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Effects of *Moringa oleifera* leaf meal (MOLM) based diets on carcass composition and hematology of *Labeo rohita* fingerlings

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## Abstract

A 70 - day feeding trial was carried out to study the effect of replacing the dietary fish meal with *Moringa oleifera* leaves meal (MOLM) on the carcass composition and hematological indices of *Labeo rohita* fingerlings. Five isonitrogenous diets were formulated with MOLM replacing 0%, 10%, 20%, 30% and 40% of fish meal in the diets. Fish were fed at 5% of their body weight two times daily. Fingerlings (avg. wt.  $6.61\pm0.053g$ ) were randomly distributed into tanks having 15 fish in each replicate. Results from proximate analysis showed that replacement of fish meal with MOLM upto 10% increased crude protein and crude fat in fish body as compared to fish fed on control (0%), 20%, 30% and 40% MOLM based diet, respectively. The hematological parameters of fingerlings fed 10% MOLM based diet were found to be significantly different (p<0.05) from the fish fed control diet. The red blood cells and hemoglobin of fish showed a significant (P<0.05) inverse correlation with increase in MOLM in diets. The present study showed that MOLM has good prospective for use as fish meal replacement in *L. rohita* diet up to 10% level without compromising fish performance whereas the hematological investigation indicated that inclusion of MOLM above 20% in the diets showed the hematological disturbance. On the base of this study it was concluded that fish meal can be replaced with MOLM up to 10% in the diets to increase the nutritive values of *L. rohita* fingerlings.

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## Introduction

Fish is a relatively economical source of animal protein and other essential nutrient required in human diet. The nutritional value of fish flesh comprises of moisture, dry matter, protein, lipids, vitamins, minerals and caloric value of the fish (Steften, 2006) and this is the major reason why fish is a favorite food for the entire society (Ojewole and Ammah, 2006). Fish are source of high quality protein, vitamins and essentials minerals and a rich source of omega-3 long chain poly unsaturated fatty acid (Dahi et al., 2006). Fish meat is generally a good source of vitamin B and in the case of fatty acids and vitamins (A and D). Proximate composition of food is of growing interest to consumers because of the effect of the various levels of protein, lipids, water and ash have on the storage and texture of fish. Besides being used as food, fish is also in increasing demand for use as livestock feed (Odedeyi, 2014).

One of the problem facing fish culturists is the need to obtain a balance between rapid fish growth and optimum use of the supplied feed (Gokcek et al., 2008). It means that nutritionally well-balanced diets and adequate feeding are the main requirements for successful culture operations (Aderolu et al., 2010). Protein is the most costly component of fish feeds. Therefore, efforts to reduce feed expenses have resulted in increased use of plant proteins as replacements for expensive animal ingredients, especially fish meal in diet formulations (Ping et al., 2010). It is therefore necessary to practically search, explore, identify and utilize other plant protein source which could be cost effective, less competitive, not relatively in high demand and resistant to drought compared to fish meal which is expensive and highly competitive in utilization. In past the fish feed was dependant on the use of fishmeal as a source of essential nutrients and growth factors (Zhou et al., 2004) but now increasing demand, rising prices and unstable supply of the fish meal made it necessary to search for alternative protein sources for fish feed industry (Pham et al., 2008; Lech and Reigh, 2012).

The effect of various plant sources as supplementary feed with fishmeal in aquatic diets has been investigated by a number of researchers.

Products from moringa have a wide range of applications in aquaculture, agricultural and industrial industry. Moringa leaves have a relatively high crude protein content which varies from 25% (Makkar and Becker, 1996) to 32% (Soliva et al., 2005). A high proportion of this protein is potentially available for digestion due to a high proportion of pepsin soluble nitrogen (82-91%) and low proportion (1-2%) of acid detergent insoluble protein (Makkar and Becker, 1996). The protein contains high levels of sulphur containing amino acids and compares well with soybean, which is usually regarded as a source of high quality plant protein (Francis et al., 2002). Its crude lipid fraction has a high proportion of n-3 fatty acids in the form of linolenic acid which account for almost 67% of total fatty acids (Soliva et al., 2005). The leaves of Moringa oleifera are rich in carotenoids, minerals, ascorbic acid and iron is commonly known as "The Miracle Tree; having an impressive range of medicinal uses with high nutritional value throughout the world (Martin et al., 2007).

In fish culture the fish hematology is gaining importance of fish production because of its significance in monitoring the health condition of fish (Hrubec et al., 2000). Hematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye et al., 1998). Hematological distinctiveness of most fish has been studied with the plan of establishing normal value range and difference from it may indicate a trouble in the physiological process (Rainza-paiva et al., 2000). Labeo rohita is widely considered to be one of the most important major carp (Hussain et al., 2015). It is column feeder, high growth rate and has high commercial value due to consumer's choice in subcontinent like Pakistan and India.

Less information is available for the formulation of artificial feeds for commercially important stomachless fish such as *Labeo rohita* (Cao *et al.*, 2007). Therefore, the present research was focused to determine the ability of *Moringa oleifera* leaf meal to provide as source of protein in formulated diets for *Labeo rohita* and also to assess its effects on carcass composition and hematological parameters of *Labeo rohita* fingerlings.

#### Material and methods

The present research work was carried out to study the effects of varying levels of *Moringa oleifera* leaf meal (MOLM) based diets on carcass composition and hematological parameters of *Labeo rohita* fingerlings. The experiment was conducted in the Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad.

#### Processing of Moringa oleifera leaf meal

Moringa leaves were obtained from the Garden of University of Agriculture, Faisalabad. The leaves were soaked overnight in a tank to remove saponins and other water soluble anti-nutritional factors. Soaked leaves were placed on a wire mesh to drain excess water and then spread on plastic sheets to dry under shade to avoid loss of vitamins through photodynamic damage/oxidation. The dried leaves were threshed from stalks to reduce crude fiber content in the meal. The dried leaves were then grinded into a fine powder using a hammer mill (Lab Mill, screen size 0.2 mm) and were stored in plastic bags at room temperature.

| Table 1. Ingredients Com | position (%) | test Diets |
|--------------------------|--------------|------------|
|--------------------------|--------------|------------|

## Fish and experimental conditions

Labeo rohita fingerlings were obtained from Government Fish Seed Hatchery, Faisalabad and kept in V-shaped tanks (designed for the collection of feces having 70 L water capacity) for two weeks to acclimatize them with experimental conditions. Before the start of feeding trial, Labeo rohita fingerlings were treated with NaCl (5g L-1) to make sure that the fingerlings are free from ectoparasites and to prevent further fungal infection (Rowland and Ingram 1991). During this acclimatization period the L. rohita fingerlings were given reference diet once daily to apparent satiation (Allan and Rowland 1992). Water quality parameters were monitored throughout the experimental period on daily basis. Aeration (24h) was provided to all the experimental tanks through capillary system.

#### Experimental design

*Moringa oleifera* leaf meal (MOLM) was used as test ingredient to replace costly fish meal and formulation of test diets on varying levels of replacement. The feed ingredients were bought from a commercial feed mill and analyzed for chemical composition following (AOAC, 1995) prior to the formulation of the experimental diet. The feed ingredients were grinded and sieved to require particle size before incorporation formulation of experimental diet (Table 1).

| Ingredients       | Test Diet-I<br>MOLM 0% | Test Diet-II<br>MOLM 10% | Test Diet-III<br>MOLM 20% | Test Diet-IV<br>MOLM 30% | Test Diet-V<br>MOLM 40% |
|-------------------|------------------------|--------------------------|---------------------------|--------------------------|-------------------------|
|                   | (control)              |                          |                           |                          |                         |
| Moringa leaf meal | 0                      | 10/3                     | 20/6                      | 30/9                     | 40/12                   |
| Fish meal         | 40/20                  | 34/17                    | 28/14                     | 22/11                    | 16/8                    |
| Wheat flour       | 34.50                  | 30.50                    | 26.50                     | 22.50                    | 18.50                   |
| Corn gluten (60%) | 16.6                   | 16.6                     | 16.6                      | 16.6                     | 16.6                    |
| Fish oil          | 4.9                    | 4.9                      | 4.9                       | 4.9                      | 4.9                     |
| Vitamin Premix    | 1.0                    | 1.0                      | 1.0                       | 1.0                      | 1.0                     |
| Mineral Premix    | 1.0                    | 1.0                      | 1.0                       | 1.0                      | 1.0                     |
| Ascorbic acid     | 1.0                    | 1.0                      | 1.0                       | 1.0                      | 1.0                     |
| Chromic oxide     | 1.0                    | 1.0                      | 1.0                       | 1.0                      | 1.0                     |
| Total             | 100                    | 100                      | 100                       | 100                      | 100                     |

MOLM was used in the test diets by replacing fishmeal at the rate of 0%, 10%, 20%, 30% and 40% of test diets and were fed to five fish groups stocked in water tanks having 70 L water capacity. Three replicates having 15 fingerlings in every replicate were used for each treatment. V-shaped tanks were specially designed for the collection of fecal material from water media. Fingerlings were provided with 12 h dark and night period throughout the trial. Total duration of experiment was 70 days. Carcass composition and hematological parameters of Labeo rohita fingerlings fed on MOLM based diets were compared with each other and the fish fed on control diet in relation to determine optimum level of fish meal replacement by using Completely Randomized Design (CRD).

Feed ingredients and formulation of experimental diets

The feed ingredients were purchased from a commercial feed mill and were analyzed for chemical composition following AOAC (1995) prior to the formulation of the test diets. The basal diet was prepared to supply sufficient levels of required nutrients for normal fish growth (Table 1). *Moringa oleifera* leaf meal will be added in the diets by replacing the fishmeal at the levels of 0%, 10%, 20%, 30% and 40% of test diets. All ingredients were mixed in an electric mixer for 10 minutes and fish oil was gradually added during mixing of diet. Chromic oxide (1%) was incorporated as inert marker. During mixing of ingredients 10-15 % water was also added to prepare suitable texture (Lovell 1989).

| Table 2. | Chemical | composition | (%) of t | feed ingr | edients (I | Dry matter basis). |
|----------|----------|-------------|----------|-----------|------------|--------------------|
|          |          |             | . ( )    |           |            |                    |

| Ingredients       | Dry<br>matter<br>(%) | Crude<br>Protein<br>(%) | Crude Fat Crude Fiber Ash<br>(%) (%) (%) |       | Carbohydrates | Gross Energy<br>(kcal g <sup>-1</sup> ) |      |
|-------------------|----------------------|-------------------------|--|-------|---------------|---|------|
| Moringa leaf meal | 92.84                | 27.69                   | 6.54                                     | 7.89  | 12.34         | 45.54                                   | 2.39 |
| Fish meal         | 91.63                | 47.25                   | 7.23                                     | 1.12  | 25.56         | 18.84                                   | 4.67 |
| Corn gluten 60%   | 92.06                | 58.79                   | 4.28                                     | 1.37  | 1.65          | 33.91                                   | 4.57 |
| Wheat flour       | 93.04                | 11.23                   | 2.44                                     | 3.09  | 3.16          | 80.08                                   | 3.25 |
| Rice polish       | 94.86                | 13.48                   | 13.17                                    | 12.70 | 11.09         | 49.56                                   | 3.59 |

These mixed feed ingredients were extruded to form floating pellets (3mm) through Lab Extruder (SYSLG30-IV Experimental Extruder). All diets were equally treated in the given extruder to formulate one control diet and four MOLM based test diets. All the prepared diets were dried and stored at 4°C until use.

#### Feeding Protocol

The fingerlings of *Labeo rohita* were fed at the rate of 5% of live wet weight on their prescribed diets. For each test diet, triplicate tanks were used with stocking density of fifteen fish in each.

#### Carcass Analysis

Three fish were chosen randomly at the end of the experiment randomly from each tank and subjected to chemical analysis of whole fish body. The samples of fish were homogenized by using mortar and pestle. These were analyzed by standard methods (AOAC 1995). Moisture contents were determined by ovendrying at 105°C for 12 hours whereas crude protein (N × 6.25) by micro kjeldahl apparatus. Ether extract (EE) was extracted by petroleum ether extraction method through Soxtec HT2 1045 system; crude fiber (CF) as loss on ignition of dried lipid-free residues after digestion with 1.25% H<sub>2</sub>SO<sub>4</sub> and 1.25% NaOH, whereas ash by ignition at 650°C for 12 hours in electric furnace (Eyela-TMF 3100) to constant weight. Total carbohydrates (N-free extract) were calculated by difference i.e., Total carbohydrate % = 100-(CP%+ EE%+ CF%+ Ash%+ Moisture).

## Blood collection and haematological analysis

Fish were tranquilized with 150 mg/1 solution of tricane methanesulfonate (MS222) (Wagner *et al.*, 1997) for blood collection. Blood samples were collected after 70 days of the experiment.

The blood samples were taken to the Molcare Lab, of Department Biochemistry, University of Agriculture, Faisalabad, Pakistan for haematological analysis. Haematocrit (PCV) was determined with micro haematocrit centrifuge by the Wintrobe and Westergreen method as described by Blaxhall and (1973) with commercially Daisley available heparinized capillary tubes of 25 mm. Red Blood Cell (RBC) and White Blood Cell (WBC) counts were determined with a haemocytometer with improved Neubauer counting chamber as described by Blaxhall and Daisley (1973). Haemoglobin (Hb) concentration estimates were determined as described by Wedemeyer and Yastuke (1977). The following parameters where calculated: mean corpuscular haemoglobin concentration (MCHC); mean corpuscular haemoglobin (MCH) and mean cell volume (MCV).

MCHC = Hb/PCV x 100 MCV = PCV/RBC x 10 MCH = Hb/RBC x 10

#### Statistical analysis

The results of carcass composition and hematological parameters were subjected to one way analysis of variance (ANOVA) (Steel *et al.*, 1996). Significant differences between means were determined by Tukey's Honesty Significant Difference Test and will be considered significant at p<0.05 (Snedecor and Cochran, 1991). The Co-Stat computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) will be used for statistical analysis. Table 3 shows the proximate composition of Labeo rohita (Rohu) fed varying levels of MOLM incorporated into feed in a 70 days trial. There were significant differences (p<0.05) among the fish in terms of crude protein, crude fat, ash, crude fiber and carbohydrate contents. The carcass results showed that the fish fed on dietary level of 10% MOLM based diet had highest level of crude protein (63.58%) whereas second higher value (61.89%) was recorded in fish fed at 20% replacement level of MOLM as compared to fish fed on control (59.46%). On the other hand the lowest value was recorded in fish fed on test diet 5 (56.92%). It was noted that 10% level of MOLM is able to improve the carcass protein as compared to fish fed on control diet. Lowest % crude protein and lipid was recorded in fish fed 40% of MOLM (Test diet 5). The crude fat in all fish fed were significantly different (p<0.05) from each other except fish fed on test diet 4 and fish fed on control. The highest value was observed in fish fed on10% MOLM with the value of (9.73%) as compared to fish fed on control diet (8.09%) while the lowest value was recorded in fish fed on test diet 5 (7.40%). The crude fiber in the fish fed on diets 1, 4 and 5 had no significant difference (p>0.05) while crude fiber in fish fed on 2 and 3 were significantly different (p<0.05), the lowest value was recorded in fish fed on test diet 2 (2.14%) with the highest value of (2.90%) in fish fed on diet 5. The total ash in fish fed on diet 1 and 4 showed no significant difference (p>0.05) while in fish fed on 2, 3 and 5 were significantly different (p<0.05), the best value recorded was in fish fed on diet 2 (6.98%) with the highest value of (7.83%).

## Results

**Table 3.** Carcass composition of *Labeo rohita* fingerlings fed varying levels of *Moringa oleifera* leaf meal based diets.

| Diets        | Replacement<br>Levels  | Moisture (%)                  | Protein (%)                    | Fat (%)                   | Ash (%)                       | Crude fiber<br>(%)          | Carbohydrate             |
|--------------|------------------------|-------------------------------|--------------------------------|---------------------------|-------------------------------|-----------------------------|--------------------------|
| Test Diet-I  | MOLM (0%)<br>(Control) | 9.40±0.091 <sup>c</sup>       | 59.46±0.226 <sup>c</sup>       | $8.087 \pm 0.055^{\circ}$ | 7.57±0.078°                   | $2.87 \pm 0.042^{cd}$       | 12.63±0.202 <sup>c</sup> |
| Test Diet II | MOLM (10%)             | $8.69 \pm 0.062^{a}$          | $63.58 \pm 0.328^{a}$          | 9.73±0.060 <sup>a</sup>   | $6.98 \pm 0.048^{a}$          | $2.14 \pm 0.066^{a}$        | 8.90±0.523 <sup>a</sup>  |
| Test Diet II | IMOLM (20%)            | $8.99{\pm}0.036^{\mathrm{b}}$ | $61.89{\pm}0.130^{\mathrm{b}}$ | $8.80{\pm}0.070^{b}$      | $7.26{\pm}0.048^{\mathrm{b}}$ | $2.44 \pm 0.052^{b}$        | $10.64 \pm 0.098^{b}$    |
| Test Diet IV | /MOLM (30%)            | $9.31{\pm}0.048^{c}$          | $58.16{\pm}0.206^{\rm d}$      | 7.96±0.060°               | $7.51 \pm 0.042^{\circ}$      | $2.70{\pm}0.086^{c}$        | $14.38 \pm 0.107^{d}$    |
| Test Diet V  | MOLM (40%)             | $9.67{\pm}0.130^{\rm d}$      | $56.92 \pm 0.114^{e}$          | $7.40{\pm}s0.098^{d}$     | $7.83{\pm}0.057^d$            | $2.90{\pm}0.062^{\text{d}}$ | $15.29 \pm 0.364^{e}$    |

Table 4 revealed the hematological indices of fingerlings fed Moringa oleifera leaf meal based diet during the experiment. The red blood cells (RBC) showed a decreasing trend as MOLM increased in the diet. The fish fed 10% MOLM based diet showed the highest value (2.95×106 mm<sup>-3</sup>) whereas the second highest value was recorded fish fed on control diet (2.74×103 mm<sup>-3</sup>). On the other hand the minimum value (1.50×103 mm<sup>-3</sup>) was observed in fish fed 40% MOLM based diet. The best value noted in fish fed on10% MOLM based diet was significantly different (P>0.05) from fish fed diet containing 0%, 20%, 30% and 40% MOLM. Higher white blood cells (WBC) were found in fish fed 40% to 30% MOLM based diet than fish fed 0%, 10% and 20% MOLM diet. The highest value of WBC (7.83 ×103 mm<sup>-3</sup>) was recorded in fish fed diet containing 40% MOLM which was not statistically significant (P>0.05) from the WBCs of fish fed all the remaining diets. Results of hemoglobin (Hb) showed that the fish fed 10% MOLM based diet had highest value (8.91 g/100 ml)

whereas the second higher value (8.53 g/100 ml) was noted in fish fed on 20% MOLM diet. The highest value of Hb was noted in fish fed on 10% MOLM based diet was significantly different (P>0.05) from fish fed diet containing 0% and 20%. The highest value (28.01%) of packed cell volume (PCV) was found in fish fed diet containing 20% MOLM based diet whereas the second highest value (27.77) was noted in fish fed control diet. These values were significantly different (p>0.05) from fish fed on other diets. The fish fed diet containing 30% to 40% MOLM diet showed a decreasing trend in the PCV values. The highest value (36.60%) for MCHC was recorded in fish fed diet containing 40% MOLM diet and the lowest value (27.73%) was noted in fish fed control diet. Similarly, the results obtained for MCH and MCV showed that the fish fed diet containing 40% MOLM had the highest values of 72.47 pg and 209.03 fl, respectively whereas the least values 26.71 pg and 86.49 fl were recorded in fish fed MOLM 10% diet.

**Table 4.** Hematological parameters of *Labeo rohita* fingerlings fed varying levels of *Moringa oleifera* leaf meal based diets.

| Diets            | Replace-<br>ment Levels | RBC<br>(10 <sup>6</sup> mm <sup>-3</sup> ) | WBC<br>(103mm <sup>-3</sup> )        | PLT                     | Hb<br>(g/100ml)        | PCV (%)                  | MCHC (%)                | MCH (pg)                | MCV (fl)                      |
|------------------|-------------------------|--|--------------------------------------|-------------------------|------------------------|--------------------------|-------------------------|-------------------------|-------------------------------|
| Test             | MOIN                    |  |                                      |                         | - 0                    |                          |                         |                         |                               |
| (Control)        | MOLM (0%)               | 2.74±0.01                                  | <sup>o</sup> 7.08±0.006 <sup>e</sup> | 57.92±0.07 <sup>e</sup> | 7.80±0.01ª             | 27.77±0.01 <sup>b</sup>  | 27.73±0.01 <sup>e</sup> | 27.47±0.01ª             | 97.39±0.01ª                   |
| Test Diet<br>–II | MOLM (10%)              | 2.95±0.01                                  | <sup>a</sup> 7.36±0.006 <sup>d</sup> | 63.31±0.04 <sup>d</sup> | 8.91±0.01 <sup>a</sup> | 27.00±0.01 <sup>c</sup>  | 31.60±0.02°             | 26.71±0.02 <sup>e</sup> | 86.49±0.03 <sup>e</sup>       |
| Test<br>Diet–III | MOLM<br>(20%)           | 2.04±0.01                                  | <sup>c</sup> 7.43±0.006 <sup>c</sup> | 65.40±0.02 <sup>b</sup> | $8.53 \pm 0.02^{b}$    | 28.01±0.01 <sup>a</sup>  | 33.47±0.01 <sup>b</sup> | 28.00±0.01 <sup>c</sup> | 110.08±0.03 <sup>c</sup>      |
| Test<br>Diet–IV  | MOLM<br>(30%)           | 1.77±0.01                                  | <sup>1</sup> 7.68±0.015 <sup>b</sup> | 64.70±0.05 <sup>c</sup> | 7.86±0.01 <sup>c</sup> | $25.01{\pm}0.01^{\rm d}$ | 29.42±0.01 <sup>d</sup> | $52.37\pm0.02^{b}$      | $142.84{\pm}2.5^{\mathrm{b}}$ |
| Test Diet<br>–V  | MOLM<br>(40%)           | $1.50 \pm 0.05$                            | <sup>e</sup> 7.83±0.010 <sup>a</sup> | 68.90±0.01ª             | 7.21±0.01 <sup>c</sup> | 22.01±0.01 <sup>e</sup>  | 36.60±0.01ª             | 72.47±0.03ª             | 209.03±2.6ª                   |
| RBC = Re         | ed Blood Cell,          | WBC = W                                    | hite blood cell                      | , PLT = Plate           | elet, Hb = h           | emoglobin co             | ncentration,            | PCV = Packe             | ed cell volume                |

MCHC = Mean corpuscular hemoglobin concentration, MCH = Mean corpuscular hemoglobin, MCV = Mean corpuscular volume.

## Discussion

The demand for fish is continually increasing with increase in population and the health benefits of eating fish. As a result, the aquaculture industry is becoming the fastest growing food producing sector in the world (FAO, 2006). As fish farming intensifies, fish feed industry is being challenged with providing feed that are nutritionally balanced for the utmost growth of cultured fish. The major ingredients in fish feeds are protein and energy supplement in form of fish meal. Due to high demand and low availability of fish meal, it is necessary to search alternatives of fish meal that will be locally available, lower price and has wide availability to replace costly conventional feed stuffs. *Moringa oleifera* leaf meal is used as a plant protein source as alternative of fish meal. MOLM based protein source is not only of considerably least cost than fish meal but also easily available. About 30% protein is reported in MOLM, therefore moringa is being used as an alternative plant protein source feed ingredient for fish. In our study, the proximate composition of fish fed varying levels of MOLM based diet showed that 10% MOLM based diet had highest level of crude protein (64%) as compared to control (60%) whereas the lowest value was recorded in 40% MOLM based diet (57%) which indicated that 10% MOLM based diet is good alternative source of protein. Similarly Ganzon-Naret (2014) studied different (0, 10, 20 and 30%) inclusion levels of MOLM as plant protein source and observed significant differences in crude protein and crude ash of sea bass among the various treatments. They found higher protein and ash contents in fish fed on 10% MOLM based diet. In contrast, fish fed dietary levels of 5% and 12.5% MOLM based diet had highest level of crude protein whereas fish fed on 7.5% level of MOLM inclusion also was relatively next in value (Olanivi et al., 2013). Moreover, Thiam et al. (2015) also noted higher protein contents in the fish fed on 10% MOLM based diet and that was significantly different from the fish fed on other diets.

In case of crude fat the highest value was observed in fish fed 10% MOLM with the value of (10%) as compared to value (8%) by fish fed on control diet (0% MOLM) while the lowest value (7%) was recorded in fish fed 40% MOLM level based diet. Similarly maximum fat was observed in the fish fed on 10% MOLM based meal followed by 5% MOLM based diet and different from the fish fed on other test diets (Thiam et al., 2015). In contrast Ganzon-Naret (2014) found increasing trends of fat contents in the fish as increasing MOLM levels. The carcass crude lipid showed an increasing trend as inclusion level of moringa was increased from 20 to 30 % to replace fish meal protein. According to the Madalla et al. (2013) lipid content probably decreased due to poor feed intake which resulted in starvation and in turn led to mobilization of body lipid reserves to meet energy requirements for vital body functions. In our results crude fiber in the test diets MOLM 0% (control), 30% and 40% had no significant

difference while 10% and 20% MOLM based diets were significantly different from each other. The lowest value (2%) was recorded in fish fed 10% MOLM based diet while the highest value in fish fed 40% MOLM based diet. On contradictory, higher crude fiber was recorded in Clarias gariepinus fed on 10% MOLM based diet and the lowest fiber contents were found in fish fed on 5% MOLM based diet (Olaniyi et al., 2013). In the case of total ash the lowest value (7%) was recorded was in diet 10% level of MOLM based diet with the highest value (8%) at 40% level of MOLM based diet. Lowest moisture content was found in fish fed 10 % MOLM based diet and increasing with the increase in MOLM levels. Our study is supported by Madalla et al. (2013) who observed higher body moisture content of fish when fed high levels of MOLM. Similar to our findings Olaniyi et al. (2013) observed lowest moisture contents in Clarias gariepinus fed on 10% MOLM level based test diet.

Fish hematology is gaining importance in aqua culture since of its consequence in monitoring the fish health (Hrubec et al., 2000). Hematological characteristics of most species of fish have been studied (Rainza et al., 2000). The Labeo rohita fed 10% MOLM based diet showed the highest red blood cells (RBC) (2.95×106 mm-3) whereas the minimum value (1.50×106 mm<sup>-3</sup>) was observed in fish fed 40% MOLM based diet. The best value noted in fish fed on10% MOLM based diet was significantly different (P>0.05) from fish fed on 0%, 20%, 30% and 40% MOLM. Similarly Bello et al. (2013) found decrease in RBC with increase in MOLM levels in fish feed. Reason might be the higher concentration of antimetabolite especially tannin in the diets containing more MOLM. RBC count was greater than  $1.00 \times 10^6$ mm<sup>-3</sup> 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). White blood cells (WBC) and lymphocytes are the defense cells of the body. Douglass and Janes (2010) WBC plays an important role in immune responses and increases the

ability of the animal to fight infection. In our study white blood cells (WBC) showed a decreasing trend as MOLM increased in the diet. High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system (Oyawoye and Ogunkunle, 1998). Similar results were also reported in different studies (Bello *et al.*, 2013; Dienye and Olumuji, 2014).

In our study haemoglobin (Hb) analysis showed that the fish fed 10% MOLM based diet had significantly highest value whereas the second higher value was noted in fish fed on 20% MOLM diet and was significantly different from fish fed diet containing 0% and 20%. The haemoglobin results showed a decrease as the MOLM increased in the diet. The reason for decrease in the level of haemoglobin as MOLM increased in the diet could imply that diets having higher MOLM had negative effect on the blood (Dienye and Olumuji, 2014). The highest value of packed cell volume (PCV) in current work was found in fish fed diet containing 20% MOLM based diet whereas PLT, MCHC, MCH and MCV was recorded maximum at 40% MOLM based diet. Minimum values were observed at 0% (PLT and MCHC) and 10% (MCH and MCV) MOLM based diet and are comparable with value ranges reported by previous workers (Adedeji et al., 2000; Anyanwu et al., 2011). Bello et al. (2013) and Dienye and Olumuji (2014) found similar results when fish fed on higher levels of MOLM based diets. 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).

It was concluded from the study that costly fish meal can be replaced with cost effective MOLM based diet upto 10% without any negative effect on carcass composition and hematology of fish. Best results of carcass composition and hematological indices were observed in *Labeo rohita* fingerlings fed on 10% MOLM based diet

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