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Use of Mahua (*Bassia latifolia*) Oil Cake as Non-Conventional Fish Feed Ingredient

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ABSTRACT

The present study was conducted to explore the possibility to use mahua (*Bassia latifolia*) oil cake (MOC) as a non-conventional fish-fed ingredient. For this purpose, an experiment was designed using five graded levels of MOC. The experiment was conducted in 15 FRP tanks of 0.75 m^3each in triplicate for a period of 42 days. The fingerlings of rohu (*Labeo rohita*) were fed on MOC supplemented diets (i.e. 0, 10, 20, 30, and 40 % in basal diet). The experimental fish was fed at 3 % of body weight per day. The supplementation of MOC had no significant impact on water quality as the values of selected water quality parameters remained more or less identical in different treatments. However, the weight gain, gain in percent SGR and FCR were significantly improved in treatments as compared to the control. Results revealed that the highest weight gain (5.92 gm), percent weight gain (63.65%), length increment (1.47cm), percent length increment (31.68%) SGR (1.18) and better FCR (1.98) were recorded in T2. Considering the higher growth rate, it is recommended to use MOC (20 %, 200 g/kg diet) as a non-conventional fish feed ingredient.

INTRODUCTION

The fish production has steadily increased over the last few decades and contribution from aquaculture to total fish production has risen to more than 35 percent. Inland sector contributes biggest part of the production. It is again dominated by inland aquaculture. The rapid growth and higher productivity from inland aquaculture sector is an outcome of quality inputs and more importantly the use of aqua-feeds. The fast growing aquaculture and more demand of feed strongly indicate that the crises will be precipitated in the aquaculture feed industries in the near future. This will be due to both high cost and shortage of quality fish feed ingredients, especially fish meal and oil cakes.

According to the FAO, the costs of fish feed ingredients have increased as much as 50 percent in recent years. Significant progress has been made to reduce the dependency on costly feed ingredients. In this respect, fishmeal and fish oil have been substituted with proteins and oils of plant origin. In the recent past, a number of studies have been conducted to replace the use of costly fish feed ingredients **(Floreto *et al.*, 2000; Daniel, 2016; Rath *et al*., 2017; Sharda *et al*., 2017)**.

Traditionally, the mahua (*Bassia latifolia*) oil cake (MOC) has been used as a pesticide in addition to industrial applications as foaming and surface active agents. In mahua producing countries, mahua cake is used mainly as a fertilizer and to a limited extent as feed because of its protein content. The heat treatment can be used for detoxification but the digestibility decreases significantly through the heat treatment. From mahua cake, the saponins level can also be reduced by treatment with isopropanol. Thus, detoxified cake appears to be a good source of protein for food and feed. Mahua oil cake is basically a non-conventional feed ingredient, which can stimulate fish growth at a particular level and also support the immune system of an animal. In this context, the present study was conducted to investigate the practical utility of MOC in the use of carp diet as non-conventional feed-staff.

MATERIALS AND METHODS

To assess the impact of mahua oil cake (MOC) supplanted diet on *Labeo rohita* growth, an experiment was conducted during March- June 2019. The procedures followed for conducting this experiment are described below:

Preparation of basal diet

The basal diet was prepared by mixing groundnut oil cake, rice bran, wheat flour and mineral mixture in 40: 40: 19: 1 ratios, respectively

Preliminary experiment

To work-out the safe and optimum range of mahua oil cake inclusion in carp diet, a preliminary 15 days- experiment was conducted. This experiment was performed in 22 glass aquaria of 56 liters capacity each. The aquaria were initially washed with tap water and disinfected with $KMnO_4$. Each glass aquarium was filled with clean water up to 50 liters. In each aquarium, 10 healthy fingerlings (41.5±0.72g) were stocked. A total number of 11 graded levels {0(T_0), 10(T_1), 20 (T_2), 30 (T_3), 40 (T_4), 50 (T_5), 60 (T_6), 70 (T_7), 80 (T_8), 90(T_9), 100 % (T_{10})} of MOC were mixed in basal diet and fed to the experimental fish @ 3% of their body in two split doses. The fecal matter and un-eaten feed were daily siphoned from the aquaria. The mortality in different treatments, if any, was recorded every day in the morning and evening. At the end of the 15 days, the data collected for fish survival/mortality were analyzed using second order polynomial regression analysis to obtain the safe level of MOC supplementation level in rohu diet.

The outcome of the second order polynomial regression analysis between MOC supplementation rate and fish survival are presented in Fig (1). The regression curve undoubtedly describes that the inclusion level of 35 % of MOC is the safest with respect the viewpoint of fish survival (Fig. 1).

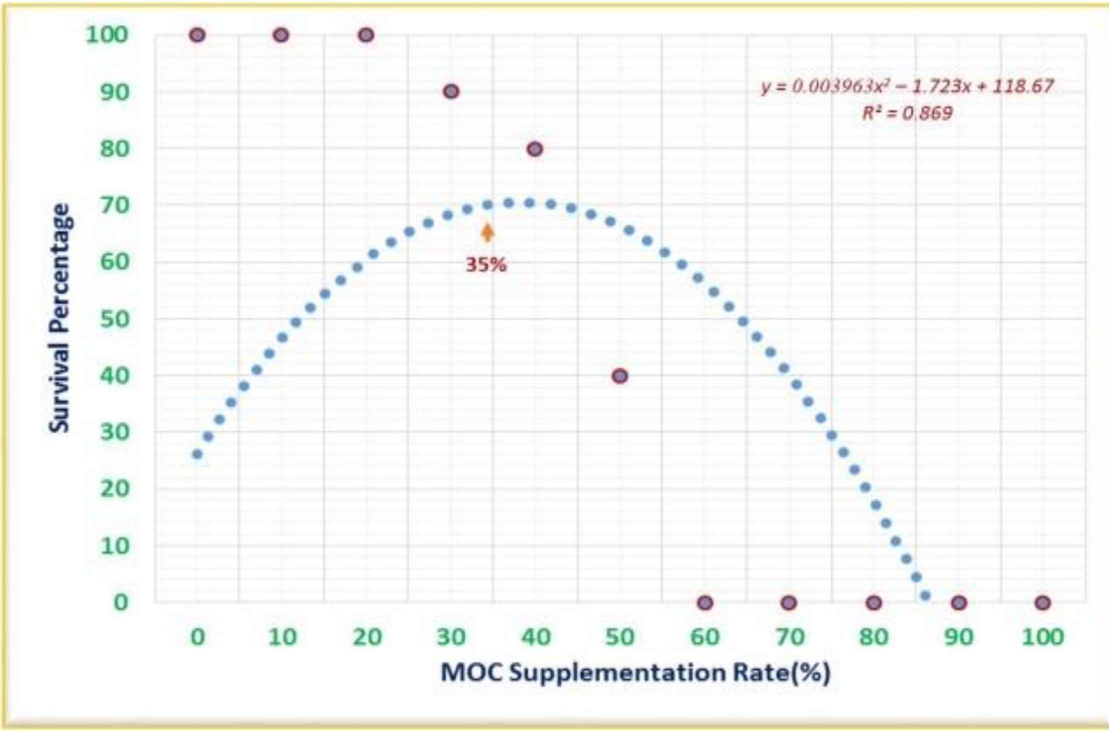

Fig.1. Second order polynomial regression curve

Preparation of experimental diet

To select the safe level of MOC, a preliminary experiment (using 11 graded levels, 0 - 100% of MOC) was conducted. On the bases of the preliminary experiment**,** 35% MOC dose was calculated as the 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.

For preparing experimental diet, all the needed ingredients (Table 1) were separately grounded to form fine powder in an electrical mini lab grinder. After this, it was thoroughly mixed in the desired quantity and moistened with water to form 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 **AOAC (2005)** and results are presented in Table (1).

Table 1. Experimental diet ingredients and proximate composition

S.No	**Particulars**	**Experimental Diets**				
A	Diet Ingredients (g/100g)	**T0**	**T1**	**T2**	**T3**	**T4**
1	Basal Diet*	100	90	80	70	60
2	Mahua 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 brawn (40%) + wheat flour (19%) + mineral mixture (1%)

Final experiment

To assess the effect of mahua oil cake supplementation on fish growth, an experiment was conducted in Wet Lab of Aquaculture Research & Seed Unit, MPUAT and Udaipur. For this purpose, 15 FRP tanks of 0.75 m^3 were used. Initially, all the experimental tanks were cleaned, disinfected with $KMnO_4$ and dried. Later the tanks were filled with 500 liters of clean filtered well water. All the tanks were divided in five [T_0 (control), T_1 (10 % MOC), T_2 (20% MOC), T_3 (30% MOC) & T_4 (40 % MOC)] groups following CRD, and each group had three replicates. Each tank was stocked with 10 healthy fingerlings (Pre-conditioned for 7 days under laboratory conditions) of rohu having a mean weight of 9.205±0.29g. Before stocking, the initial length and weight of each fingerling were recorded. The experiment was conducted for a period of 42 days during which fish was fed with an experimental diet at 3% of their body weight. In the experimental systems, selected water quality parameter (i.e. temperature, pH, dissolved oxygen, TDS, salinity and EC) were monitored on the initial day and subsequently at an interval of 7 days following **APHA (2005)**. At the end of every seven days, the weight of fish from each experimental tank was recorded using an electronic weight balance. Similarly, the length of the experimental fish was also recorded using digital Vernier Caliper at weekly intervals. On the basis of length, weight and feed, the data were determined following the growth performance parameter,and their values were calculated following the standard methods of **Biswas (1992):**

a) **Net weight gain (g)** = $W_f - W_i$

Where, W_f: final weight (g) & $W_{i:}$ initial weight (g)

b) **Net length gain (cm)** = $L_f - L_i$

Where, $L_{f:}$ final length (cm) & L_i : initial length (cm)

c) Percent weight gain (PWG %) = $\frac{W_t - W_o}{W_o} \times 100$

Where, $W_{t:}$ final weight (g) & W_O : Initial weight (g)

d) **Percent length gain (%)** = $\frac{L_t - L_o}{L_O} \times 100$

Where, $L_{t:}$ final length (cm) & L_O : Initial length (cm)

e) Specific growth rate (%) = $\frac{L_n W_t - L_n W_o}{t} \times 100$

Where, W_t: final weight (g), W_O: Initial weight (g) & t: time duration (days)

f) **Survival rate (%)** = $\frac{\text{final number of fish}}{\text{intial number of fish}} \times 100$

g) **Food conversion ratio (FCR %)** = $\frac{\text{Feed intake}}{\text{Weight gain}}$

The data recorded for the evaluation of different treatments were statistically analyzed using the standard procedure for analysis of variance (ANOVA) in complete randomized design (CRD) in order to test the significance of experimental results. Besides, the standard error was also calculated and results were expressed as mean ± SEM (standard error of the mean). For the analysis of statistical parameters, the standard methods of **Steel and Torrie, (2009)** were followed.

RESULTS

The supplementation level of mahua oil cake in carp diet did not affect the water quality parameters. Thus, the levels of selected water quality parameters, such as water temperature, pH, dissolved oxygen, electric conductivity, TDS, hardness and salinity remained more or less the same in different treatments (Table, 2).

The supplementation of MOC in fish diet had significant impact on fish growth parameters. In treatments, the weight gain, length increment and SGR were significantly better than the control. However, the survival rate was cent per cent in all the treatments and the control (Table 3).

Table 2. Range & mean values of selected water quality parameters

Treatment		Parameters					
		DO (ppm)	pH	Temp (°C)	TDS (ppm)	Ec (mS/cm)	Hardness (ppm)
T_0	**Mean**	6.71	7.68	23.12	417.57	851.00	167.29
	Maximum	7.38	7.93	24.90	440.00	863.50	176.00
	Minimum	5.31	7.29	20.55	409.00	840.50	163.60
T_1	**Mean**	6.84	7.92	22.81	418.64	850.50	167.26
	Maximum	8.02	8.40	24.60	444.00	864.00	177.60
	Minimum	5.31	7.62	20.90	410.50	841.00	164.20
T_2	**Mean**	6.75	8.08	22.85	419.00	849.35	167.60
	Maximum	7.80	8.60	24.75	441.00	862.50	176.40
	Minimum	5.36	7.87	20.95	413.50	840.50	165.40
T_3	**Mean**	7.09	7.82	22.98	417.92	848.28	167.54
	Maximum	7.54	8.25	24.85	440.00	860.00	176.00
	Minimum	5.78	7.47	20.80	411.50	842.50	164.60
T_4	**Mean**	6.43	7.73	23.02	417.50	850.35	167.51
	Maximum	7.52	7.94	24.75	438.00	858.00	175.20
	Minimum	5.10	7.40	20.90	407.50	843.00	164.80
The salinity was 0.4 ppt in all the treatments							

The data pretending to weight gain in experimental fish are presented in Table (3) and Figs. (2 - 5). It is evident from Table (3**)** that the higher growth rate was recorded in treatments compared to the control. Still, the highest weight gain was recorded in T_2 (5.92g). The minimum (3.35g) weight gain was noticed in the control (T_0).

Statistically, the initial weight in all the treatments and the control were statistically non-significant at 5% level of probability (Table 3). The analysis of variance (ANOVA) for weight gain showed that all the treatments were significant as compared to the control. Furthermore, the Duncan's New Multiple Range Test suggested that the weight gains in T_2, T_1 and T_0 were significantly different ($p \leq 0.05$). However, weight gain differences in T_3, T_4 and control were statistically non-significant ($p > 0.05$).

The calculated percent weight gain values varied between 63.65-36.44% (Table 3). The respective lowest (36.44%) and highest (63.65%) weight gain percent was recorded in control (T_0) and T_2. The percent weight gain in other treatments were 49.21, 31.38, and 36.37% in T_1, T_3, & T_4, respectively (Table 3). The statistical analysis (ANOVA) revealed that the treatments were significantly different from the control.In addition, the Duncan's test proved that the percent weight gain between treatments T_3 and T_4 was not significantly different. Similarly, the differences between T_0 (control) and both T_3& T_4 was not significant ($p>0.05$).

Table 3. Growth summary of experimental fish, *L rohita* fed on experimental diet

Treatment	Weight (g)				Length (cm)				
	Initial	**Final**	**Net Gain**	**Gain %**	**Initial**	**Final**	**Net Gain**	**Gain %**	**Survival (%)**
T_0	9.21^{a}	12.56^{c}	3.35^{c}	36.44^{c}	4.65^{a}	5.16^{b}	0.51^{c}	10.97^{c}	100^{a}
T_1	9.59^{a}	14.31^{ab}	4.72^{b}	49.21^{b}	4.45^{a}	5.84^{ab}	1.39^{a}	31.24^{a}	100^{a}
T_2	9.30^{a}	15.22^{ab}	5.92^{a}	63.65^{a}	4.64^{a}	6.11^{a}	1.47^{a}	31.68^{a}	100^{a}
T_3	9.47^{a}	13.01^{bc}	3.54^{c}	37.38^{c}	4.57^{a}	5.74ab	1.17^{ab}	25.60^{ab}	100^{a}
T_4	9.10^{a}	12.41^{c}	3.31^{c}	36.37^{c}	4.40^{a}	5.48^{b}	1.08^{ab}	24.54^{ab}	100^{a}
The values superscript with same letter are statistically non-significant at 5% of probability level									

On the first day, the length of experimental fish in different treatments was statistically non-significant. After the rearing period of 42 days, the length increment in different treatments varied from 1.47 to 0.51cm. In general, the length increment/gain was significantly higher in all the treatments as compare to the control. Still the highest gain was recorded in T_2 followed by T_1, T_3& T_4. Further, the minimum length gain of 0.51cm was noticed in the control (T_0). ANOVA proved that the treatments were significantly different from the control. Additionally, the Duncan's test suggested that the control was significantly different from treatments. However, treatments T_1& T_2 and T_3& T_4 were statistically non-significant ($p\geq0.05$)(Table 3).

The values of the percent length gain are presented in Table (3). It was noticed that, the length gain was higher in the treatments than the control. However, the highest length gain in percentage was calculated in T_2 (31.68%) while the lowest (10.97%)was in the control. The percent length gain in treatments was significantly different as compared to the control. Nevertheless, the difference between T_2& T_1 and T_3& T_4 were not significant ($p>0.05$).

Results obtained for SGR are presented in Fig. (4). Significant impact of MOC supplemented diet was visible from the higher levels of SGR in the treatments compared to the control. The highest (1.18%) value of SGR was found in $T_{2;}$ followed by T_1 (0.95%), T_3 (0.75%) and T_4 (0.74%) while the lowest (0.68%) was recorded in the control. Statistically, the values of SGR in the treatments were significantly different ($p<0.05$) except for T_4 and the control. The difference between T_3&T_4 were also non - significant ($p>0.05$).

The results on food conversion ratio (FCR) of experimental fish are presented in Fig. (5).The mean value of FCR was the highest (3.80) in the control (T_0).Whereas, the minimum value of FCR was recorded in T_2 (1.98). The values of FCR in other treatments were 2.56 (T_1), 3.37 (T_3) and 3.46 (T_4). The growth rate (Table 3) and FCR (Fig. 5) showed an inverse relationship between these two parameters as the values of FCR decreased with higher growth rate. It is worthy to mention that, the values of FCR between control and treatments were significantly different except for T_3&T_4.

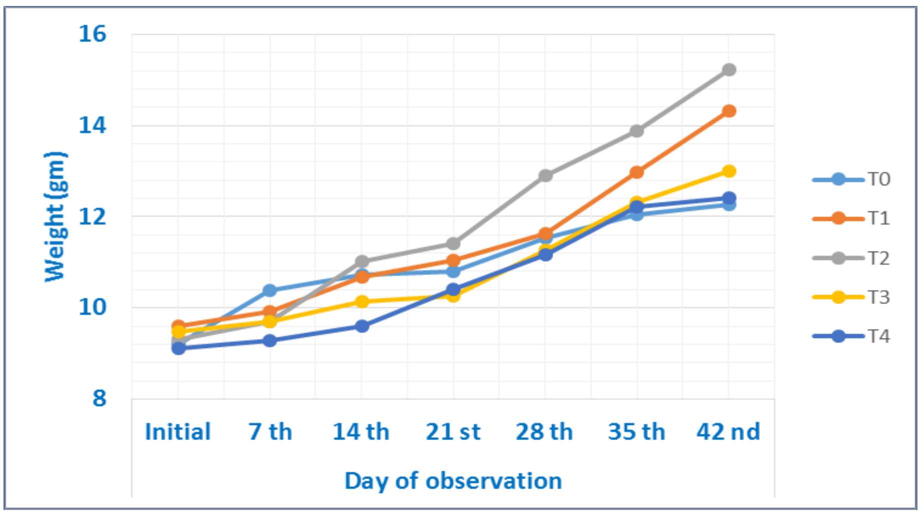

Fig. 2. Weekly weight gain of fish

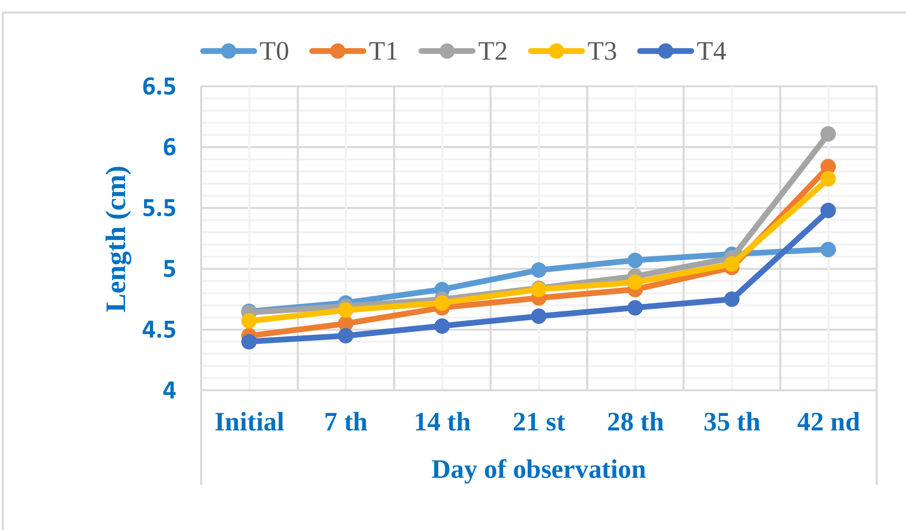

Fig.3: Weekly length gain of fish

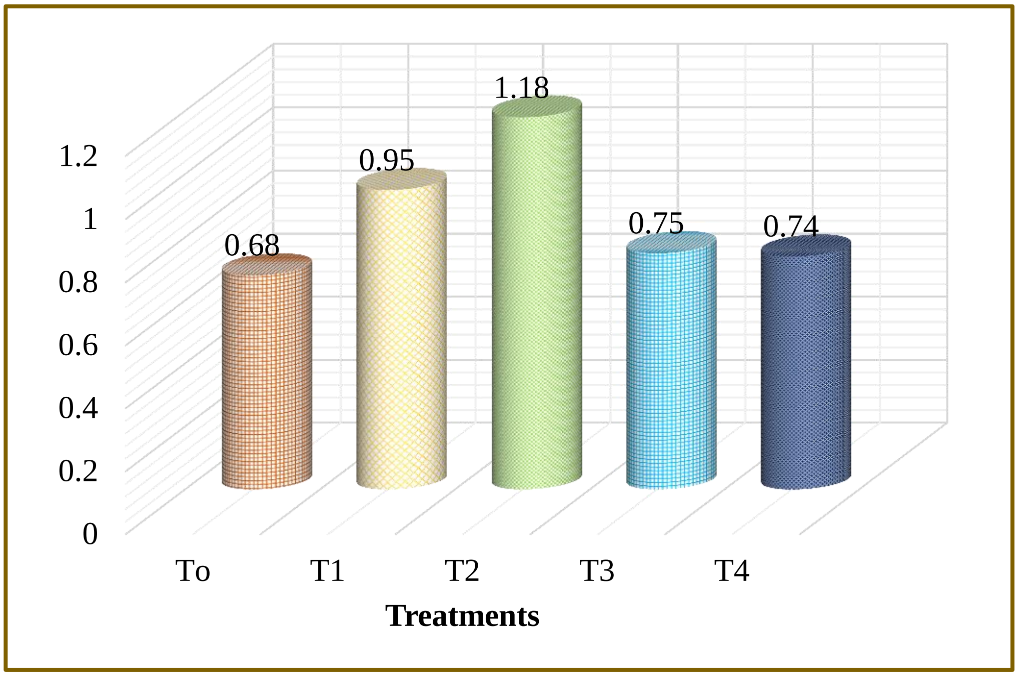

Fig.4. SGR (%) of experimental fish

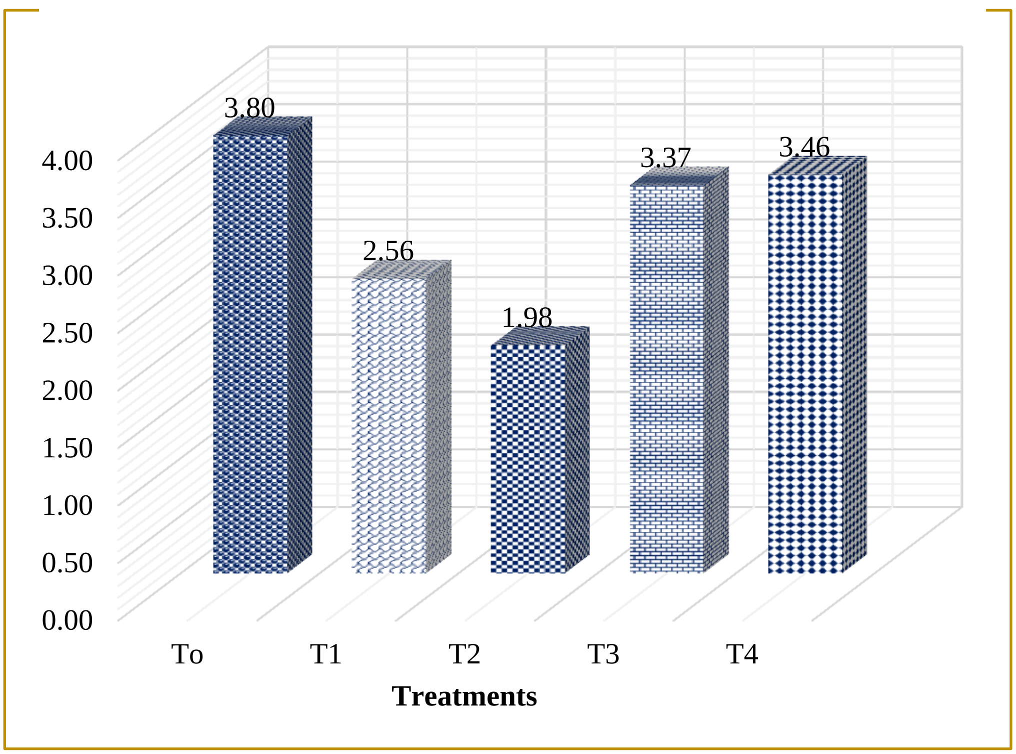

Fig.5: FCR of experimental fish

DISCUSSION

The results of the present investigation revealed the significant impact of MOC supplemented diet on fish growth parameters. However, the water quality parameters remained unaffected. Water quality parameters studied during the experiment under all the treatment conditions were within the optimum range, suitable for the rearing of carps, especially *Labeo rohita*. The water temperature in experimental tanks ranged between 20.55°C to 24.90°C. The mean temperature ranged between 22.81°C and 23.12°C. **Larsson and Berglund (2005)** have suggested that in the temperature range tolerated by fish, growth rates increase with increasing temperature and show a parabolic pattern. When experimental temperature reaches the upper extreme of the tolerance range, performance of growth decreases. This depression of growth is due to the higher energy cost for maintenance metabolism and seems to be related mainly to a loss of appetite.

The dissolved oxygen ranged between 5.10 and 8.02 ppm. Total dissolved solids ranged from 407.50- 440.00 ppm in treatments and 409.00-440.00 ppm in the control. The variations in electrical conductivity were 841.00 -862.50 mS/cm in treatments and 840.50- 863.50 mS/cm in the control. The mean pH ranged between 7.13 and 8.08 in both the treatments and the control. These results are in accordance with the findings of **Ghosh and George (1989)** who reported that in the Indian subcontinent, most of the water bodies had their temperature lying between 7.8°C and 38.5°C. **Mahboob and Sheri (1998)** reported that the dissolved oxygen had an inverse relationship with water temperature in fish ponds. In general, no significant input of water quality on fish growth was noticed, and water quality remained congenial for the growth of experimental fish **(Boyd, 1997; Phelps & Popma, 2000)**. Thus, the significant variations in growth rate in different treatments could be due to the addition of MOC in carp diet.

The effects of MOC have been studied in fish and other animals. Improvement of the growth has been reported by feeding of MOC supplemented diet **(Ojha *et al,* 2013; Jacob *et al.*, 2014; Rath *et al.*, 2017)**. In the present study, the growth of *Labeo rohita* was increased significantly with MOC supplemented diet. Such increase in the growth of fish fed with MOC supplemented diet was also reported by **Rath*et al*. (2017)**. The previous authors have suggested that the MOC can be incorporated in *Labeo rohita* diet at 30%. However, in the present study, the highest growth was obtained with 20 % MOC supplementation level. A further increase in MOC level resulted in significantly reduced growth. Thus, suggesting that the high concentrations of MOC supplemented in diets may not further promote the growth of *Labeo rohita* in this study is attainable

The present findings (higher growth and survival in MOC supplemented diet) assessed that the MOC would be a good source of non-conventional fish feed ingredient. The similar findings were also reported by **Rath*et al.* (2017)**. For further comparison of the present findings, no other literature is available for fish. However, a number of good studies were conducted on other animals. The better growth performance of crossbred calves with 10 % of MOC supplemented diet was reported by **Ojha *et al.* (2013)** and **Jacob *et al.* (2015)**. However, for the same animal (crossbred calves), a 20 % MOC supplementation level was suggested by **Tiwari *et al.* (1996)**. Similarly, **Khaing *et al*. (2015)** have also suggested the use of 30 % MOC in goat diet.

Remarkably, the saponin is one of the most important components of MOC. The higher weight gain, SGR and better FCR recorded in MOC supplemented diet might be the result of saponin content. The enhanced growth of the fish fed saponin supplemented diet was also reported by **Francis *et al*. (2005)**. An increased average body weight of *Cyprinus carpio* in saponin supplemented diet was also noticed **(Serrano, 2013)**. It has been opined by **Francis *et al* (2002)** that the dietary saponin increases permeability of intestinal membrane to the digested dietary components which ultimately enhance the feed utilization efficiency. The increased activity of amylase and trypsin in the gut of fish fed saponin- supplemented diet (carp species) was noticed by **Serrano *et al.* (1998)**. Therefore, the enhanced growth rate in fish fed MOC supplemented diet is justified from the findings of the afore- mentioned authors, because MOC is a good source of saponin which promotes feed utilization efficiency.

Feed conversion efficiency of experimental fish increased with MOC supplemented diet. The increased levels of MOC supplementation from 0 to 20% was significant ($p<0.05$) but the further increases in MOC supplementation rate (>20%) of 30 and 40 % were not significant. Thus, the best fed conversion ratio (FCR) was recorded for the 20% MOC supplemented diet. **Rath*et al.* (2017)** showed that the feed conversion ratio values for *Labeo rohita*, ranging from 2.02 to 3.13, were significantly affected by the inclusion of mahua oil cake as protein source. In the present study, feed conversion ratio (FCR) was not statistically different among treatments T3 & T4 ($p>0.05$). However, the best FCR value (1.98) recorded in T2 (diet with 20% MOC) was significantly different from both other treatments and the control. **Atack *et al.* (1979)** reported FCR of 2.50 when 30% crude protein diet for mirror carp (*Cyprinus carpio*) was used. In conclusion, under the experimental conditions, the results obtained not only supported the use of MOC for better growth but also improved the feed utilization. To elevate the growth ability of *Labeo rohita*, the MOC supplementation at 200 g/ kg diet (20%) is an optimal dose, determined by growth, SGR and survival. However, further study on different fish species and field trials are proposed for the development of POP (Package of Practices) for aquaculture.

CONCLUSIONS

The results of this study clearly depicted that the supplementation of MOC had no significant impact on water quality of culture systems. However, the growth parameters of the experimental fish (weight gain, SGR and FCR) were improved in treatments as compared to the control. Considering the highest weight gain (5.92 gm), per cent weight gain (63.65%), length increment (1.47cm), per cent length increment (31.68%) SGR (1.18) and better FCR (1.98) it is recommended to use MOC @ 20 % (200g/kg diet) as non-conventional fish feed ingredient.

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