusion), D4 (30% inclusion), D5 (40% inclusion)and D6 (50% inclusion).

Table 1: Formulation of the experimental diet and proximate composition of the experimental diet for hybrid catfish fingerlings (g/kg).

Ingredients	D1	D2	D3	D4	D5	D6
FM	448.80	373.50	298.20	222.80	147.50	72.30
SBM	100.00	100.00	100.00	100.00	100.00	100.00
GNC	100.00	100.00	100.00	100.00	100.00	100.00
ZVG	0.00	100.00	200.00	300.00	400.00	500.00
MM	25.00	25.00	25.00	25.00	25.00	25.00
Millet	25.00	25.00	25.00	25.00	25.00	25.00
Starch	45.00	45.00	45.00	45.00	45.00	45.00
Cellulose	189.20	164.70	140.50	116.30	91.90	67.50
Vitamin premix	20.00	20.00	20.00	20.00	20.00	20.00
SBO	27.00	26.80	26.30	25.90	25.60	25.20
Mineral	20.00	20.00	20.00	20.00	20.00	20.00
Total	1000.0 0	1000.0 0	1000.0 0	1000.0 0	1000.0 0	1000.0 0
Moisture (%)	6.25	6.56	5.82	6.45	6.74	6.85
Crude protien (% d.b.*1)	37.32	37.15	37.48	37.43	37.31	37.03
Crude lipid (% d.b.*1)	9.62	9.78	9.77	9.62	9.76	9.84
Ash (% d.b.*1)	9.27	9.87	10.45	10.65	10.76	10.84
Crude fibre (% d.b.*1)	4.14	4.25	5.26	5.43	5.43	5.43
AIA (% d.b.*1)	4.66	4.67	4.75	4.22	5.51	5.64

The experimented fish, hybrid catfish (Heteroclarias) fingerlings (average weight of 1.38± 20.16g), were purchased from Pearl Catfish Hybridization Centre, Lagos State. The fish were transferred in a well-oxygenated water plastic container from the hatchery to the Department of Water Resources, Aquaculture and Fisheries Technology experimental fish farm, Federal University of Technology, Bosso campus, Minna where the feeding trial was conducted. Upon arrival they were acclimatized in a transitional tank in the farm for four days and were fed commercial feed (coppense feed) at 40% crude protein once a day before the experiment commenced. Eighteen net hapa (0.5x0.5x1m) were suspended in two outdoor concrete tanks (8mx5mx1.5m) with the aid of kuralon twine tied to plastic poles. The concrete tanks were filled to 5/6 of its volume (40m^3) with filtered and dechlorinated tap water. Twenty (20) fish were accommodated in each hapa and each treatment was randomly allocated to three hapas. Photoperiod depends on the natural light, and water temperature was monitored daily. The water quality parameters in the system were monitored weekly, the temperature ranged between 24°C-29°C while the concentration of dissolved oxygen ranged between 5.94-7.82 mg/L and the pH values of the treatments ranged from 7.18-7.60. No critical values were detected for nitrite and nitrate. The fish were fed three times daily at 5% of body weight at 09:00 am, 12:00pm and 16:00pm for 70 days. Feeding rate was subsequently adjusted according to their growth rates per hapa. The uneaten and faecal matters were siphoned out of the hapa every morning before feeding, and 45 minutes after the fish have been fed. The fish were denied feed 24 h prior to sampling. Five fish were randomly sampled on weekly basis, and weights were measured using a digital electronic weighing balance

(*CITIZEN MP-300*) model.

About 10g initial sample and 15g of final samples from each hapa were pooled separately and then homogenized using laboratory mortar and pestle. The major ingredient used for the diet; the formulated diet and the fish body samples were subjected to chemical analysis. The proximate composition analysis was determined according to AOAC procedures (AOAC 2002). Moisture content was determined by drying samples at 105±2°C until a constant weight was obtained. Dried samples were used for determination of crude fat, protein and Ash contents. Crude fat was measured by solvent extraction method in a soxhlet system where n-hexane was used as solvent. Crude protein content was calculated by using nitrogen content obtained by Kjeldahl method. A conversion factor of 6.25 was used for calculation of protein content according to AOAC (2002). Growth performance and diet nutrient utilization were analyzed in terms of weight gain (WG), feed efficiency (FE), specific growth rate (SGR), feed intake (FI) and protein efficiency ratio (PER). The following formulae were used:

Feed efficiency (%) = (weight gained (g) / feed fed (g)) x 100

Feed intake (mg/fish/day) = dry feed (mg) fed / number of fish / feeding period (day)

Protein efficiency ratio = wet body weight gain (g) / protein intake (g)

The determination of the protein and lipid digestibility coefficient was done according to Jimoh *et al.* (2010) which was calculated based on the percentage of AIA in feed and in faeces and the percentage of nutrient on diets and faeces.

Blood samples were collected in triplicate following the procedure of Klontz and Smith (1968) and Wedemeyer and Yasutake (1977), and subsequently taken to the Laboratory of the Department of Biochemistry Federal University of Technology Nigeria Minna for haematological analysis. At the laboratory the clear fluid sample which is the serum was pipetted out into a clean and sterilized bottle for haematological parameters analysis (Ogbu and Okechukwu, 2001). The direct measurement of erythrocyte values packed cell volume (PCV), haemoglobin (Hb), and Red blood cell (RBC) and absolute erythrocyte indices (MCH, MCV and MCHC) were calculated. The white blood cell and differential count (neutrophils and lymphocytes) were analysed as described by Dacie and Lewis (2001).

MCV=PCV/ Erythrocytes count ×10

MCH= Haemoglobin/ Erythrocytes count ×10

MCHC= Haemoglobin /PCV ×100

Data were analyzed using one-way analysis of variance (ANOVA) using Statistica 8.0 (Stat-Soft, Inc., Oklahoma, USA). Differences between treatments were compared by Tukey's test. Level of significance was tested at $P<0.05$.

RESULTS

Table 1 showed the ingredient profile and nutrient composition of the six experimental diets. The diets were similar in all the nutrient composition.

Nutrient utilization and apparent digestibility coefficient of hybrid catfish fed experimental diets for 70days is given in Table 2. The result showed that fish fed D4 had the highest value in all nutrient utilization parameters recorded and was significant (P<0. D5). Although D6 had the lowest total feed in take (TFI), there was no significant difference (P>0. 05) between D6 and D5. Fish fed D1, D2, and D3 were not significantly (P>0. 05) different from each other in all the nutrient utilization parameters measured however they were significantly (P<0. D5) higher than fish fed D5 and D6. Fish fed D4 had the highest apparent digestibility co-efficient of crude protein and was significantly different from others, except for D3. D6 had the lowest significant value but was not significantly different from D5. D3 had the highest apparent digestibility co-efficient of crude lipid however it was not significantly different (P>0. 05) from D1, D2 and D4, but they were significant higher (P<0. D5) than D5 and D6.

Table 2: Nutrient utilization and apparent digestibility coefficient of hybrid catfish of fed experimental diets for 70 days.

Diet code	Total feed intake (g)	Feed efficiency	Protein efficiency ratio	Protein retention (%)	ADC of crude protein	ADC of crude lipid	ADC of crude fiber
D1	22.29±0.57 ^{b}	0.82±0.02 ^{b}	2.21±0.04 ^{b}	38.58±0.65 ^{b}	87.86±2.56 ^{b}	84.09±1.54 ^{a}	73.10±1.11
D2	22.24±0.32 ^{b}	0.82±0.01 ^{b}	2.21±0.16 ^{b}	38.78±0.40 ^{b}	87.71±2.61 ^{b}	84.08±1.66 ^{a}	73.10±1.23 ^{b}
D3	22.89±0.10 ^{b}	0.82±0.01 ^{b}	2.20±0.11 ^{b}	39.56±1.04 ^{ab}	88.23±1.25 ^{ab}	84.95±2.87 ^{a}	73.17±1.54 ^{b}
D4	24.52±0.35 ^{a}	0.86±0.06 ^{a}	2.30±0.03 ^{a}	40.74±0.49 ^{a}	89.92±1.33 ^{a}	83.95±1.29 ^{a}	75.73±1.67 ^{a}
D5	20.35±0.31 ^{c}	0.78±0.02 ^{c}	2.08±0.08 ^{c}	36.48±0.73 ^{c}	85.80±2.35 ^{c}	79.65±1.43 ^{b}	70.29±1.84 ^{c}
D6	19.98±0.27 ^{c}	0.75±0.01 ^{d}	2.03±0.24 ^{c}	35.62±0.43 ^{c}	85.16±3.47 ^{c}	78.80±1.77 ^{b}	70.27±1.55 ^{c}

Values in the same column with different superscript letters are significantly different ($p<0.05$) from each other.

The haematological indices of fish fed graded levels of ZVM based diet is shown in Table 3. The PCV result showed that fishes fed the experimental diet including the control diet D1 all increased in the PCV value. Except for fish fed D5 and D6 which were slightly significantly lower than the other fish fed other experimental diets, there was no significant ($P > 0.05$) difference among fed D1, D2, D3 and D4. The results obtained for the white blood cell (WBC) showed that there was an increase as ZVM increased in the diet however fish fed D1, D2, D3, D4 were significantly lower than those fed D5 and D6. The result of RBC shown in Table 3 indicated that RBC value increased among all the fish fed the experimental diets and there was no significant difference among them. Except for D6 that was slightly significantly lower than the rest the haemoglobin (Hb) result showed that there was no significant difference between fish fed D1, D2, D3, D4 and D5. The MCHC value obtained followed the same pattern as the RBC, while the mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) value obtained followed the same pattern as the PCV.

Table 3. Haematological parameters of hybrid catfish fingerling fed experimental diets for 70 days

ood Parameter	Initial	Final*1					
		D1	D2	D3	D4	D5	D6
V (%)	31.32	35.37±0.36 a	35.58±0.22 a	35.68±0.33 a	35.85±0.51 a	34.62±1.43 b	33.42±1.67
BC (10^3 mm^{-3})	5.45	6.25±0.18 b	6.44±0.72 b	6.32±0.75 b	6.27±0.24 b	8.25±0.68 a	8.52±0.56
C (10^6 mm^{-3})	2.88	3.12±0.11 a	3.18±0.54 a	3.16±0.17 a	3.18±0.46 a	3.12±0.53 a	3.05±0.42
(g/100 ml)	9.32	10.76±0.21 a	10.87±0.33 a	10.76±0.29 a	10.96±0.30 a	10.45±0.42 a	10.08±0.78
MPH (100)	60.84	61.48±0.26 a	61.25±0.68 a	61.58±0.13 a	61.85±0.55 a	61.32±0.66 a	61.15±0.24
HC (%)	29.76	30.42±0.45 a	30.55±0.21 a	30.16±0.44 a	30.57±0.42 a	30.18±0.18 a	30.16±0.42
H (pg)	32.36	34.49±0.66 a	34.18±1.06 a	34.05±0.58 a	34.47±0.37 a	33.49±1.17 b	33.05±1.27
V (fl)	108.75	113.37±1.38 a	111.89±1.43 a	112.91±1.27 a	112.74±1.63 a	110.96±1.15 b	109.57±1.44

V, packed cell volume; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; LYMPH, lymphocyte; MCHC, mean rpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin concentration; MCH, mean corpuscular emoglobin; MCV, mean corpuscular volume.

Values in the same row with different superscript letters are significantly different ($p<0.05$) from each other (n=3).

DISCUSSION

This study revealed the possibility of utilising ZVM in the diet of hybrid catfish fingerlings. The nutrient utilization, diet digestibility and haematological indices were slightly influenced by the increase in the inclusion level of ZVM.

This study showed that acceptability may have affected the feed intake and the nutrient utilization of the experimental diets, the fish fed 30% inclusion of ZVM gave a better nutrient utilization in term of FE, PR and TFI however at a higher inclusion levels of 40-50%, the nutrient utilization and digestibility of the diets tend to reduce slightly.This could be related to the increased content of indigestible materials like chitin and ash. Olsen *et al.* (2005) reported that at high levels of chitin could result in indigestibility of a diet. The digestibility of individual ingredient in feeds according Desilva et al. (1996), Fagbenro *et. al* (2002) and Bake *et.al* (2014) has been known to influence nutrient utilization and diet digestibility in fish. In this study, growth rate decreased significantly ($P< 0.05$) with an increase in the inclusion level of VGM above 30% inclusion. PER recorded in this study exhibited no significant difference. The importance of feed intake of fish as a determinant of fish performance has been strongly emphasized (Preston and Leng, 1987; Faturoti, 1989; Pillay, 1990). The high in feed intake in this study could be attributed to the probable palatability of the ZVM included in the diets.The decrease in nutrient utilization and digestibility values of the groups fed high levels of variegated grasshopper meal agrees with the finding of Shiau and Yu (1999) and Olsen *et al.* (2006).

Chitin is a non- starch polysaccharide and is considered to be a component of dietary fiber; according to Tharannathan and Kiltur (2003), chitin normally display the main characteristics of dietary fibre like water –binding capacity and poor nutrient digestibility. Chitin has been associated with poor lipid digestibility and increase feacal lipid content. Tharanathan and Kiltur (2003) noted that lipid hydrolysis by lipase could be hampered by the tendency of chitin to manifests as dietary fibre that can bind lipid and bile, thus, lowering absorption in mammals, thus these has been found to be the cause of high feacal lipid output and lower carcass lipid according to (Lindsay et al. 1984; Shiau & Yu 1999).

All the haematological parameters measured in this study were within the recommended physiological ranges reported for catfish species. Blaxhall and Daisley (1973) reported the essence of using haematocrit to detect anaemic condition in fishes. The packed cell volume PCV range 31.32 to 35.85% observed in this study is within the range of 20 to 50% reported by Pietse *et al.* (1981) and rarely do values above 50% being reported (Clarks et al., 1976; Etim et al., 1999). Though, a decrease was observed in the level of PCV as the level of ZVM increased above 30% in the diet. Reduction in the concentration of the PCV in the blood usually suggests the presence of toxic factor example of which is haemagglutin which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). The decreasing trend observed in the PCV of this study may be attributed to the presence of some anti-metabolites such as chitin which present in the exoskeleton of grasshoppers. White blood cells (WBC) results recorded in this study showed an increase as the level of VGM inclusion above 30% in the diet. The highest value of 8.52×10^3 mm^{-3} for WBC was recorded in fish fed diet containing 50% VGM (D6). White blood cells (WBC) are the defense cells of the body. Douglas and Jane (2010) demonstrated that the amount has implication in immune responses and the ability of the animal to fight infection. High WBC count is usually associated with microbial infection or the circulating system (Oyawoye and Ogunkunle, 1998). The value range of 6.20×10^3 to 8.02×10^3 mm^{-3} recorded in this study for WBC was lower compared to 16.13 × 103 to 16.39 × 103 mm-3 reported by Sotolu and Faturoti (2009). The increase in RBC may be ascribed to the lower concentration of anti-metabolites especially chitin in the diet containing ZVM. There was a steady increase in the RBC as the level of ZVM inclusion increases and there was no significant difference among all the fish fed all the experimental diets. The range of RBC (3.05×10^6 to 3.18×10^6 mm^{-3}) recorded in this study is fairly comparable with (1.70×10^6 to 4.00×10^6 mm^{-3}) Bhasker and Rao (1990) and (2.24×10^6 to 2.49×10^6 mm^{-3}) Sotolu and Faturoti (2009). The RBC in this study is considered high and indicative of high oxygen carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and with high metabolic activity (Lenfant and Johansen, 1972). The haemoglobin result showed a decrease as the M. olifera leaf meal increased in the diet. The haemoglobin range (10.08 – 10.96g/100ml) recorded were high and fell within the range (5.6 to 15.8 g/100 ml) reported by Mulcahy, (1970) it also compared well with 8.70 g/100 ml for *C. gariepinus* (Sowunmi, 2003). These values were also higher than 4.46 g/100 ml reported for *Heterotis niloticus* (Fagbenro *et al.,* 2000). The range of haemoglobin concentration recorded in this study is quite high and can be

related to large anaerobic metabolism capacity of hybrid catfish. The decrease in the level of haemoglobin as ZVM increased in the diet above 30% could imply that diets having higher ZVM had negative effect on the blood. The mean corpuscular volume (MCV) range (109.96 - 112.74 fl) recorded in this experiment was higher than (79.20 to 105.32 fl) reported for Heteroclarias (Anyanwu *et al.*, 2011), meanwhile the mean corpuscular haemoglobin concentration (MCHC) range (30.16 to 30.57%) recorded in this study for fish fed ZVM diet compared fairly well with (30.70%) reported for *C. gariepinus* from Asejire dam (Adedeji and Adegbile 2011).

The study revealed that up to 30% ZVM can be included in the diet of hybrid cat fish fingerlings without compromising the fish nutrient utilization and having negative impact on the haematological indices of the fish.

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