Effects of Dietary Protein Levels on Proximate, Haematological and Leukocyte Compositions of *Clarias gariepinus*  

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**ABSTRACT:** Study was conducted to evaluate effects of feeding different dietary protein levels on haematological profile and leucocyte population of *Clarias gariepinus* using net-hapa system. Catfish fingerlings (mean weight 4.50±0.01g) were randomly stocked at 20 fish per net-hapa (1m³). Five experimental diets with crude protein of 40.00%, 42.50%, 45.00%, 47.50% and 50% were formulated and fed to the fish for 24 weeks. Blood samples were collected and examined for white blood cell (WBC), red blood cell (RBC), haemoglobin (HB), haematocrit (HCT) mean corpuscle volume (MCV) mean corpuscle haemoglobin (MCH) mean corpuscle haemoglobin concentration (MCHC), Platelet (PLT), leucocytes, lymphocytes, neutrophils, monocytes, eosinophils and basophils. Results revealed a reverse relationship between haematological indices of fish and dietary protein inclusion levels. The best WBC (207x10^3/μl), RBC (4.9x10^6/μl), Hb (19.0g/dl), HCT (41.0%), MCV (149.0fl), MCH (49.35pg), MCHC (40.6g/dl) and PLT (134.0x10^3/μl) were presented in fish fed 40% protein diets. Results of leucocyte population did not follow any particular trend. A high positive correlation (r=0.9000; p<0.05) existed between the treatments in RBC, WBC, and Hb. The results conclude that 40% dietary protein inclusion is recommended for *C. gariepinus* for sound and healthy condition in floating net-hapa system.

**DOI:** DOI: [https://dx.doi.org/10.4314/jasem.v2i11.25](https://dx.doi.org/10.4314/jasem.v2i11.25)

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**Dates:** Received: 07 October 2019; Revised: 11 November 2019; Accepted: 24 November 2019

**Keywords:** Dietary protein, haematology, leucocyte, catfish health.

Dietary protein is described as the building block nutrient of the body and is given the most prominent consideration in the formulation of fish feeds. Lack of good quality feed for economic production adversely affects growth rates, disease manifestation and total harvest of fish (Alatise *et al*., 2006). The use of haematological techniques in fish study is gaining importance for toxicological research (Adewoye, 2010), environmental monitoring (Adeyemo *et al*., 2003) and assessment of fish health conditions (Ayoola, 2011) among other uses. Blood reflects both physical and chemical changes occurring in an organism. A number of haematological indices such as haematocrit, haemoglobin and total erythrocyte counts are used to assess the functional status and oxygen carrying capacity of the blood (Shah and Alhindag, 2004). Health and wellness of fish had often been reported in terms of the relationship between the length and weight increases (Keke and Anene, 2011) as well as growth performances (Effiong *et al*., 2014). Therefore, haematological analysis will enhance fish cultivation by facilitating early detection of situations of stress and or diseases that could affect production performance (Tavares-Dias *et al*., 2005). This study was therefore carried out to evaluate effects of different dietary protein levels on haematological parameters and leucocyte population of *Clarias gariepinus*. Thus justifying these effects on the health status of catfish.

**MATERIALS AND METHODS**

An outdoor concrete tank (L x B x H: 8m x 5m x 1.65m) situated at the Vika Farms Limited, Mbak Eto, Uyo, Akwa Ibom State (geographical coordinates of Latitude: 5° 30' North and Longitude: 7° 56’ East) was used for the production stage of the study. This tank was equipped with both inlet and outlet facilities and a 5,000 litre capacity overhead tank served as water reservoir. The experimental design was made up of a module consisting of 8.5 x 6.5m bamboo raft with fifteen 1.5m x 1.5m apartments fitted with fifteen 1m x 1m net-hapas constructed and placed to fit on the concrete tank. The net-hapas had top covers which prevent the caged fish from jumping out and also protect the fish from being preyed upon by aerial predators. Before the experiment commenced, the tank was properly washed and filled with water to a depth of about 1.2m. Fifteen net-hapas were fitted to the compartments representing five treatments with three replicates each. Each hapa was rigged and suspended.
at a depth of 0.75m in water. The float lines were tied
to the four corners of each compartment using kuralon
rope (No 15) as described by Orubusin (2000).

Diets Preparation and Fish Rearing: Five (5)
experimental diets with varying dietary protein levels
namely 40.00%, 42.50%, 45.00%, 47.50% and
50.00% (Table 1) were prepared based on proximate
composition of the various feedstuffs. All ingredients
were procured at the same time to avoid variations
associated with batch differences. They were carefully
weighed out, mixed, made into pellets using 2mm meat
mincer, air-dried and labelled separately according to
diets. Fingerlings of Clarias gariepinus (mean weight
of 4.5±0.10g) were obtained from the breeding tanks of
the farm, randomly selected and stocked at 20 fish
per rearing net-hapa. Fish pellets were crushed into
smaller sizes and fed five times their maintenance
requirement (3.2 x 5 x (fish weight (g)/1000)⁸
(Kumar et al., 2011) daily. This amount was divided
into three equal portions and fed at 8.00hr, 13.0hr and
18.0hr. Feeding trials lasted for twenty-four (24)
weeks after which fish were harvested and taken for
various examinations.

Table 1: Composition (%/100g) of experimental diets containing varying protein levels

| Ingredients            | 40.0% | 42.5% | 45.0% | 47.5% | 50.0% |
|------------------------|-------|-------|-------|-------|-------|
| Fishmeal               | 17.21 | 18.60 | 20.00 | 21.53 | 23.06 |
| Soybean meal           | 17.21 | 18.60 | 20.00 | 21.53 | 23.06 |
| Corn flour             | 23.97 | 18.19 | 12.43 | 06.73 | 01.12 |
| Groundnut cake         | 34.51 | 37.50 | 40.46 | 43.10 | 45.65 |
| Fish oil               | 07.00 | 07.00 | 07.00 | 07.00 | 07.00 |
| Lysine                 | 0.030 | 0.030 | 0.030 | 0.030 | 0.030 |
| Methionine             | 0.030 | 0.030 | 0.030 | 0.030 | 0.030 |
| Fish Premix*           | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 |

*1kg fish premix contains: Vitamin A=10,000,000 IU; D3=2,000,000 IU; E=23,000mg; K3=3,000mg; B1=3000mg; B2=6,000mg;
niacin=50,000mg; calcium pantothenate=10,000mg; B6=5000mg; B12=25,000mg; folic acid=1,000mg; biotin=50.0mg; cholinechloride=400,000mg; manganese=120,000mg; iron=100,000mg; copper=8,500mg; iodine=1,500mg; c
obalt=300mg; selenium=120mg; antioxidant=120,000mg.

Proximate and Physico-chemical Analysis: Proximate
analysis of feed ingredients, experimental diets, and
fish carcass was done according to standard method
(AOAC, 2004). Crude protein by micro – kjeldahl
method; crude fat by soxhlet extraction; total ash by
muffle furnace combustion; crude fiber by trichloroacetic acid method; moisture by oven – drying
to a constant weight; carbohydrate by 100 – (% protein
+ % fat + % fiber + % ash + moisture) and gross energy
using physiological fuel values of 0.2364KJ/g, 0.3954KJ/g and 0.1715KJ/g for protein, carbohydrates
and lipid, respectively (Henken et al., 1986). Physico-
chemical variables of tank water were monitored using
standard water analysis kit.

Haematological Examination: Five specimens were
randomly collected from each hapa for blood analysis.
A 5 – 10 ml blood per fish was collected from vertebral
blood vessel using 2ml EDTA treated disposable
syringes and needle. The method of blood sampling
and analysis followed the method described by
Svobodova et al. (1991). All haematological
parameters were estimated at Haematological Unit of
the University of Uyo Teaching Hospital, using
automated haematology analyzer (SYSMEX, model:
KX-21N, USA, 2012) based on the reference method
described in International Federation of Clinical
Chemists (Schwartz et al., 1985). This analyzer
provides a complete blood count with seventeen (17)
reportable parameters and white blood cells
differential, which include absolute neutrophils,
lymocytes, eosinophils, monocytes, lymphocytes and
basophils. All haematological analyses were done
within two (2) hours of blood collection.

Statistical Analysis: The SPSS Version 20.0 statistical
software was used for statistical analysis (SPSS Inc.,
Chicago, IL, USA). Data was statistically analyzed for
means ± standard error.

RESULTS AND DISCUSSION
The results in Table 2 showed haematological profile of
catfish after dietary treatment with different protein
diets. The counts of WBC, RBC, HB, HCT, MCV, MCHC and PLT all decreased with increase in protein
concentrations in diets. At the end of the feeding trial,
the values of all the haematological parameters
measured were statistically higher (p < 0.05) than the
initial levels. The group fed 40% protein diet had the
highest levels of all the aforementioned
haematological indices while 50% protein group had
the least values. No significant variations (p > 0.05)
were observed in WBC counts among fish treated with
42.5%, 45.0%, 47.5% and 50.0% protein diets. Similar
performances were observed in RBC, MCV and PLT
counts. The results of bivariate analysis indicated a
strong positive correlation between MCHC and WBC
(r = 0.904); HGB (r = 0.944); HCT (r = 0.927) and
between RBC and MCV (r = 0.966); PLT (r = 0.991)
and between HCT and HGB (r = 0.912). This may
signify that there was a positive influence of dietary protein on the hematological profile of catfish. The results of proximate compositions are presented in Table 3. Carcass protein of fish fed all the test diets were a reflection of protein contained in respective diets with significant differences (p < 0.05) among treated groups. The highest level (20.5%) was recorded in group fed 50% protein level. Carcass lipid increased linearly with associated increase in dietary protein from 3.52% in 40% protein diet to 5.83% in 50% protein diet. In contrast, whole-body moisture was highest (75.07%) in 40% protein diet and lowest (69.45%) in 50% protein diet. Ash content of fish tissue did not show any particular relationship with protein levels in diets. The result of bivariate analysis of the whole body composition of catfish revealed a strong positive correlation between whole body moisture and crude protein (r = 0.967), tissue lipid (r = 0.855) and gross energy (r = 0.960) at 5% probability level.

### Table 2: Haematological profile of Clarias gariepinus fed diets containing varying protein levels.

| Parameters | Initial | 40% | 42.5% | 45% | 47.5% | 50% |
|------------|---------|-----|-------|-----|-------|-----|
| WBC (x10³/µl) | 156.5±0.2⁹ | 207.5±0.3⁹ | 206.5±0.3⁹ | 202.5±0.2⁹ | 200.6±0.6⁹ | 187.5±4.55⁹ |
| RBC (x10³/µl) | 2.48±0.01⁹ | 4.90±0.00⁹ | 4.85±0.05⁹ | 4.75±0.05⁹ | 4.00±0.80⁹ | 3.05±0.05⁹ |
| Hb (g/dl) | 10.6±0.1⁹ | 19.0±0.3⁹ | 18.05±0.15⁹ | 16.85±0.55⁹ | 14.85±1.55⁹ | 13.3±10⁹ |
| HCT (%) | 35.0±0.00⁹ | 41.0±0.4⁹ | 40.05±0.35⁹ | 39.05±0.25⁹ | 38.05±0.65⁹ | 35.95±0.55⁹ |
| MCV (fL) | 120±0.3³ | 149.0±0.3³ | 146.8±1.6⁰ | 143.9±3.6⁰ | 135.8±7.5⁰ | 119.85±1.5⁰ |
| MCH (pg) | 44.53±0.05⁹ | 49.35±0.05⁹ | 47.8±0.5⁰ | 46.35±0.95⁹ | 49.7±2.4⁰ | 45.45±2.0⁵ |
| MCHC (g/dl) | 36.05±0.01⁹ | 40.6±0.1⁹ | 40.05±0.25⁹ | 39.45±0.25⁹ | 38.6±1.1⁹ | 36.00±0.20⁹ |
| PLT (x10³/µl) | 134.0±1.0⁹ | 132.15±0.85⁹ | 129.0±1.0⁹ | 117.5±12.5⁹ | 103±2.0⁹ |

Values are mean ± standard error. Means with different superscript letters within a row are significantly different (p < 0.05).

### Table 3: Proximate carcass composition (% wet weight) of experimental fish

| Parameters | Different crude protein diets | 40% CP | 42.5% CP | 45% CP | 47.5% CP | 50% CP |
|------------|-----------------------------|--------|----------|--------|----------|--------|
| Moisture   | 75.4⁴ | 74.0±0.42⁴ | 73.02±0.36⁴ | 72.23±0.2⁴ | 71.21±0.4③ | 69.45±0.5③ |
| Ash        | 3.42⁻ | 3.13±0.22⁻ | 2.87±0.08⁻ | 2.82±0.13⁻ | 2.42±0.02⁻ | 3.16±0.4±⁻ |
| Protein    | 15.3⁵ | 16.83±0.27⁵ | 17.68±0.33⁵ | 17.68±0.3⁵ | 18.88±0.3⁵ | 20.25±0.4³ |
| Lipid      | 3.34⁶ | 3.52±1.0⁶ | 4.45±0.12⁶ | 5.01±0.14⁶ | 5.72±0.08⁶ | 5.83±0.07⁶ |
| Fibre      | 2.3⁷ | 1.36±0.03⁷ | 1.41±0.21⁷ | 1.38±0.20⁷ | 1.25±0.29⁷ | 1.05±0.12⁷ |
| Carbohydrate | 0.09 | 0.75±0.24 | 0.31±0.16 | 0.81±0.27 | 0.76±0.24 | 0.78±0.36 |
| Energy     | 4.96⁸ | 5.50±0.08⁸ | 5.99±0.07⁸ | 6.31±0.03⁸ | 6.86±0.05⁸ | 7.02±0.04⁸ |

Values are mean ± standard error. Means with different superscript letters within a row are significantly different (p < 0.05).

### Table 4: Variations in absolute values of white cells (x10³/µl) of African catfish fed diets containing varying protein levels.

| White cells | 40% | 42.5% | 45% | 47.5% | 50% |
|-------------|-----|-------|-----|-------|-----|
| Leukocytes  | 187.5±4.55⁹ | 200.6±0.6⁹ | 202.5±2.5⁹ | 206.5±0.5⁹ | 207.5±0.5⁹ |
| Lymphocytes | 97.85±0.6⁵ | 98.5±0.00 | 98.55±0.15 | 98.8±0.10 | 98.8±0.1 |
| Neutrophils | 0.7±0.00 | 0.80±0.20 | 0.75±0.30 | 0.85±0.15 | 1.20±0.20 |
| Monocytes   | 2.0±0.01 | 1.85±0.03 | 1.02±0.01 | 1.31±0.01 | 1.73±0.04 |
| Eosinophils | 0.62±0.30 | 0.71±0.21 | 0.82±0.41 | 0.87±0.20 | 0.90±0.32 |
| Basophils   | 0.06±0.01 | 0.06±0.02 | 0.07±0.03 | 0.08±0.01 | 0.09±0.02 |

Values are Mean ± standard error. Means with different superscript letters within a row are significantly different (p < 0.05).

The present study revealed an increased values of leucocyte counts with increase in protein levels (Table 4). From the results, all other parameters showed no significant variations (p<0.05) among test, groups. The results of the physicochemical variables revealed dissolved oxygen to range from 5.15 to 6.90 mg/l, pH 6.50 to 8.10 and water temperature between 26.8 to 28.3°C.

The present study showed that different diets produced various effects on proximate composition, haematology and leucocytes population of catfish. This might have happened possibly due to differences in dietary protein levels. Inverse relationship was observed between body moisture and lipid content. This was consistent with other reports, regardless of fish species (Ng et al., 2001 and Schulz et al., 2007). In earlier study, FAO (1999) reported that moisture and lipid contents in fish fillets are inversely related and their sum is approximately 80%. These results showed that gross body composition was influenced by the experimental diets. In contrast the study Alvarez-González et al. (2001) found that dietary protein levels had no significant effects on proximate body composition in Florida pompano, Trachinotus carolinus, and spotted sand bass respectively. A significant trend for increasing whole-body protein with increasing dietary protein levels up to 42.5%
protein was observed in the current study. This agreed with the work of Khan et al. (1993) who reported that the whole-body protein content of the Asian red tail catfish increased significantly with increasing dietary protein levels up to 42% and then decreased when fish were fed with higher protein levels. However, the opposite effect was found by (Schulz et al., 2007). Although 7% fish oil was added to each of the diets as the main lipid source, proximate analysis of the diets showed that their lipid contents increased with protein contents up to about 2%. This might have contributed to the observed linear increase in whole-body lipid levels in association with increasing dietary protein. Also, the nature of the experimental fish (being a fatty fish), has the ability to deaminate and store excess dietary protein as lipid. Similar observation was reported by Khan et al. (1993) on Asian red-tail catfish. The present study has showed that, whole body composition of African catfish could be significantly influenced by feeding with different crude protein diets. The white blood components, such as leukocytes, lymphocyte, monocytes, and neutrophil of fish or any animal is a function of the immunity and the animal's resistance to some vulnerable diseases. The levels of these parameters obtained in this study have not been associated with any detrimental health effect in catfish. This explained the efficacy of different protein diets in maintaining good and healthy condition in catfish. The counts of white cells obtained in this study were significantly higher (p < 0.05) than 52.00 x 10⁶/µl documented by (George et al., 2012) for Clarias gariepinus. The RBC is a function of oxygen absorption and transportation within a living cell, and depletion in the count may weaken and lead to death in fish. Catfish fingerlings fed all protein diets from this study, had correspondingly high RBC counts which were higher but not significantly different (p > 0.05) from 3.6 x 10⁶/µl presented by George et al. (2012) for Clarias gariepinus. Haemoglobin concentration reflects the oxygen supply in the blood and a decrease level had been associated with developing anaemia. Hence, haemoglobin concentration in the blood is a rapid method of detecting disease conditions in fish. The study showed that mean haemoglobin concentrations were high. Haematological indices (MCV, MCH and MCHC) have been reported to indicate secondary responses of an organism to irritants (O’Neal and Weirich, 2001). MCV is useful in the estimation of size of red blood cell while MCH are used to estimate the concentration of haemoglobin in fish blood and MCHC, a good indicator of red blood cell swelling (Wepener et al., 1992). A low level of MCV, MCH and MCHC signifies normal condition of the blood of the fish. In this study, the values of MCV, MCH and MCHC decreased slightly with increasing protein levels. This indicated that high protein levels favoured these indices. Thus, this study is in line with the general phenomenon that protein molecules are building block nutrients as well as excellent blood cells builder.

Conclusion: Evidences were found from the present study to suggest that at any dietary protein feeding level, there was a positive influence on the haematological profile, leucocyte population and proximate compositions of the African catfish.

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