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International Journal of Fisheries and Aquaculture

*Full Length Research Paper*

# Effects of protease enzyme supplementation on protein digestibility of legume and/or fish meal-based fish feeds

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**Improving protein digestibility in nutrient poor fish feeds through incorporation of dietary enzymes is expected to be achieved with protease. Understanding the role of other dietary enzymes was therefore evaluated to guide appropriate use for optimal fish growth. Protein digestibility of 30, 35, 50 and 55% crude protein (CP) diets was determined with catfish gut enzyme extract, sprouted sorghum, protease and phytase both singly and in a mixture of 500 units of protease and phytase using the pH drop method *in vitro*. Significant ($p<0.05$) digestibilities were recorded in 30 and 35% CP diets incorporated with phytase and in 50 and 55% CP diets incorporated with protease singly. These results showed that protein digestibility was more efficient with protease enzyme in high protein diets while phytase was efficient in low protein diets. This implied that the use of protease was more beneficial in catfish starter feeds and phytase in grower/finisher diets and provided a basis for enzyme selection for production of cost-effective catfish diets.**

**Key words:** *In-vitro* protein digestion, catfish gut enzyme extract, phytase, protease, sprouted sorghum.

## INTRODUCTION

Feed account for 60 to 70% of operating costs in farming of high value fed species like Clarias gariepinus, and without it stock productivity and profitability will remain a cherished desire (World Bank, 2007).

Fish feed quality is compromised by limited use of fishmeal, the most nutritive and digestible protein ingredient traditionally used in fish diets, due to its high cost (US$2/Kg) (World Bank, 2013), associated food insecurity and aquatic degradation (FAO, 2009). This has intensified use of plant protein instead (Gabriel et al., 2007) as they are more accessible and fairly priced (Hecht, 2006). However, almost all practical plant feed ingredients contain invariable amounts of antinutrients of which phytic acid is considered most detrimental. It forms indigestible complexes with nutrients including protein, reducing their utilisation by fish for growth (Gabriel et al., 2007; GarcõÂa-Estepa et al., 1999; Gilani et al., 2005; Hidvegi and Lasztity, 2002; Kumar et al., 2012b).

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**Table 1.** Ingredients used in formulation of experimental diets.

| Ingredient | 30% | 35% | 50% | 55% |
|---|---|---|---|---|
| DCP | 4 | 4 | 0 | 0 |
| Cassava flour | 6 | 4 | 0 | 0 |
| Wheat pollard | 8 | 8 | 10 | 2 |
| Whole grain maize | 10 | 10 | 0 | 0 |
| Fish meal | 20 | 26 | 72 | 96 |
| Soy bean | 22 | 26 | 13 | 1 |
| Bush beans | 15 | 8 | 0 | 0 |
| L-Lysin | 3.5 | 4 | 3 | 1 |
| DL-Methionine | 1.5 | 2 | 2 | 0 |
| Cotton seed cake | 7 | 5 | 0 | 0 |
| Nile perch oil | 3 | 3 | 0 | 0 |
| Salt | 0.001 | 0.001 | 0.001 | 0.001 |
| Fish vitamin and mineral premix | 0.002 | 0.002 | 0.002 | 0.002 |
| **Total** | **100.003** | **100.003** | **100.003** | **100.003** |

They are difficult to suppress cost effectively using conventional pre-treatments like heating, soaking and germination (Afify et al., 2011). Due to this use of dietary exogenous digestive enzymes especially phytase has been explored as a cheaper pre-treatment strategy of improving protein digestibility in low value plant based feeds for enhanced fish growth (Abdoulaye et al., 2011; Bedford and Partridge, 2010; Gabriel et al., 2007; Kim et al., 2006; Kumar et al., 2012a; Kumar et al., 2012b; Reddy et al., 1989; Serraino and Thompson, 1984; Wheeler and Ferrel, 1971). It has already been proven that the use of phytase in fish diets improves phosphorus absorption by fish reducing water pollution (Tudkaew et al., 2008). However, its contribution to protein digestibility which directly translates into fish growth is not established. As use of multiple enzymes in a single diet gets common in an effort to improve feed efficiency and enterprise profitability (Bedford and Partridge, 2010), the efficiency of other enzymes including protease on improving protein digestibility in phytic acid loaded plant based diets needs to be investigated as there can be antagonistic or additive effects (Dechavez and Serrano, 2012). This study determined rapid protein digestibility in legume based grow out diets (30 and 35% CP) and in fish meal-based larval diets (50 and 55% CP) subjected to catfish gut enzyme extract, sprouted sorghum, protease and phytase enzymes. Information generated provided an insight on the probable appropriate exogenous enzyme for incorporation in fish feeds at different stages of development for improved growth.

## MATERIALS AND METHODS

### Study area

The *in-vitro* analysis experiment was conducted at the Bioscience laboratory of the National Crop Resources Research Institute (NaCRRI) situated in Namulonge, Wakiso District in Uganda.

### Feed formulation and diet development

Four experimental diets were formulated to contain 30, 35, 50 and 55% crude protein with Feedwin software (Table 1). Feeds were pelleted to be stable in water using a pelleting machine locally fabricated in Kampala, Uganda.

Diet proximate composition was cross-examined/confirmed at Makerere University, School of Agriculture and Environmental Sciences, Animal science laboratory following standard procedures of the Association of Analytical Chemists (AOAC, 2002) and presented in Table 2.

### Preparation of enzyme extracts (solutions)

#### *Catfish feeding and preparation of digestive enzyme extracts*

Fish in four MBAZARDI ponds were fed on diets with crude protein graded at four levels (30, 35, 50 and 55% crude protein diets) for a week. Five catfish (weight 284 ± 4.5 g and length 35.2 ±1.20 cm) were captured randomly using a seine from each of the four pond treatments 30 min after feeding. The caught fish were humanly killed after anesthetizing them with excess clove oil (2.5 ml/L of water) according to guidelines of death as end point by (Homeoffice, 2014). They were then dissected; gut removed together with its contents and kept in a refrigerator at -4°C until when enzyme extraction was conducted according to the flow chart used by Sultana et al. (2010).

Catfish digestive enzyme extraction was conducted following the procedure of (Ali et al, 2009; Sultana et al., 2010). The guts were thawed to 40°C, the region encompassing the stomach and small intestines were cut out and chopped into small sections of 1 to 2 cm long. These small sections from each fish were ground in a beaker placed on ice with an ultra sonic cell lyser (model -150 V/T Biologics Inc) at 60 pulses per minute for 10 min. The slurry formed was diluted with distilled water chilled to 4°C at a ratio of 1:10 (weight/volume). It was then poured into 1.5 ml micro tubes (eppendrof) and centrifuged in a refrigerated centrifuge for 15 min at 12000 RPM. A transparent lipid layer formed on top of the

**Table 2.** Proximate composition of experimental diets as after independent verification at Makerere University.

| Diet description | Dry matter | Ash | Crude protein | Crude fibre | Crude fat | Gross energy (Kcal/Kg as is) |
|---|---|---|---|---|---|---|
| 55% CP diet | 90.18 ± 0.13 | 11.01 ± 0.17 | 54.86 ± 0.35 | 5.81 ± 0.146 | 7.71 ± 0.15 | 4778 ± 0.88 |
| 50% CP diet | 93.42 ± 0.02 | 12.80 ± 0.71 | 50.17 ± 0.72 | 3.93 ± 0.92 | 5.45 ± 0.08 | 4447 ± 18.33 |
| 35% CP diet | 91.53 + 0.10 | 8.92 ± 0.28 | 35.33 ± 0.59 | 4.41 ± 0.16 | 7.276 ± 0.97 | 4549 ± 15.54 |
| 30% CP diet | 91.65 ± 0.03 | 9.07 ± 0.24 | 30.76 ± 0.17 | 4.35 ± 1.09 | 9.00 ± 1.3 | 4551 ± 414 |

**Table 3.** Amount of protease enzyme dissolved in 1000 ml of distilled water to make a stock solution from which 10 ml worth corresponding activity units was with drawn into 20 ml of feed substrate suspension.

| Protease | Enzyme quantity (g) worth 750 activity units | Enzyme quantity (g) worth 1000 activity units | Enzyme quantity (g) worth 1250 activity units |
|---|---|---|---|
| 30% CP diet | 0.0066 | 0.009 | 0.0011 |
| 35% CP diet | 0.0058 | 0.0078 | 0.0097 |
| 50% CP diet | 0.004 | 0.0054 | 0.0067 |
| 50% CP diet | 0.0036 | 0.0049 | 0.0061 |

**Table 4.** Amount of phytase enzyme dissolved in 50 and 5 ml of distilled water to make a stock solution from which 2 ml worth corresponding activity units was with drawn into 20ml of feed substrate suspension.

| Phytase | Enzyme quantity (g) worth 750 activity units | Enzyme quantity (g) worth 1000 activity units | Enzyme quantity (g) worth 1250 activity units |
|---|---|---|---|
| 30% CP diet | 0.008 | 0.0011* | 0.0013 * |
| 35% CP diet | 0.0069 | 0.0092 | 0.0012* |
| 50% CP diet | 0.0048 | 0.0064 | 0.008 |
| 50% CP diet | 0.0044 | 0.0058 | 0.0073 |

*Dissolved in 5 ml from which 2 ml were withdrawn.

supernatant was removed using plastic pipettes and discarded. The supernatant was collected in a glass bottle of 50 ml and stored in a deep freezer at -20°C until it was used.

***Preparation of sprouted sorghum solution***

Sprouted sorghum (*S. bicolor*) grains were dried and ground into flour of fine particles of less than 0.02 mm. An amount of flour equivalent to 10% of the feed used to get 160 mg of crude protein under each category of feed was determined and weighed using a digital scale (Denver Instruments, Germany Model TP-3002). This flour was made into a suspension with distilled water which was mixed with the pre-soaked feed suspension and incubated at 26°C for 10 min.

***Preparation of phytase and protease enzymes***

The amount of enzyme worth 750, 1000 and 1250 activity units of protease *(fungus Trichoderma reesei*) and phytase (bacteria *Bacilus lincheniformis*) were calculated based on the manufacturer's prescriptions of the enzyme activity (that is, 1 g of protease contained 600,000 activity units and 1 g of phytase contained 5000 activity units).

The amount of enzyme used was measured by sensitive digital scale (Denver Instruments, Germany Model TP-3002). For protease enzyme, 1000 ml of stock solution was made with distilled water at 4°C from which 10 ml worth 750, 1000 and 1250 protease activity units were withdrawn and put into 20 ml of pre-soaked feed substrate following the Tocris morality (Table 3). For phytase enzyme, stock solutions of 50 and 5 ml were made with chilled distilled water from which 2 ml worth corresponding activity units was drawn (Table 4).

**Determination of protein digestibility**

The pH drop method was used following the procedure described by (Sultana et al., 2010) as adopted from Chisty et al. (2005). Four diets of 30, 35, 50 and 55% crude protein were ground and an amount that provided 160 mg of crude protein weighed (based on proximate composition, that is, 0.53 g for 30%, 0.46g for 35% CP,0.32 g for 50% CP and 0.29g for 55% CP diets. The mount of feed for each protein level was soaked overnight in 20 ml of distilled water at 4°C with casein from bovine milk (90% crude protein, C7078, Sigma-Aldrich, St. Louis, MO, USA) as the standard protein. The 160 mg protein from each diet including casein (in 20 ml) was incubated at 26°C in a water bath (Grant TXF 200) for 3 min. In each case, the suspension pH was first adjusted to pH 8 (optimal pH of protease and phytase enzymes used) using ether Sodium hydroxide or hydrochloric acid as would be appropriate.

**Table 5.** pH values recorded after every one minute interval during incubation of 1250 protease activity units with 50% crude protein feed suspension substrate in three replicates.

| Protease 1250 | | | | | |
|---|---|---|---|---|---|
| **Casein** | **50%CP** | **Casein** | **50%CP** | **50%CP** | **Casein** |
| 8.09 | 8.08 | 8.07 | 8.00 | 8.07 | 8.00 |
| 8.02 | 8.05 | 8.01 | 7.98 | 8.06 | 7.93 |
| 8.00 | 8.03 | 8.00 | 7.96 | 8.05 | 7.93 |
| 8.00 | 8.01 | 7.99 | 7.95 | 8.03 | 7.92 |
| 8.00 | 8.00 | 7.98 | 7.87 | 8.02 | 7.92 |
| 8.00 | 7.97 | 7.97 | 7.82 | 8.01 | 7.92 |
| 8.00 | 7.94 | 7.97 | 7.71 | 7.81 | 7.92 |
| 8.00 | 7.93 | 7.97 | 7.64 | 7.81 | 7.92 |
| 8.00 | 7.79 | 7.97 | 7.62 | 7.8 | 7.92 |
| 8.00 | 7.73 | 7.97 | 7.62 | 7.8 | 7.92 |
| 8.00 | 7.73 | 7.97 | 7.62 | 7.8 | 7.92 |

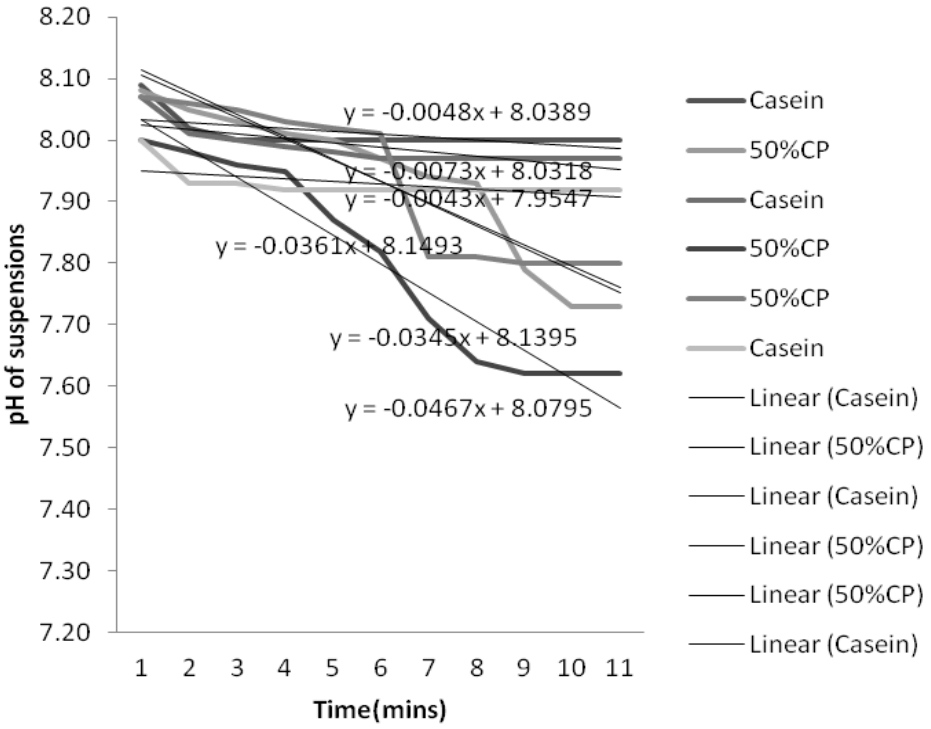


**Figure 1.** Rates of pH change in casein and diet suspensions per minute (for 10 min).

All the prepared catfish digestive enzyme extracts from each fish (5) and sprouted sorghum (worth 10% of the feed substrate feed), 750, 1000 and 1250 activity units of phytase (*Bacilus lincheniformis bacterium)* and protease (*Trichoderma reesei fungus)* were added to feed substrate suspensions (Table 3 for protease and Table 4 for phytase respectively). The pH readings in each enzyme-feed substrate were in all cases recorded after an interval of one minute for 10 min using a digital pH meter with a protected tip (pH 211, Labor-pH/mV/°C- Meter unit Microprocessor, HANNA instruments), sample data in Table 5.

A graph of pH values for enzyme-casein substrate was plotted against pH values of the enzyme – diet substrate and the slope of the graph used as the rate of pH change with time (Figure 1).The rapid protein digestibility (RPD) was calculated as the ratio of percentage of pH change (-Δ pH) in the enzyme-diet substrate to pH change of enzyme-casein substrate following a formula adapted from that of (Lazo, 1994) as:

$$RPD\ (\%) = [(((-\Delta pH\ diet\ /-\Delta pH\ Casein) * 10\ )/160) * 100$$

Where 10 = Number of incubation minutes; 160 = Amount of protein (mg) in feed substrate (Table 6).

**Table 6.** How protein digestibility was determined from changes in pH of casein and that of the diets (in triplets).

| Change in casein pH | Change in diet pH | Ratio of change in diet pH to change in casein pH | Estimated diet digestibility (%) |
|---|---|---|---|
| 0.0048 | 0.0361 | 7.520833 | 47.00521 |
| 0.0073 | 0.0467 | 6.39726 | 39.98288 |
| 0.0048 | 0.0345 | 7.1875 | 44.92188 |

**Table 7.** Digestibility regression of diets incorporated with sprouted sorghum, protease, phytase and a combination of 500 units of phytase and 500 units of protease with the fish gut enzymes as the explanatory variable.

| Enzyme type | Protein digestibility (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 30 % | | 35% | | 50% | | 55% | |
| | Mean | P-value | Mean | P-value | Mean | P-value | Mean | P-value |
| Fish enzyme | 3.07 | - | 3.13 | - | 11.57 | - | 21.20 | - |
| Sprouted sorghum | 14.23 | 0.144 | 16.93 | 0.276 | 13.97 | 0.822 | 27.37 | 0.654 |
| Protease | 28.76 | <0.001 | 43.61 | <0.001 | 54.44 | <0.001* | 59.86 | 0.002* |
| Phytase | 85.16 | <0.001* | 76.43 | <0.001* | 43.84 | 0.001* | 33.20 | 0.290 |
| Protease and phytase | 13.67 | 0.165 | 17.67 | 0.252 | 7.20 | 0.682 | 20.57 | 0.963 |

*Significant at $\alpha \leq 0.05$.

### Statistical analysis

All data was first entered into Microsoft Excel and later imported into STRATA statistical soft ware (version 14). A simple linear regression (ANOVA) analysis of protein digestibilities was conducted with fish gut enzyme as explanatory variable. Statistical differences were declared at 95% confidence interval ($p \leq 0.05$).

## RESULTS

Protein digestibility was significantly higher for Phytase incorporated legume based diets (30 and 35% crude protein) and protease incorporated fish meal based diets (50 and 55% crude protein) than in catfish gut enzyme extract. Protein digestibility in diets incorporated with sprouted sorghum and a mixture of protease and phytase combined was not significantly different from that of catfish gut enzyme extract (Table 7).

Generally there was higher protein digestibility in 55% crude protein diets incorporated with protease and 35% crude protein diets incorporated with Phytase enzyme (Figure 2).Protein digestibility in protease incorporated diets increased with increasing protein concentration while digestibility in diets incorporated to phytase had general decline with increasing protein (Figures 3 and 4 respectively). However the highest digestibility (88.9 and 88.4%) was recorded with 750 units/kg of phytase followed by 70.1% in protease incorporated diets with 1000 units (Figures 3 and 4, respectively).

## DISCUSSION

Significantly high protein digestibility in Phytase incorporated legume based diets (30 and 35% crude protein) and protease incorporated fish meal based diets (50 and 55% crude protein) was attributed to limited interference from impurities and antagonistic reactions of other enzymes that could have been present in the crude enzyme extracts from the catfish gut and sprouted sorghum extracts. The recorded protein digestibility with phytase and protease enzymes was however higher than those observed by Ali et al (2009); the while protein digestibility of diets incorporated with fish gut enzyme extract (3.07-21.20%) was lower than what he observed for fish meal (78.08%), soy bean meal (76.08%) and rice polish (35.86%) and Thai koi (*Anabas Testudineus*) gut enzyme extract. This was attributed to differences in gut physiology and composition of test diets with regard to ingredients, nutrient and antinutrient composition of diet ingredients (soy bean, common beans, wheat pollard, cassava, cotton seed cake).

Lack of significance on rapid protein digestibility recorded with incorporating sprouted sorghum and a mixture of protease and phytase at all diet protein levels compared with the catfish gut enzyme extract was thought to be due to antagonistic or proteolytic digestion of phytase by protease enzyme. Similar reports on reduced efficiency of protease in presence of phytase and xylanase enzymes were reported by Ravindran (2013) and Sultana et al. (2010). Degradation of phytase by proteases such as pepsin and trypsin-like enzymes in the fish stomach enzyme extract was also reported for most enzymes except for *Aspergillus niger*, *Escherichia coli* and some *Bacillus* species of which it is not clear whether *Bacilus lincheniformis* is among (Kumar et al.,

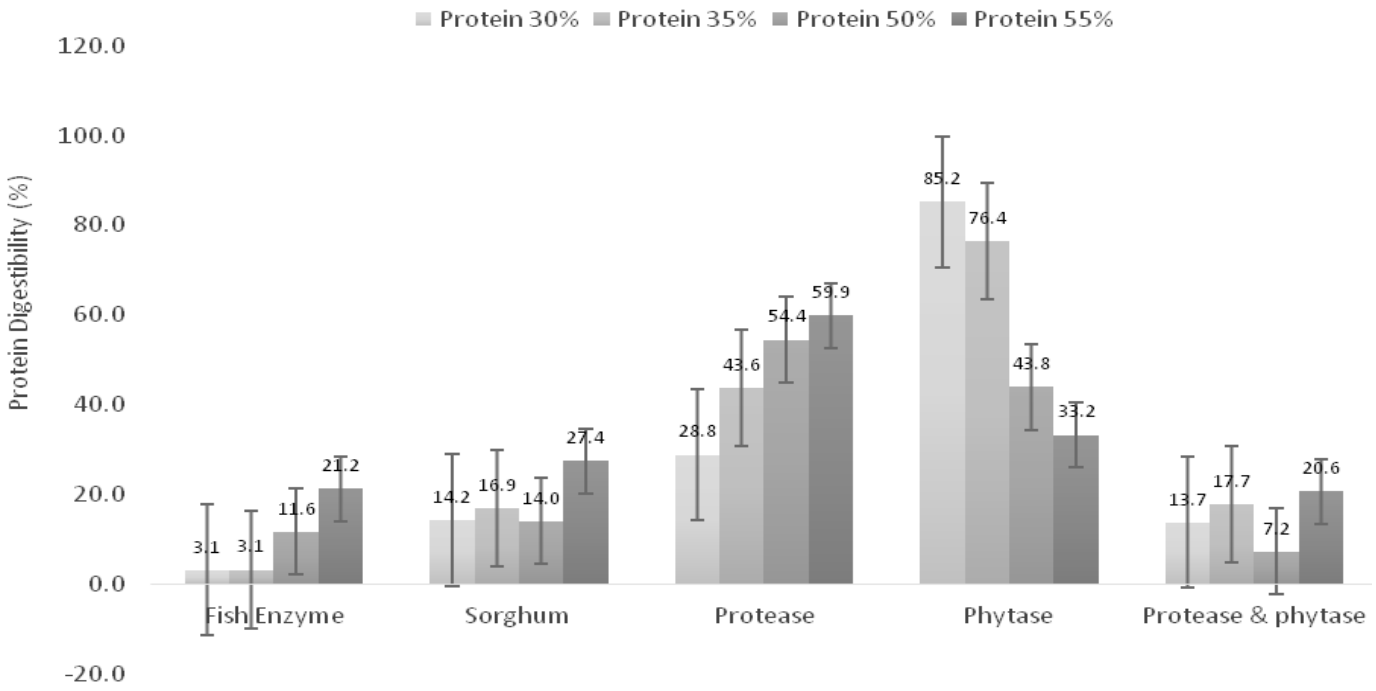


**Figure 2.** Rapid protein digestibility coefficients (%) of diets incorporated with catfish gut enzyme extract, sprouted sorghum (S. bicolor), protease (fungus *Trichoderma reesei*), phytase (bacteria *Bacillus lincheniformis)* and a combination of 500 units of phytase and protease.

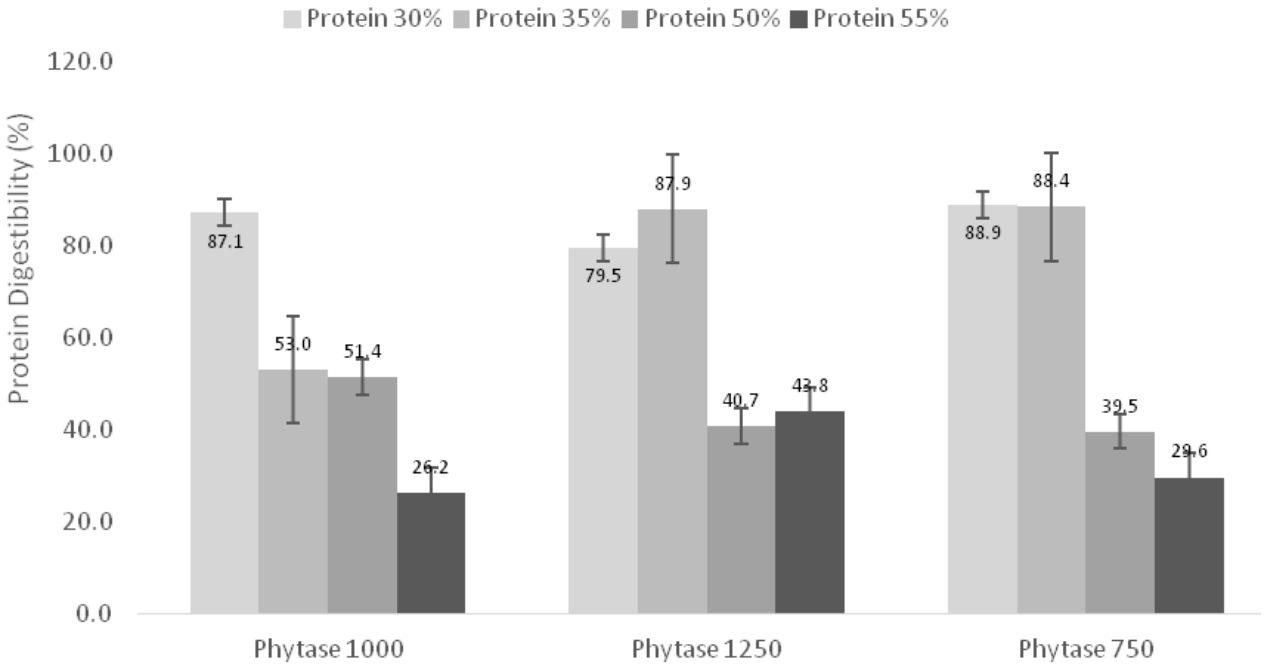


**Figure 3.** Mean protein digestibility of experimental diets incorporated with 750, 1000 and 1250 activity units of phytase enzyme.

2012b).

This implied that combining enzymes reduces efficiency than when used singly and required to be guided by such limitations or by compressive research on enzyme complementarily to maximize economic benefit of their applications.

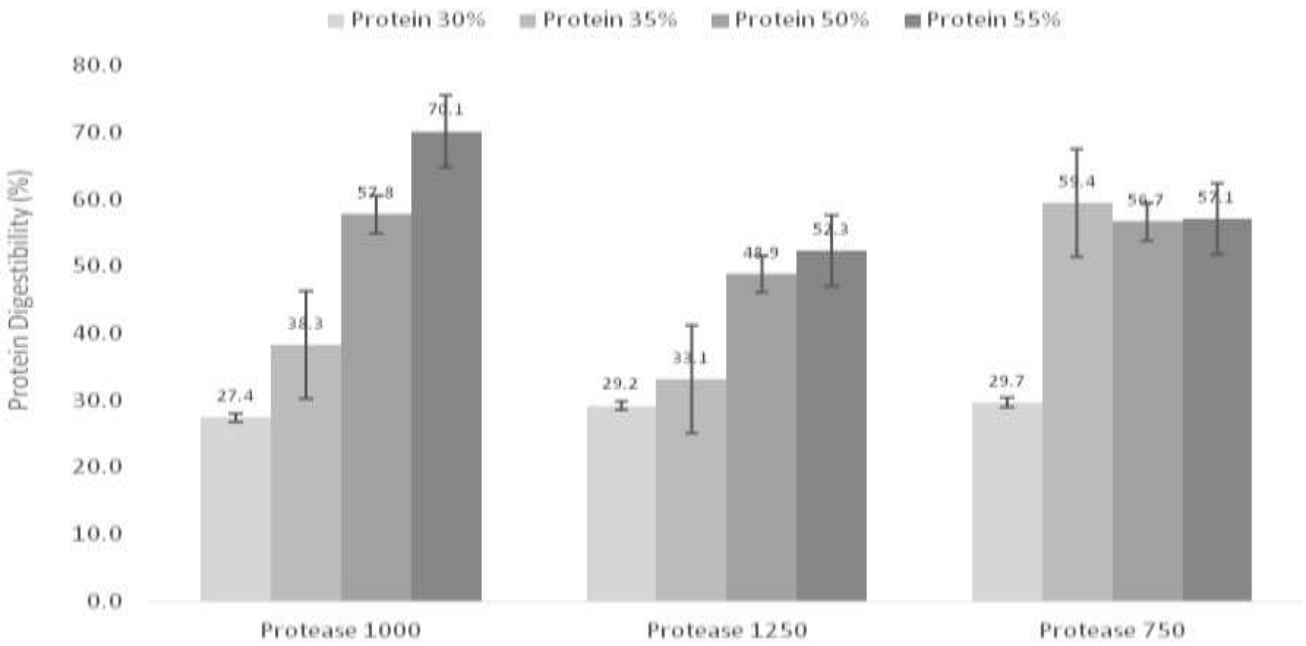


**Figure 4.** Mean protein digestibility in experimental diets incorporated with 750, 1000 and 1250 activity units of protease enzyme.

The significantly high protein digestibility in the fish meal based 50 and 55% crude protein diets incorporated with protease enzyme was attributed availability of sufficient dietary protein as substrate for protease enzyme than at low protein levels in grow out plant-based feeds (30 and 35%.CP). However, the percentage of protein that remained undigested could have been hindered by antinutrients in the plant protein/material that were included in these diets.

Conversely, the significantly high protein digestibility recorded in legume based grow out diets (30 and 35% crude protein) incorporated with phytase enzymes was attributed to high content of phytic acid bond protein in legume seeds which provided sufficient substrate for phytase enzyme. Most of the protein portions in dicotyledonous legumes which dominated these grow out diets are known to be closely linked to phytic acids with which they form inseparable/indigestible complexes unlike in monocots like corn and wheat where phytic acid is concentrated in germ and aleuronic layer (Chow and Schell, 1980). Breakdown of phytic acid by phytase enzyme should have been responsible for the more protein digestibility than with the case of protease which could have not got sufficient free protein to reduce into amino acids. This is in line with the theory of substrate-enzyme reaction which states that “at relatively low concentrations the rate of enzyme catalyzed reaction increases linearly with substrate concentration but is asymptotic at relatively higher substrate concentrations” (Sousa et al., 2015).

This implied that incorporation of protease is more beneficial in high protein catfish diets such as starter feeds while phytase is beneficial in low crude protein diets such as grower and finisher diets.

## Conclusions

Incorporation of exogenous digestive enzymes generally increased protein digestibility in all fish diets than the catfish gut enzyme extract. Incorporation of phytase and protease enzymes however recorded significantly high protein digestibility if incorporated in legume based diets (30 and 35% crude protein) and in fish meal based diets (50 and 55% crude protein) respectively. Mixing protease and phytase enzymes into a single diet significantly lowered protein digestibility than using each enzyme singly. These results demonstrated that protein digestibility was more efficient with protease enzyme in high protein diets while phytase was efficient in low protein ones. This implied that use of protease was more beneficial in catfish starter feeds and phytase in grower/ finisher diets. They therefore provided a basis for selection of appropriate enzymes for production of cost-effective catfish diets at different growth stages.

## Recommendations

For practical applicability, results of the study require confirmation with an *in-vivo* catfish feeding experiment with diets used here incorporated with sprouted sorghum; protease and phytase. Research on phytase and protease activities in the catfish enzyme extract and sprouted

sorghum need to be determined for in depth understanding about the low protein digestibility.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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### REFERENCES

Abdoulaye C, Brou K, Jie C (2011). Phytic Acid in Cereal Grains: Structure, Healthy or Harmful Ways to Reduce Phytic Acid in Cereal Grains and their Effects on Nutritional Quality. Am. J. Plant Nutr. Fertilization Technol. 1:22.

Afify AEMM, El-Beltagi HS, El-Salam SMA, Omran AA (2011). Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. Plos one 6(10):e25512.

Ali H, Haque MM,Chowdhury MMR, Shariful MI (2009). In vitro protein digestibility of different feed ingredients in Thai koi (Anabas testudineus). Bangladesh Agric. Univ. 7:205-210.

AOAC (2002). Official Methods of Analysis of the OAOC. 17th ed., Gaitihersburg ,Maryland,USA.

Bedford MR, Partridge GG (Eds.) (2010). Enzymes in Farm Animal Nutrition, 2nd Edition, CAB International

Chow KW, Schell WR (1980). The minerals. In Aquaculture Development and Coordination Program.Fish feed technology, Lectures presented at the FAO/UNDP Training Course in Fish Feed Technology, Seattle, Washington, FAO, ADCP/REP/80/11. pp. 400.

Dechavez RB, Serrano Jr. AE (2012). *In vivo* and *in vitro* digestibility of plant ingredients and diets by Bacillus phytases in tilapia, *Oreochromis mossambicus*. Extreme Life Biospeol. Astrobiol. 4:48-55.

FAO (2009). Fish as feed inputs in Aquaculture: practices,sustainability and implications: a global synthesis. FAO, Rome,Italy.

Gabriel UU, Akinrotimi OA, Bekibele DO, Onunkwo DN, Anyanwu PE (2007). Locally produced fish feed: potentials for aquaculture development in sub-saharan Africa. Afr. J. Agric. Res. 2:287-295.

Gilani GS, Cockell KA, Sepehr E (2005). Effects of antinutritional factors on protein digestibility and amino acid availability in foods. J. AOAC Int. 88:967-87.

Hecht T (2006). Regional Review on Aquaculture Development 4. Sub-Saharan Africa -2005, FAO Fisheries Circular Rome FAO 96 p.

Hidvegi M, Lasztity R (2002). Phytic acid content of Cereals and Legumes and Interactions with Proteins. Periodica Polytechnica Ser .Chem. Eng. 40:59-64.

Homeoffice (2014). Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 Crown copyright, London, Uk.

Kim T, Mullaney EJ, Porres JM, Roneker KR, Crowe S, Rice S, Ko T, Ullah AHJ, Daly CB, Welch R, Lei XG (2006). Shifting the pH profile of Aspergillus niger PhyA phytase to match the stomach pH enhances its effectiveness as an animal feed additive. Appl. Environ. Microbiol. 72:4397-403.

Kumar V, Barman D, Kumar K, Kumar V, Mandal SC, De Clercq E (2012a). Anti-nutritional factors in plant feedstuffs used in aquafeeds. World Aquac. 43(3):64.

Kumar V, Sinha K, Makkar HPS, De Boeck G, Becker K (2012b). Phytate and phytase in fish nutrition. Nutr. 96:335-364.

Lazo JP (1994). Evaluation of several in vitro enzyme assays for estimating *in vivo* apparent protein digestibility by the Pacific white shrimp (Penaeus cannamel), Institute Technologic de Estudios Superiores Monterrey, Mexico. P 63.

Ravindran V (2013). Feed enzymes: The science, practice and metabolic realities. Poultry Science Association, Inc. DOI: 10.3382/japr.2013-00739.

Reddy NR, Pierson MD, Sathe SK, Salunkhe DK (1989). Phytates in Cereals and Legumes. CRC Press, Inc.Boca Raton Florida. P 158.

Serraino MR, Thompson LU (1984). Removal of Phytic Acid and Protein-phytic acid Interactions in Rapeseed. J. Agric. Chem. 32:38-40.

Sultana Z, Ahamed MS, Iqball MS, Chisty MAH (2010). Determination of in-vitro Protein Digestibility of different Feed ingredients for Nile Tilapia (Oreochromis nilotica). Bangladesh Res. Publication J. 4:87-94.

Tudkaew J, Gabaudan J, Phromkunthong W (2008). The supplementation of phytase RONOZYME P on the growth and the utilisation of phosphorus by sex-reversed red tilapia (*Oreochromis niloticus Linn.*). Songklanakarin J. Sci. Technol. 30:17-24.

Wheeler EL, Ferrel RE (1971). A method for phytic acid determination in Wheat and Wheat Fractions, in: A. R. S. Western Region Research Laboratory, US Department of Agriculture,Albany,Calfonia. (Ed.), 54th Annual meeting, American Association of Cereal Chemists, Chicago III. pp. 312-320.

World Bank (2007). Changing the Face of the Waters. The promise and challenges of Sustainable Aquaculture., Agriculture and Rural Development ,Washington, DC.

World Bank (2013). FISH TO 2030. Prospects for Fisheries and Aquaculture, World Bank report Number 83177-GLB,Agriculture and Enviromental Services Discussion Paper 03, World Bank. P 102.

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*Full Length Research Paper*

# Haematological profile of *Heterobranchus bidorsalis* fingerlings fed processed *Delonix regia* seeds at different inclusion levels of diets

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**This study aimed at investigating the haematological profile of *Heterobranchus bidorsalis* fingerlings fed processed *Delonix regia* seeds at different inclusion levels of diets. Ten isonitrogenous diets (40% crude protein) were formulated with processed *D. regia* seed at 0% (Control), 10, 20 and 30% inclusion, respectively. The parameters analysed were pack cell volume (PCV), red blood cell (RBC), white blood cell (WBC), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC). Different among the groups were tested using analysis of variance. Raw *Delonix regia* seed meal had significant effect (P<0.05) on RBC, HB, MCV, MCH and MCHC respectively across the dietary treatments. RBC, MCV, MCH and MCHC differs significantly (P<0.05) across the dietary treatments for fish fed fermented *D. regia* seeds. All the haematological parameters differ significantly (P<0.05) across the dietary treatments with the exception of PCV, MCV and MCHC respectively for fish fed cooked *D. regia* seeds. It is therefore concluded that significant variations exist among the processing methods on the health status of the fish. It is recommended that inclusion of *D. regia* up to 20% will have no deleterious effect on their health status.**

**Key words:** Haematological profile, *Delonix regia*, processing methods, *Heterobranchus bidorsalis*, inclusion levels.

## INTRODUCTION

Fish is an important source of high quality protein, providing approximately 16% of the animal protein consumed by the world's population (FAO, 1997). Fish evolved after several years of genetic improvement, and their relevance and success as a relatively cheaper and steady source of animal protein hinges on their higher carcass yield. Much progress in the productivity indices of fish are now achieved through improvement of several environmental factors regarding their growth, health and maintenance. Among the Clariidae family, *Heterobranchus bidorsalis* is the second most important aquaculture species in Nigeria (Vanden Bossche and Bernacsek, 1990). *H. bidorsalis* is endemic to Africa and recent interest in culturing its species has been rising. *H.*

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bidorsalis, an active swimmer and predator (Fagbenro, 1992) which like all chariid catfishes is capable of aerial respiration. This species is found in turbulent or fast running streams, it feeds on fish, molluscs and alarming number of people, mostly in developing crustaceans. It attains a considerable size of over 120 cm. Physiological, including hematological, behavioral and biochemical parameters are useful diagnostic tools in the practice of veterinary medicine (Lemma and Moges, 2009). Haematological parameters are good indicators of the physiological status of animals under different conditions (Ambore et al., 2009). Heamatological studies are important when evaluating fish health diagnostically just as it is important in human health. Sampath et al. (1993) observed that studies on fish blood could reveal conditions within the body of fish long before an outward manifestation of disease or stress condition. Many hematological parameters can be used to assist in providing evidence and possible identification of any abnormality or disease condition. Hematological parameters have been acknowledge as valuable tools for monitoring fish health, confirming maturation and monitoring any changes in the quality of feed, water and related soil (Kumar et al., 2011). Low heamatological indices are indications of anemic conditions (Haruna and Adikwu, 2001). The quest for more economically viable, palatable and environmentally friendly feed among the fish farmers is highly desirable. This has redirected research interests toward the use of unconventional protein sources especially from plant products like leaves, seeds and other agricultural by products (Ali et al., 2003; Bake et al., 2009). In Nigeria, the high cost of formulated commercial fish feed is a major constraint to the growth and expansion of the aquaculture sector and this has prompted a concerted effort to seek for alternative feed ingredients. Hence, the objective of this study was to evaluate the haematological profile of *H. bidorsalis* fingerlings fed processed *Delonix regia* seeds at different inclusion levels of diets.

## MATERIALS AND METHODS

The study was conducted at the aquaculture production technology unit of the skill acquisition and development centre, National Agricultural Extension and research Liason Services, Ahmadu Bello University, Zaria, located at latitude 11° 09 45.2 N and longitude 7° 38 17.9 E.

Matured and dry pods of *D. regia* containing the seeds were collected from the annex campus of Nuhu Bamalli Polytechnic Zaria. Seeds were collected by opening the pods manually.

### Fermentation of *D. regia* seeds

The seeds were soaked in water for 12 h. The drained soaked seeds were allowed to ferment naturally by tying in polythene bag and kept in a dark cupboard for 72 h without the addition of yeast (Udensi et al., 2006). The fermented seeds were allowed to air dry for two days before grinding into homogenous powder using a hammer mill.

### Cooking of *D. regia* seeds

The seeds were boiled at 100°C for 80 min and were allowed to cool by sun drying and later grounded to homogenous powder using a hammer mill (Bake et al., 2013).

### Raw *D. regia* seeds

The raw seeds were sundried for two days and were milled into a homogenous powder using a hammer mill.

### Determination of haematological parameters of experimental fish

At the end of 26 weeks of feeding trials, a total of ninety fishes were randomly selected from the ten treatments used in this study. Nine fish was selected per treatment. The blood was collected from live fish by putting it on a tray. It was handled carefully to minimize stress. A damp cloth was used to cover the head of the fish. Blood was collected in the morning hours to avoid diurnal variation. The blood was drawn from the caudal vein using syringe. The collected blood was transferred from the syringe into an anti- coagulant, ethylene diaminetetraacetic acid (EDTA) bottles for heamatological analysis.

### Heamatological procedures

All the heamatological parameters were determined using standard techniques. The heamatological parameters determined include red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV) and hemoglobin (HB).

### Determination of red blood cell rbc and white blood cell (WBC) counts

Red blood cell counts (RBC) and white blood cell count (WBC) were determined by use of the Neubeur improved counting chamber (Kelly, 1979). The blood was diluted 1:200 with Dacies fluid (99 ml of 3% aqueous solution of sodium citrate and 1 ml of 40% formal dehydrate). This keeps and preserves the shape of red blood cell which was then estimated using the counting chamber for RBC. For the total white blood cell count the dilution was 1:20 using 2 to 3% aqueous solution of acetic acid to which a tinge of Gentian violet was added. The blood smear was stained using Wright-Giemsa stain, a total of 100 white blood cells were enumerated and differentiated (Schalm et al., 1975).

### Determination of packed cell volume (PCV)

The packed cell volume was determined using a micro heamatocrit centrifuge. The blood was placed into capillary tubes and filled to ¾ of the tubes; one end was sealed with plasticine. They were centrifuged for 5 min at 12,000 rpm. The PCV was read by the use of heamatocrit reader.

### Estimation of heamoglobin (HB)

The heamoglobin was estimated using the Cyanomethaemoglobin method as described by Schalm et al. (1975) and Kelly (1979). 0.02 ml of blood was added to 4 ml of modified Dabkin's solution (Potassium ferricyanide - 200 mg; potassium cyanide - 50 mg, potassium dihydrogen phosphate - 140 mg).

**Table 1.** Means ±standard error of heamatological profile in *Heterobranchus bidorsalis* fed raw *Delonix regia* seeds meal at different inclusion levels of diet.

| Parameter | 0% (Control) | $R_{10}$ | $R_{20}$ | $R_{30}$ | LOS |
|---|---|---|---|---|---|
| PCV (%) | 51.00±1.00$^{a}$ | 49.6±0.10$^{a}$ | 49.4±0.53$^{a}$ | 49.0±2.65$^{a}$ | 0.41$^{ns}$ |
| RBC ($10^{6}mm^{-3}$) | 4.20±0.20$^{a}$ | 3.13±0.15$^{b}$ | 3.00±1.00$^{b}$ | 2.60±0.20$^{b}$ | 0.03* |
| WBC ($10^{9}$/L) | 6.80±0.60$^{a}$ | 6.00±1.00$^{a}$ | 6.23±0.25$^{a}$ | 6.87±0.35$^{a}$ | 0.31$^{ns}$ |
| HB (g/dL) | 16.00±1.00$^{a}$ | 14.80±0.20$^{ab}$ | 14.40±0.40$^{b}$ | 14.20±0.80$^{b}$ | 0.05* |
| MCV (fl) | 121.43±0.77$^{c}$ | 160±10.00$^{b}$ | 164.67±6.42$^{b}$ | 188.46±0.04$^{a}$ | <0.0001** |
| MCH (Pg) | 38.10±0.10$^{c}$ | 47.74±0.02$^{b}$ | 48.00±2.00$^{b}$ | 54.62±0.02$^{a}$ | <0.0001** |
| MCHC (g/dL) | 31.37±0.03$^{a}$ | 29.84±0.03$^{b}$ | 29.15±0.03$^{c}$ | 28.98±0.03$^{d}$ | <0.0001** |

$^{abc}$Means with different superscripts across the treatments differs significantly (P<0.05). ns, Not significant; PCV, packed cell volume; WBC, white blood cell; RBC, red blood cell; HB, haemoglobin; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

The volume was made up to 1 L with distilled water. The mixture was allowed to stand for 3 min and the HB concentration was read photonutrically by comparing with a cyanomethaemoglobin standard with a yellow- green filter at 625 mm.

Erythrocytes indices which include mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC) were calculated using the following formulae (Jain 1986; Adeyemo et al., 2007).

$$\text{MCV (fl)} = \frac{\text{PCV}}{\text{RBC}} \text{ X } 10$$

$$\text{MCH (pg)} = \frac{\text{Hb}}{\text{RBC}} \text{ X } 10$$

$$\text{MCHC (\%)} = \frac{\text{Hb (100mg blood)}}{\text{PCV}} \text{ X } 100$$

**Determination of physicochemical parameters of experimental water**

Water temperature was recorded daily in the morning using thermometer. Hydrogen ion concentration (pH) was taken with a pH meter model pH - 009(111). Dissolved oxygen (DO) was recorded using a dissolved oxygen model meter - DO 510. Turbidity of the water was determined by the method of AOAC (2003).

**Statistical model**

Model equation for analysis of variance used in this study includes:

$Y_{ijkl} = \mu + T_i + W_{ij}$

Where, $\mu$ is the effect population mean; $T_i$ is the effect of treatments (Processing methods = cooking and fermentation), and $W_{ij}$ is the random error associated with the record heamatology of the $l^{th}$ fish.

**Data analysis**

Data obtained were subjected to one way analysis of variance (ANOVA) using general linear model (GLM of SAS 9.2, 2008). Duncan multiple range test (DMRT) was used to test difference between levels of means and mean separation was considered significant at P<0.05.

## RESULTS

Table 1 shows the effect of raw *D. regia* at different inclusion levels of diets on the haematological parameters of *H. bidorsalis* fish. Raw *D. regia* seed meal had significant effect (P < 0.05) on RBC, HB, MCV, MCH and MCHC respectively across the dietary treatments. PCV and WBC were statistically similar (P > 0.05) across the dietary treatments. The fish fed control diet had higher numerical values (51.00 ± 1.00%) as compared to other dietary treatments for packed cell volume. The control group had significantly (P < 0.05) the highest volume of red blood cell as compared to $R_{10}$, $R_{20}$ and $R_{30}$ respectively. Fish fed raw *D. regia* seed meal at 30% inclusion level had highest concentration of WBC (6.87± 0.35) while the least concentration was recorded in $R_{10}$ (6.00 ± 1.00 $10^{9}$/L).

Haemoglobin levels were higher in fish fed the control and $R_{10}$ diets (16.00 ± 1.00 and 14.80 ± 0.20 g/dl) which were statiscally different (P < 0.05) from fish fed raw *D. regia* at 20 and 30% inclusion levels 14.40 ± 0.40 and 14.20 ± 0.80 g/dl). Fish fed raw *D. regia* at 30% inclusion level had the highest quantity of MCV (188.46±0.04 fl) while the control group recorded the least value (121.43 ± 0.77 fl). $R_{30}$ had the highest concentration of MCH (54.62 ± 0.02) which was statistically significant (P < 0.05) from the control, $R_{10}$ and $R_{20}$ respectively. Fish fed the control diet (31.37 ± 0.03 g/dl) had the highest concentration of MCHC which was statistically different from control, $R_{10}$, $R_{20}$ and $R_{30}$, respectively.

The effect of fermented *D. regia* seed meal at different inclusion levels of diet on the haematological parameters of *H. bidorsalis* are shown in Table 2. RBC, MCV, MCH and MCHC differs significantly (P < 0.05) across the dietary treatments. PCV, WBC and HB were statistically similar across the dietary treatments.

Fish fed fermented *D. regia* seed meal at 10% inclusion level had higher numerical concentration of PCV (51.50 ± 0.20%), WBC (7.00 ± 1.00 $10^{9}$/L) and HB (17.27 ± 0.35 g/dL) across the dietary treatments, though they were similar (P > 0.05) across the treatments.

**Table 2.** Means ±standard error of heamatological profile in *H. bidorsalis* fed fermented *D. regia* seeds meal at different inclusion levels of diet.

| Parameter | 0% (Control) | $F_{10}$ | $F_{20}$ | $F_{30}$ | LOS |
|---|---|---|---|---|---|
| PCV (%) | 51.00±1.00$^{a}$ | 51.50±0.20$^{a}$ | 51.10±0.10$^{a}$ | 50.00±5.00$^{a}$ | 0.90$^{ns}$ |
| RBC ($10^6$mm$^{-3}$) | 4.20±0.20$^{a}$ | 4.27±1.15$^{a}$ | 4.20±0.10$^{a}$ | 3.60±0.05$^{b}$ | <0.0001** |
| WBC ($10^9$/L) | 6.80±0.60$^{a}$ | 7.00±1.00$^{a}$ | 6.67±0.35$^{a}$ | 6.00±0.50$^{a}$ | 0.34$^{ns}$ |
| HB (g/dL) | 16.00±1.00$^{a}$ | 17.27±0.35$^{a}$ | 16.60±0.40$^{a}$ | 16.33±0.65$^{a}$ | 0.19$^{ns}$ |
| MCV (fl) | 121.43±0.77$^{b}$ | 119.77±0.03$^{c}$ | 121.67±0.03$^{b}$ | 138.89±0.04$^{a}$ | <0.0001** |
| MCH (Pg) | 38.10±0.10$^{d}$ | 40.23±0.07$^{b}$ | 39.52±0.08$^{c}$ | 45.28±0.04$^{a}$ | <0.0001** |
| MCHC (g/dL) | 31.37±0.03$^{c}$ | 33.59±0.50$^{a}$ | 32.29±0.03$^{b}$ | 32.63±0.06$^{b}$ | <0.0001** |

$^{abc}$Means with different superscripts across the treatments differs significantly (P<0.05). ns, Not significant; PCV, packed cell volume; WBC, white blood cell; RBC, red blood cell; HB, haemoglobin; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

**Table 3.** Means ±standard error of heamatological profile in *H. bidorsalis* fed cooked *D. regia* seeds meal at different inclusion levels of diet.

| Parameter | 0% (Control) | $C_{10}$ | $C_{20}$ | $C_{30}$ | LOS |
|---|---|---|---|---|---|
| PCV (%) | 51.00±1.00$^{b}$ | 52.50±0.25$^{a}$ | 52.37±1.00$^{a}$ | 52.00±1.00$^{a}$ | 0.01** |
| RBC ($10^6$mm$^{-3}$) | 4.20±0.20$^{a}$ | 4.40±0.30$^{a}$ | 4.30±0.20$^{a}$ | 4.30±0.20$^{a}$ | 0.82$^{ns}$ |
| WBC ($10^9$/L) | 6.80±0.60$^{a}$ | 6.40±0.20$^{a}$ | 6.77±0.25$^{a}$ | 6.90±0.20$^{a}$ | 0.39$^{ns}$ |
| HB (g/dL) | 16.00±1.00$^{b}$ | 17.6±0.40$^{a}$ | 17.6±1.00$^{a}$ | 17.20±0.30$^{ab}$ | 0.09$^{ns}$ |
| MCV (fl) | 121.43±0.77$^{ab}$ | 119.32±0.03$^{c}$ | 121.86±0.04$^{a}$ | 120.93±0.03$^{b}$ | 0.0002** |
| MCH (Pg) | 38.10±0.10$^{a}$ | 40.0±5.00$^{a}$ | 40.93±0.04$^{a}$ | 40.0±1.00$^{a}$ | 0.60$^{ns}$ |
| MCHC (g/dL) | 31.37±0.03$^{d}$ | 33.52±0.03$^{b}$ | 33.59±0.02$^{a}$ | 33.08±0.02$^{c}$ | <0.0001** |

$^{abc}$Means with different superscripts across the treatments differs significantly (P<0.05). ns, Not significant; PCV, packed cell volume; WBC, white blood cell; RBC, red blood cell; HB, haemoglobin; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Fish fed the control diet was significantly ($P < 0.05$) higher (4.20 ± 0.20 $10^6$ mm$^{-3}$) than fish fed fermented *D. regia* seed meal at 10, 20 and 30% inclusion levels respectively. Fish fed 30% inclusion level of fermented *D. regia* seed meal had the highest concentration of MCV (138.39) and MCH (45.28 ± 0.04 Pg) which differs significantly from other dietary treatments. Fish fed 10% inclusion level of fermented *D. regia* seed meal had significantly ($P < 0.05$) higher concentration (33.59 ± 0.50 g/dL) of MCHC from fish fed control diet, $F_{20}$ and $F_{30}$ respectively.

The effect of cooked *D. regia* seed meal at different inclusion levels of diets on the haematological parameters of *H. bidorsalis* are presented in Table 3. All the haematological parameters differs significantly ($P < 0.05$) across the dietary treatments with the exception of PCV, MCV and MCHC respectively. The highest concentration of PCV was recorded in the fish fed cooked *D. regia* seed meal at 10, 20 and 30% inclusion levels respectively which were statistically different ($P < 0.05$) from the control (51.0± 1.00%). $C_{20}$ and the control group had the highest levels of MCV (121.86 ± 0.04 and 121.43 ± 0.77 fl) while $C_{10}$ recorded the least concentration (119.32± 0.03 fL). Fish fed cooked *D. regia* seed meal at 20% inclusion levels had the highest quantity (33.59 ± 0.02 g/dL) of MCHC which was statistically different ($P < 0.05$) from fish fed diets containing cooked *D. regia* seed meal at 0, 10 and 30% inclusion levels.

## DISCUSSION

The use of heamatological parameters for on the spot assessment of health status of few tropical African catfish species are well documented (Fagbenro et al., 2013; Etim et al., 1999). The result of PCV (49.0 to 52.50%) obtained in this study were higher than the 28.3 to 29.5% reported by Gayatri and Prafulla (2012) for *Claris batrachus.* The difference could be due to differences in species and *H. bidorsalis* seems to have more blood volume than the other species. The result of the RBC count of this work which ranged between 2.60 × $10^6$/mm$^3$ and 4.40 × $10^6$ mm$^3$ was within the range of 2.41 to 2.89 × $10^6$ mm$^3$ reported by Gayatri and Prafulla (2012) but lower than the range of 5.05 ± 0.17 × $10^6$ to 5.2 ± 0.26 × $10^6$ mm$^3$ as reported by Onyia et al. (2013).

The higher values of RBC count in the fish fed cooked and fermented *D. regia* seeds could be linked to the

higher activity of the seeds during processing which degraded the antinutritional factors in the seed. The white blood cell count in this study (6.00 - 7.00 × $10^3$/mm of blood) was lower than the range of 8.59 ± 0.27 × $10^3$ and 9.71 ± 0.43 × $10^3$/mm of blood reported by Gayatri and Prafulla (2012) in *Clarias betrachus* (Linnaeus 1758).

The result of Hb count (14.20 - 17.60 × $10^3$/mm of blood was higher than the 8.70 g/100 ml for *Clarias gariepinus* (Sowunmi, 2003; Gayatri and Prafulla, 2012). The MCV value reflects the size of red blood cells by expressing the volume occupied by a single red blood cell. The higher MCV and MCH values in fish fed raw *D. regia* seed meal as compared to fish fed cooked and fermented *D. regia* seed meal indicates higher likelihood of occurrence of macrocytic anaemia in fish fed raw *D. regia* seed. The range of 28.98 to 33.59 g/dl in HB was similar to the range of 32.41 ± 0.40 to 32.79 ± 0.59) in male and female *C. batrachus* as reported by Gayatri and Prafulla (2012). The higher concentration of MCHC in the fish fed cooked and fermented *D. regia* seeds implies more HB in a unit of RBCs (Robbins, 1974) as compared to fish fed raw *D. regia* seed meals.

## Conclusion

It is therefore concluded that significant variations existed among the processing methods on the health status of the fish, though values were within the normal range reported for healthy fish. It is recommended that inclusion of *D. regia* up to 20% will have no deleterious effect on their health status.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### REFERENCES


Adeyemo OK, Agbede SA, Okinyan A, Shoaga OA (2007). The haematological response of *Clarias gariepinus* to changes in acclimation temperature. Afr. J. BioI. Res. 6:105-108.

Ali A, Al-Asga NA, Al-Ogaily SM, Ali S (2003). Effect of feeding different levels of alfalfa meal on the growth performance and body composition of Nile tilapia *(oreochronius niloticus)* fingerlings. Asian F. Soc. 16:1-2.

Ambore B, Ravikanth K, Maina S, Rekhe DS (2009). Haematological profile and growth performance of goat under transportation stress. Vet. World 5:195-198.

AOAC (Association of Official Analytical Chemist) (2003). Official methods of analysis. 17th ed. Washington DC, USA.

Bake GG, Adejumo TM, Sadiku SOE (2013). Growth performance and nutrient utilization of Nile Tilapia (*Oreochromis niloticus*) fed toasted flamboyant seed meal (*Delonix regia*). Cont. J. Agric. Sci. 7(1):1-10.

Bake GG, Endo A, Akimoto A, Takwenchi T (2009). Evaluation of recycled food waste as a partial replacement of fishmeal in the diets for first feeding Nile tilapia *Oreochromis niloticus* F. Science 75:1275-1283.

Etim L, Lebo PE, King RP (1999). The dynamics of an exploited population of a siluroid catfish (*Schilbe intermedius Ruppell*, 1832) in the Cross River, Nig. Fish. Res. 40:295-307.

Fagbenro OA (1992). The dietary habits of the clarid catfish, *Heterobranchus bidorsalis* (Geoffroy St Hillaire 1809) in Owena Reservior, Southwestern Nigeria, Trop. Zool. 5(1):11-17.

Fagbenro OA, Adeparusi EO, Jimoh WA (2013). Haematological Profile of Blood of African Catfish (*Clarias gariepinus*, Burchell 1822) fed Sunflower and Sesame Meal Based Diets. J. Fish. Aqua. Sci. 8:80-86.

FAO (1997). Food and Agriculture Organization of the United Nations. Review of the State of World Aquaculture. FAO Fisheries Circular No. 886 Rev.1. Rome, Italy.

Gayatri A, Prafulla KM (2012). Heamatological and serum biochemical parameters in different sexes of walking catfish, *Clarias batrachus*. Int. J. Sci. Res. 3(8):1914-1917.

Haruna AD, Adikwu II (2001). Hematological response to non- familiar diets; A study of *Clarias gariepinus.* J. Arid Zone Fish 1:12-22.

Jain NC (1986). Schalm's veterinary Hematology 4th edition. Lea and Febiger, 600. Washington Square, Philadelphia, USA.

Kelly WR (1979).Veterinary Clinical Diagnosis (2nd Edition). Balliere, Tindall, London.

Kumar B, Rita S, Mukherjee D (2011). Geochemical distribution of heavy metals in sediments from sewage fed fish ponds from Kolkata wetlands. India. Chem. Speciation Bioavailability 23(1):24-32.

Lemma A, Moges M (2009). Clinical, haematological and serum biochemical reference values of working donkeys (*Equus asinus*) owned by transport operators in Addis Ababa, Ethiopia. Livest. Res. Rur. Dev. 12:36-38.

Onyia LU, Micheal KG, Ekoto B (2013). Haemotological profile, blood group and genotype of *Heterobranchus bidorsalis.* Net J. Agric. Sci. 1(2):69-72.

Robbins CR (1974). The validity and status of the roundscale spear fish, *Tetrapturus georgei*. Proceedings of 1st International Billfish Symposium. Pp. 54- 61.

Sampath K, Velammal S, Kennedy IJ, James R (1993). Haematological changes and their recovery in *Oreochomis mossambicus* as a function of exposure period and sublethal levels of Ekalux. Acta Hydrobiol. 35:73-83.

SAS (2008). SAS / STATE User's guide, release 9.2 edition pub. SAS Institute Inc, SAS Circle, Cary, NC.

Schalm OW, Jain ON, Carrol EJ (1975). Veterinary Haematology. 3rd Edition., Lea and Febiger, Philadelphia, pp. 144- 156.

Sowunmi AA (2003). Haematology of the African catfish, *Clarias gariepinus* (Burchell, 1812) From Eleyele Reservoir Ibadan, Southwest Nigeria. Zool. 2(1):85-91.

Udensi EA, Okonronkwo KA (2006). Effects of fermentation and germination on the physicochemical properties of *Mucuna cochinchnensis* protein isolates. Afr. J. Biol. 5(10):896-900.

Vanden Bossche JP Bernacsek GM (1990). Source book for the inland fishery resources of Africa: 2, CIFA technical paper No. 18.2. Rome: FAO, 411p.

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