Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(3): 887-890

Received: 22-03-2020
Accepted: 24-04-2020

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Effect of non-conventional feed ingredients mahua oil cake supplemented diet on body composition of *Labeo rohita*

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Abstract
The highest (15.59±0.35%), and lowest (15.20±0.29%) level of protein was in T4 and T0 (control) respectively. The respective lowest (3.77±0.34%) and highest (3.92±0.13%) fat content was recorded in T4 and T3. The contents of ash ranged between 4.42±0.25to 4.55±0.22percent with minimum in both T0 & T4 and maximum in T1. The moisture content ranges from 75.18±0.62to 75.87±0.57per cent. The maximum and minimum values of moisture were recorded in T4 and T3 respectively. Therefore, a dose of 20% (200g/kg diet) is recommended for supplementation in carp diet. Further, the trial using different species under field conditions are also recommended.

Keywords: Mahua oil cake, non-conventional feed ingredients, *Labeo rohita*

Introduction
In aquaculture, more than 60% of the input cost of production is contributed by feed [1]. Therefore, to achieve the ultimate goal of high profit from carp culture, it is most necessary to reduce the cost of feed by using some cheaper feed ingredient without compromising the growth of fish. The cake of mahua seed can be one option to reduce the cost of feed. Because this plant is easily available in TSP (Tribal Sub Plan) area of Rajasthan and the cake of this plant is mostly used for agriculture purpose which can also be a suitable option for including in carp feed. Good nutrition in animal production systems is essential to the economic production of a healthy, high-quality product. In recent years, fish nutrition has advanced dramatically due to the development of new, balanced commercial diets that promote optimal fish growth and health [2]. The artificial diets may be either complete or supplemental. The complete diets supply all the required ingredients (protein, carbohydrates, fats, vitamins, and minerals) for the optimal growth of fish. Most fish farmers use complete diets, typically made up of the following components and percentage ranges: protein, 18-50 percent; lipids, 10-25 percent and carbohydrate, 15-20 percent [3, 4]. Feed sources based on nutrient content are plant protein sources (i.e. soybean meal - high protein, potential partial replacement of fish meal) and animal protein sources (i.e. fish meal - most common protein source) and non-conventional protein sources (eg. algae, fungi (yeasts) and bacteria). The five major nutrients included in fish diet are protein, fats, carbohydrate, mineral, and vitamins. The *in vitro* digestibility of mahua seed cake after treatment with isopropanol was found to be 81%. Detoxified mahua seed flour appears to be a good source of protein for food and feed products [5, 6]. The mahua oil cake (MOC) used as a fish toxicant in India [12]. But present investigation MOC used as a non-conventional feed ingredient for *Labeo rohita*.

Materials and methods
Preparation of experimental diet
As stated earlier, a preliminary experiment (using 11 graded levels -0-100% of (Mahua oil cake) MOC)) to select the safe level of MOC was conducted. On the bases of the preliminary experiment, 35% (Mahua oil cake) MOC dose was calculated as safest by second-order polynomial regression analysis. For conducting a final trial, four (4) levels of MOC were selected for supplementation in carp feed, which included one level above safe level (40%) and three (10, 20 &30%) below the safe level of MOC.
Treatment T0 represents diet prepared without mahua oil cake (control diet); T1 represents diet incorporated with 10% of mahua oil cake; T2 represents diet incorporated with 20% mahua oil

cake; T3 represents diet incorporated with 30% mahua oil cake; T4 represents diet incorporated with 40% mahua oil cake protein. The results on proximate composition of experimental fish are presented in Table 1. The biochemical composition of the control and treatment diets are present in Table 1.

For preparing experimental diet, all the needed ingredients (as per Table 1) were separately grounded to form fine powder in an electrical mini lab grinder. After this these were thoroughly mixed in the desired quantity and moistened with water to form a dough. Thus, the prepared dough was placed in an autoclave (121°C temperature and 15 lbs/cm^2 pressure) for 15 minutes. The autoclaved material was cooled at room temperature and pelletized with a small hand operated pelletizer. The pelletized feed was air-dried at ambient temperature for 72 h, packed in air-tight containers, labeled and stored. Further, the proximate composition of the experimental diet was done following standard methods of[7] and results are presented in Table 1.

Proximate composition of diet and fish

Initially, the proximate composition of the experimental diet was performed following [7]. Similarly, at the end of the 45 days' culture period, the nutritional quality of fish was also analyzed. For this purpose, protein, fat, ash and moisture content were analyzed by[7] described below:

Estimation of moisture: A known amount of sample (4 g) was taken in a weighed porcelain crucible and kept in a preheated oven at 60 ± 2°C for at least 24 hours. The crucible then transferred directly to a desiccator, cooled and weighed immediately. The moisture i.e. weight loss was reported in percent as per the following formula:

$$\text{Moisture (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

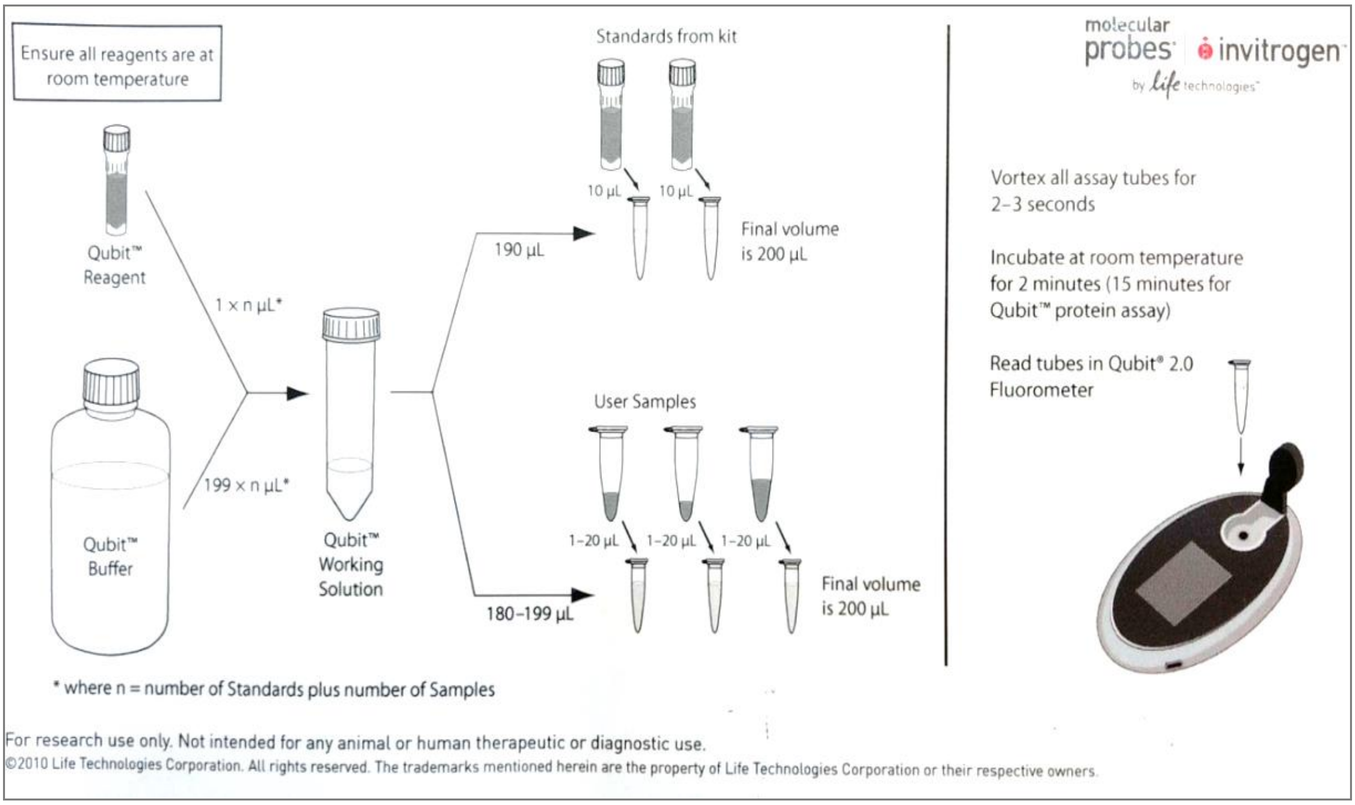
Estimation of fat: A sachet of filter paper (Whatman No. 40) was made and its weight was recorded. In this sachet one g of dried and powered sample was taken. The sample was then extracted with ether at 60-80°C in Soxhlet apparatus. The extraction was continued for six hours at condensation rate of 20-30 drops per min. After extraction, sample was dried for 30 minutes at 100°C, cooled and final weight was recorded. The difference in weight of sample before and after extraction indicated the total organic solvent soluble lipids. The fat content of the sample was expressed in percentage of dried sample.

Estimation of Ash: For the estimation of ash 100 mg dried and powdered sample was weighed in silicon crucible and incinerated in a furnace preheated to 550°C for four hours. The crucible containing fully burnt material was transferred to a desiccator, cooled and weighed. The difference between initial and final weight was noted. The ash was reported in percent as follows:

$$\text{Ash (per cent)} = \frac{\text{Ash weight (mg)}}{\text{Sample Weight (mg)}} \times 100$$

Estimation of crude protein (By qubit® 2.0 fluorometer)

The protein content in experimental diet and fish were analyzed using protein analysis kit Qubit® 2.0. (Invitrogen). The standard protocol provided with this kit was followed. The main steps are presented in the following figure.



Results

Treatment T0(Control) represents diet prepared without mahua oil cake (control diet); T1 represents diet incorporated with 10% of mahua oil cake; T2 represents diet incorporated with 20% mahua oil cake; T3 represents diet incorporated with 30% mahua oil cake; T4 represents diet incorporated with 40% mahua oil cake protein. The biochemical composition of the control and treatment diets are present in Table 1. The results on proximate composition of experimental fish are presented in Table 1. The level of protein was nearly at 22-23% among all treatment diets. The crude lipid content varied from 9.23% to 11.25%. Crude lipid content of the different diets was, T0 (11.25%), T1 (10.89%), T2 (10.34%), T3 (9.73%) and T4 (9.23%). Moisture content varied from 14.00 (T2 and T3) to 16.00 (T1) %; the ash content varied from 6.05 (T0 control) to 7.98 (T4) % among the all treatment diets.

It is evident from Table 2 that the highest (15.59±0.35%), and lowest (15.20±0.29%) level of protein was in T4 and T0 (control) respectively. The respective lowest (3.77±0.34%) and highest (3.92±0.13%) fat content was recorded in T4 and T3. The contents of ash ranged between 4.42±0.25to 4.55±0.22percent with minimum in both T0 & T4 and maximum in T1. The moisture content ranges from 75.18±0.62to 75.87±0.57per cent. The maximum and minimum values of moisture were recorded in T4 and T3 respectively. Further, it is worth mentioning here that the contents of protein, fat, ash and moisture in different treatments were statistically non-significant (*P*>0.05).

Discussion

The findings of the present study (higher growth and survival in MOC supplemented diet) have established that the MOC would be a good source of non-conventional fish feed ingredient. The similar findings were also reported by [8]. The proximate composition of the whole body of experimental fish (*Labeo rohita*) is shown in Table 2. In comparison with the control, MOC supplementation level had no significant influence on moisture content. However, protein contents in treatments were significantly higher than control except T1. Moreover, fat and ash contents were significantly (*p*<0.05) increased in treatments as compared to control. While there is no other published data on MOC effect on fish proximate composition therefore the comparisons could not be done. However, there are a number of studies related to the use of saponin in fish feed and its effect on growth and other body parameters [9]. The dietary saponin increases permeability of intestinal membrane to the digested dietary components, thereby increasing the feed utilisation efficiency [10]. The dietary quillaja saponin could significantly increase the activity of amylase and trypsin in the gut as well as lactate dehydrogenease in the liver of carp [11]. The overall results obtained in the literature on the use of saponin- rich plants in aquaculture, an improvement seems to be observed for supplementation doses lower than 0.1% saponin. It has been hypothesized that increasing growth in fish fed saponin supplemented diet could be due to intensification in the nutrient absorption from the intestine, induced by a slight permeabilization of the intestinal walls with saponins [9]. This would explain why low doses (<1000ppm/0.1%) of saponin-rich plant extracts can exert a beneficial impact on fish growth.

Conclusion

On the basis of the result obtained in the present experiment, it can be concluded that mahua oil cake supplementation has vital role in enhancing the enhance the body composition of *L. rohita* fingerling. Therefore, a dose of 20% (200g/kg diet) is recommended for supplementation in carp diet. Further, the trial using different species under field conditions are also recommended.

Table 1: Experimental diet ingredients and proximate composition

S. No.	Particulars	Experimental Diets				
A	**Diet Ingredients (g/100g)**	**Control**	**T1 (10%)**	**T2 (20%)**	**T3 (30%)**	**T4 (40%)**
1	Basal Diet*	100	90	80	70	60
2	Mahu oil cake	00	10	20	30	40
	Total	100	100	100	100	100
B	**Proximate composition (%)**					
1	Moisture	15.00	16.00	14.00	14.00	15.00
2	Crude Protein	22.43	22.29	23.05	23.62	23.78
3	Fat	11.25	10.89	10.34	9.73	9.23
4	Ash	6.05	6.21	6.37	7.6	7.98

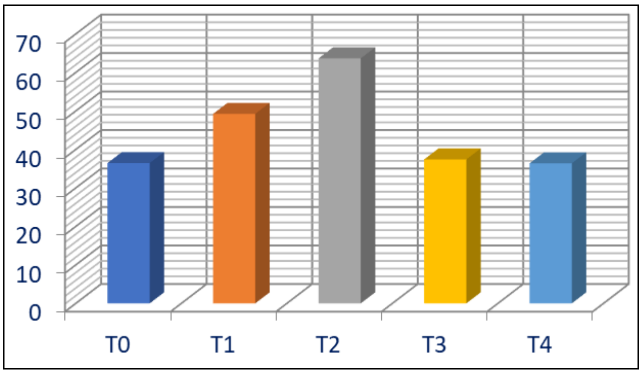
*Basal diet: Groundnut oil cake (40%) + rice bran (40%) + wheat flour (19%) + mineral mixture (1%)

Table 2: Proximate Composition fish carcass of *L. rohita* fed on MOC supplemented diet

S. No.	Treatment	Proximate composition (%)			
		Moisture	**Protein**	**Fat**	**Ash**
1	Control	75.58±0.52^a	15.2±0.29^a	3.84±0.19^a	4.42±0.25^a
2	T1	75.81±0.65^a	15.23±0.17^a	3.78±0.20^a	4.55±0.22^a
3	T2	75.18±0.62^a	15.25±0.16^a	3.88±0.26^a	4.53±0.27^a
4	T3	75.3±0.25^a	15.57±0.18^a	3.92±0.13^a	4.39±0.22^a
5	T4	75.87±0.57^a	15.59±0.35^a	3.77±0.34^a	4.42±0.21^a

Data expressed as Mean ± SE (n=3)

Mean value in the same column sharing different superscripts are significantly different (*P*<0.05)



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