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Evaluating Moringa Oleifera Leaf Meal as an Alternative to Fishmeal in the Diet of Catla Catla Fingerlings

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Abstract

Aquaculture industry is a largest industry which provides protein rich food. This industry aims to fulfill peoples dietary needs globally. Purpose of this study was to examine the effects of Moringa oleifera leaf meal (MOLM) on growth, hematological and mineral composition of Catla catla fingerlings. An experiment with 180 days feeding trials was conducted to examine the efficacy of MOLM. This experiment was performed according to semi-intensive rearing system to assess the performance of Catla catla fingerlings under MOLM diet. MOLM was used as test ingredient in experimental diets. Five varying levels of MOLM (6%, 12%, 18%, 24% and 30%) were tested to replace fishmeal. For first fifteen days, these fingerlings were placed under laboratory conditions in cemented ponds for acclimatization. When these fingerlings were fully acclimatized, then they were divided into six study groups. Among six earthen ponds, total 90 fingerlings were uniformly distributed. So, fifteen fingerlings were nourished in each pond. Data were recorded for various growth, hematological and mineral composition of fingerlings body. Analysis of recorded data revealed that 12% MOLM diet provided highest results for growth parameters as compared to controlled or other experimental diets. Highest results for hematological and body mineralization were also found in those fingerlings fed with 12% MOLM diet. In this current study, it was determined that 12% replacement of fishmeal by MOLM enhanced growth, hematological parameters and body minerals of Catla catla fingerlings.

Keywords: Aquaculture, Catla catla Fingerlings, MOLM, Alternative Fishmeal Sources.

1- INTRODUCTION

Aquaculture industry is a largest industry which provides protein rich food. This industry aims to fulfill peoples dietary needs globally [1]. Aquaculture industry grows eachyear at the rate of 5.8% which shows enormous growth of this industry [2]. Aquaculture provides 50% of food fish utilization globally that's why it is growing at faster rate [3]. As the population of developing countries increasing rapidly which lead to higher demands of healthy diet and results in expansion of this industry [4]. Fish is known as the most prominent source of best quality minerals, vitamins and its is also imortant sourece of easily digestible proteins [5]. Fish is one of the most important product of aquatic industry as it provides essential nutrients for human food [6]. Aquaculture related sectors like technological advancements, feed industry are also growing at higher rates because of higher demands of proteins obtained fro fishes [7]. Natural habitats of fish like lakes, rivers are manipulating by anthropogenic activities and these habitats are not producing enough fish to fulfill human needs. Fish provides poly unsaturated fatty acids which protects and strenthen human cardic. As compared to other food industries, aquaculture is growing at

exponential rate and trying to perform their role against global hunger and malnutrition [8].

Aquaculture fish and it's by products provide 17% of animal proteins and 7% of all proteins obtained and consumed globally. This industry not only providing food security to developed countries but also it provides nutrients and food security to developing nations as well [2].

Nutritious foods are very important for the survival, reproduction and maintenance of all life bearing organisms, including fishes as well [9]. Farmed fish proteins content is significantally affected by fish meal. Though aquaculture industry is a prominent source of protein but this industry also consumes 3.06 million tonnes of it [10]. 40-60% of total production cost consists of fish feed in aquaculture [11]. Additionally the cost of fish meal also increasing significantly from last thirty years. This significant increase in feed cost and fish oil pose challenges for aquaculture operators [12]. As aquaculture industry is growing, it cause increase in meal demand which lead to unpredictable availability. This greater value and unpredictable availability of fish meal advocates to develop new protein rich sources for aquaculture [13].

Now a days plants are widely used in fish feed industry as protein sources [14]. These protein sources are easily available and much cheaper than other protein sources of fish feed industry. These sources are sustainable and less toxic to the environment or ecosystem [15]. One of plant protein sources is Moringa oliefera, which is widely used now a days in aquaculture diets. This plant (known as miracle tree/ drumstick tree) is widely cultivated in subtropical and tropical regions. It has a lot of applications both in medicines and in industries [16, 17]. Leaves of moringa are rich in alkoids, polyphenols, glucosinolates, flavonoids, tannins, vitamins and carotenoids [18]. Moringa oleifera leaf meal (MOLM) have been used as a fish feed supplement in various research projects of aquaculture to enhance immunity and growth of aquatic life [19]. As compared to other food products like banana, carrot, yoghurt and milk moringa leaves possess 25-32% more vitamins, calcium, potassium, iron and proteins [20]. Additionally, moringa leaves also have important amino acids like lysine, cystine, tryptophan and methionine. This composition of moringa leaves advocates its importance as a potential substitute of protein source in aquaculture industry [21].

Catla catla fish is widely culture in Pakistan along with other varieties. It is known as a surface feeder as it feeds on surface of water [22]. In twenty first century, its production was increased in first decade. In 2012 its output reached roughly 2.8 millions tonnes each year [3]. On the basis of importance of Catla catla and MOLM, main objective of this experiment is to evaluate MOLM effects on growth, body composition, and minerals of Catla catla. This experiment also aims to examine capability of MOLM as an alternative protein source in fish meal.

2- MATERIALS AND METHODS

2.1 Study Area and Pond Preparation

In 2023, six-month trials (February to August) were performed in ponds according to semi-intensive rearing method at the research area of fisheries, UAF, Pakistan. At fish nutrition laboratory, analysis of feed composition and fish body were performed. Tube well ground water was used for the irrigation of ponds. Each pond has an inlet and outlet for irrigation and cleaning purposes. These ponds were rebuilt, and all fauna and flora were removed, then experimental trials were conducted. To maintain the pH of ponds, lime was applied in all ponds at the rate of 250 kg/ ha. Then both inorganic and organic fertilizers were applied in ponds to develop planktonic biomasses for these Catla catla fingerlings.

2.2 Fish and Experimental Conditions

From government fish seed hatchery Catla catla fingerlings were purchased. For first fifteen days, these fingerlings were placed under laboratory conditions in cemented ponds for acclimatization. Every day fingerlings were fed at 10:00 AM. Air was provided to fingerlings by an air pump via capillary system. When these small fishes were fully acclimatized, then they were divided into six study groups. Before the start of feeding trials NaCl was applied at the rate of 5g/L in each pond to prevent fungal infections and to destroy ectoparasites [23]. Water was changed periodically to provide air and maintain DO level in ponds. Temperature, DO, CO2, pH and all other chemical and physical attributes were monitored regularly.

2.3 Experimental Design

In this experiment MOLM was used as an experimental test unit. Among six earthen ponds, total 90 fingerlings were uniformly distributed. So, fifteen fingerlings were nourished in each pond. One of these six groups were labelled as controlled while other five ponds or groups were labelled according to their respective MOLM treatments. Five MOLM levels were: 6% MOLM, 12% MOLM, 18% MOLM, 24% MOLM and 30% MOLM. Total duration of these feeding trials were six months.

2.4 Moringa Oleifera Leaf Processing and Other Feed Ingredients

Moringa leaves also possess some anti nutritional substances such as assaponins. To reduce these assaponins crushed leaves were soaked for three days in tap water at room temperature and this mixture were stirred daily for one hour [24]. Then this mixture was squeezed through a cloth and all water was removed. Then chemical composition of this grounded material was examined and then it was blended [25]. In the experimental diets, the inert maker was chrome oxide. To

replace fish meal, six diets for test were prepared by using MOLM at 0%, 6%, 12%, 18%, 24% and 30% level.

Table 1: Ingredients Composition (%) in test diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Replacement level	0 %	06 %	12 %	18 %	24 %	30 %
MOLM	00.00	06.00	12.00	18.00	24.00	30.00
Fish meal	50.00	44.00	38.00	32.00	26.00	20.00
Wheat flour	17.00	17.00	17.00	17.00	17.00	17.00
Corn gluten	20.00	20.00	20.00	20.00	20.00	20.00
Fish oil	09.00	09.00	09.00	09.00	09.00	09.00
Vitamin premix	01.00	01.00	01.00	01.00	01.00	01.00
Mineral premix	01.00	01.00	01.00	01.00	01.00	01.00
Ascorbic acid	01.00	01.00	01.00	01.00	01.00	01.00
Chromic oxide	01.00	01.00	01.00	01.00	01.00	01.00

2.5 Formulation and Processing of Experimental Diets

Feed materials were finely grounded by a o.5 mm sieve. These all contents of feed were mixed in a mixer and after five minutes of mixing fish oil was added gradually. Then 10-15% water was added to feed ingredients, to form appropriate texture dough, these ingredients were added in a mixer. Then pellets were produced from this dough in an experimental extruder [26].

2.6 Growth Study

Before conducting trials initial weight of fingerlings were measured and then after completing the trials final weight of all fishes were measured to assess growth and weight gain.

2.7 Proximate Analysis

Motor and pestle were used to individually homogenize experimental diet and fish whole body. Kjehldahl apparatus was used for the assessment of crude proteins. While crude fats were assessed by using petroleum ether extraction process. Atomic absorption spectrophotometer was used for determining mineral ccontents of fingerlings [25].

2.8 Hematological Parameters

Blood was collected with the help of syringe from caudal vein of fingerlings. Hematocrit was assessed by micro hematocrit approach by using capillary tubes [27]. A hemocytometer having authorized Neubauer counting chamber was used for counting white blood cells and red blood cells [28]. While hemoglobin concentration was measured according to Wedemeyer and Yastuk method [29].

2.9 Data Analysis

Data was recorded for various growth, hematological and mineral composition of fingerlings. This recorded data were analyzed by a microcomputer. Data was analyzed by using one way ANOVA [30]. Tukey's HSD test was used to check differences among means of various MOLM levels.

3- RESULTS AND DISCUSSION

3.1 Growth Performance

Growth parameters of MOLM fed C. catla are represented in Table-2. 12% MOLM diet provided most significant results for growth parameters. When fingerlings were fed with 12% MOLM, the highest final weight (647.64 g) of fingerlings was obtained. Additionally, 12% MOLM also provided highest values (632.33 g) for weight gain of fingerlings. While 30% MOLM provided the lowest values for final weight and weight gain of fingerlings. For growth parameters 12% MOLM provided highest values and showed much differences as compared to controlled. Results of this experiment were in correspondence with the findings of Doctolero and Bartolome [31]. Who found that 20% MOLM improved growth values of fish. Moreover, Elabd *et al*., [32], also found same results, he concluded that Nile tilapia growth were significantally increased when fed with MOLM. Hussain *et al*., [33], also found that, growth of *Labeo rohita* were significantally enhanced on inoculation of 10-20% MOLM.

3.2 Body Composition

Table-2 shows that crude protein and crude fats of fingerlings were significantly affected by different MOLM diets. As compared to growth parameters which were highest at 12% MOLM, body composition parameters were not highest at that MOLM diet. The highest value (21.23) of crude protein was observed at 06% MOLM while lowest value of crude protein was

observed at 30% MOLM. While in case of crude fats, highest value was observed at 30% MOLM and lowest value was observed at 06% MOLM. Our results were in accordance with the previous findings. Such as, Arslan *et al*., [34], found that 10% MOLM provided highest values for crude protein as compared to controlled. While 40% MOLM provided lowest values of crude protein.

Table 2: Mean Values ± SE of Growth parameters, Crude Protein (CP) and Crude Fat (CF) on Various Levels of MOLM Diet

Treatments	Initial Weight (g)	Final Weight (g)	Weight gain (g)	CP (%)	CF (%)
0% MOLM	15.37 ± 0.17	516.47 ± 2.25	501.1 ± 2.27	19.47 ± 0.23	3.43 ± 0.16
6% MOLM	15.41 ± 0.31	617.97 ± 2.87	602.56 ± 2.77	21.23 ± 0.41	2.37 ± 0.11
12% MOLM	15.31 ± 0.22	647.64 ± 2.09	632.33 ± 2.11	19.44 ± 0.25	3.19 ± 0.21
18% MOLM	15.29 ± 0.13	561.13 ± 1.94	545.84 ± 1.87	19.14 ± 0.17	3.67 ± 0.19
24% MOLM	15.21 ± 0.31	476.67 ± 1.99	461.46 ± 2.33	17.77 ± 0.21	5.17 ± 0.25
30% MOLM	15.26 ± 0.19	437.87 ± 1.81	422.61 ± 1.77	16.55 ± 0.09	6.91 ± 0.19

3.3 Hematological Parameters

Figure-1 is representing the mean values of various hematological parameters with respect to MOLM diet. Fingerlings showed highest RBCs value (2.73×10^{6} mm^{-3}) at 12% MOLM diet, while RCBs lowest value was recorded at 30% MOLM diet. Fingerlings also showed highest WBCs value (7.76×10^{-6} mm^{-3}) at 12% MOLM diet, while WCBs lowest value was also recorded at 30% MOLM diet. RBCs and WBCs highest values at 12% MOLM diet suggests that optimum quantity of MOLM is required for proper growth. Platelets in fingerlings also showed the highest value (63.81) at 12% MOLM. Hemoglobin highest value (8.73 g/100ml) was also observed at 12% MOLM. Findings of this experiment suggests that 12% MOLM showed significant increase in all hematological parameters of fingerlings. Now a days hematological parameters are the most important factors in aquaculture because they determine health and growth of fishes [35]. Tabassum *et al*., [36], also found that 10% MOLM caused increase in hematological parameters of *Cirrhinus mrigala* but more increase of MOLM levels cause reduction in hematological parameters.

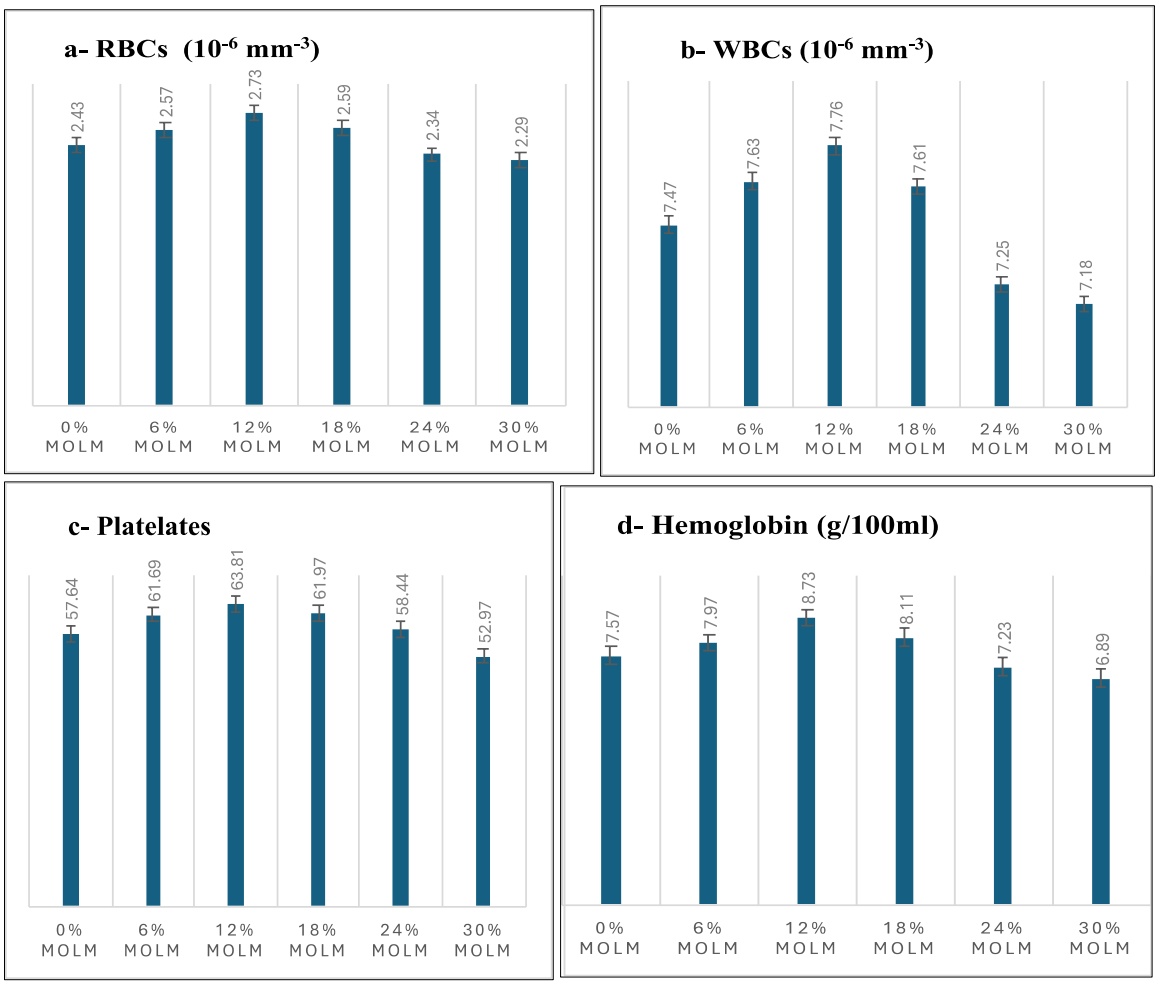

Figure-1: Graphical Representation of Mean Values of Hematological Parameters on Various Levels of MOLM Diet

3.4 Body Mineralization

For the normal functioning of body of fishes, minerals are very important. These occur naturally in the form of inorganic substances. Figure-2 shows the mineral content of Catla catla fingerlings at various levels of MOLM. MOLM diets caused significant variations in body minerals of these fingerlings. At 12% MOLM, the highest values of Zn, K, Fe, Ca and Mg (4.07 µg/g, 8.44%, 59.31 µg/g, 0.99% and 3.22%) were observed in catla fingerlings body and these values were significantly higher than fingerlings fed on other diets. While selenium showed highest deposition at 30 MOLM diet. This study shows that like all other growth and hematological parameters, body minerals were also highly deposited at 12% MOLM. Shehzad *et al*., [37], also found similar results to our study. He found that MOLM diet enhances deposition of minerals in fish body. While enhancing MOLM beyond optimum level reduces the deposition of these minerals.

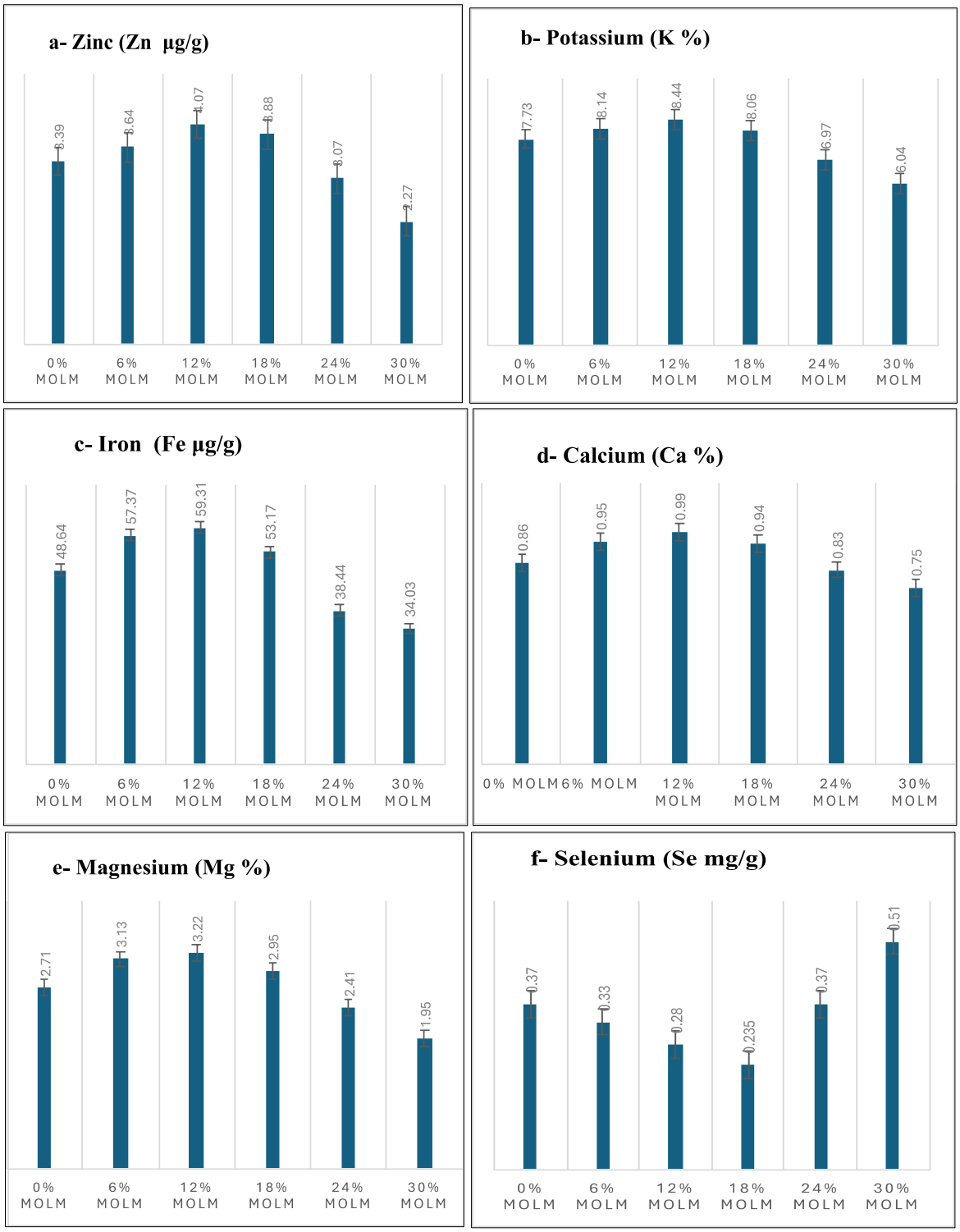

Figure 2: Graphical Representation of Mean Values of Mineral Composition of Fingerlings Body on Various Levels of MOLM Diet

CONCLUSIONS

From results of these studies, it can be concluded that optimum level of all fish meal is compulsory. Beyond their optimum levels they cause harm to fingerlings instead of benefits. In this study 12% MOLM provided significant results for all growth, hematological parameters and for mineral composition of fingerlings body. While increasing the level of MOLM after 20% did not show any positive growth responses as compared to their controlled. So from these findings, it can be suggested that 10-20% of MOLM replacement of fish meal provide significant results and these levels of replacement are cost effective as compared to expensive fish meals.

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