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Effect of *Morinda citrifolia* (Noni) fruit extract dietary supplementation in the freshwater fish, *Cyprinus carpio* L.

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ABSTRACT

Noni (*Morinda citrifolia* L.) is a renowned medicinal plant native to Southeastern Asia and the Pacific islands. This study investigated the effects of *M. citrifolia* fruit extract on the growth, body composition, and haematological parameters of one-month-old fry of common carp, Cyprinus carpio, over a 35-day period in triplicate. The fruit extract was incorporated into diets at concentrations of 0, 2.5 g/kg (0.25%), 5.0 g/kg (0.50%), 7.5 g/kg (0.75%), and 10 g/kg (1%), creating one control and four experimental diets. A total of 150 acclimated C. carpio fry were randomly divided into five experimental groups (Gr. I, II, III, IV, and V), each containing 30 fish subdivided into three replicates of 10 fish each. Group I was fed the control diet, while groups II, III, IV, and V received diets supplemented with 0.25%, 0.50%, 0.75%, and 1% M. citrifolia, respectively. Various growth, haematological parameters, and proximate composition metrics of the experimental fish were assessed. The results indicated that dietary supplementation with *M. citrifolia* significantly ($p<0.05$) improved growth performance metrics (weight gain, weight gain percentage, specific growth rate, protein efficiency ratio, and feed conversion ratio) in groups II, III, IV, and V compared to the control group (Gr. I). In terms of body composition, moisture and ash content were significantly ($p<0.05$) increased, while crude lipid content was significantly ($p<0.05$) decreased in the supplemented groups compared to the control. No significant differences were observed in crude protein content among the experimental groups. Additionally, *M. citrifolia* supplementation significantly ($p<0.05$) enhanced haematological parameters, with increased erythrocyte and leukocyte counts in the supplemented groups compared to the control. In conclusion, dietary supplementation with *M. citrifolia* fruit extract improved the fish's growth, body composition, and haematological parameters, with the 0.25% supplementation showing the best overall response, and thus considered the optimal dose.

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1. Introduction

Morinda citrifolia L. (also called 'Noni' in Polynesia and 'Indian Mulberry' in India) is a small evergreen tree (Rubiaceae) that grows in Southeastern Asia, has long been used as a traditional medicine (Palu *et al.*, 2008; Chan-blanco *et al.*, 2006). Different parts of this plant contain more than 200 phytochemicals (Singh *et al.*, 2012). The fruit extract of *M. citrifolia* is rich with flavonoids (rutin, quercetin), organic acids(ascorbic acid, linoleic acid, oleic acid), alkaloid (xeronin), lignans (americanin) and phenolic compound (Scopelitin) (Pawlus and Kinghorn, 2007; Assi *et al.*, 2017). It also contains proteins, vitamins (β-carotene, vitamin B complex, vitamin C) and minerals (Ca, P, Se) (Chan-blanco *et al.*, 2006). *M. citrifolia* is used to treat many diseases like diabetes, high blood pressure, arthritis, hypertension, heart diseases, headache and fever (Moh *et al.*, 2021). *M. citrifolia* is well known for its antioxidant (Zin *et al.,* 2002), anti-inflammatory(Wang *et al.*,2008; Dussossoy *et al.*, 2011), immunostimulatory (Hirazumi and Furusawa, 1999) and antimicrobial (Assi *et al.*, 2017) properties. It also benefits animals' growth performance (Yancey *et al.*, 2013; Sunder *et al.*, 2015; Zhang *et al.*, 2023).

The common carp, *Cyprinus carpio* L. (Cypriniformes: Cyprinidae), is a commercially important and widely distributed freshwater fish. It is also the fourth major species (7.7%) produced in world finfish aquaculture (FAO, 2018). In recent years, different plant-based dietary supplements have been used in aquaculture to enhance growth and immunity in fish and shrimp. However, the utilization of *M. citrifolia* fruit extract as dietary supplementation in fish is very limited in the literature. Thus, we investigated the effect of *M. citrifolia* fruit extract on growth performance and whole body composition and selected haematological parameters in one-month-old fry of common carp, *C. carpio*.

2. Materials and Methods

2.1. Experimental design

The study was conducted as per the guidelines of the Institutional Animal Ethics Committee, University of Calcutta. The common carp fry (Cypriniformes: Cyprinidae) of one-month-old (Fig. 1) were purchased from a fish market located in Naihati area, North 24 Parganas, West Bengal, India, and then transported to the laboratory in a large plastic bag with sufficient oxygen. Collected fries were stocked in a large glass aquarium (capacity 150 L) and acclimatized for one month with continuous aeration. During acclimation, fries were fed commercial diets @ 3% body weight daily twice daily. A total of 150 acclimated *C. carpio* fries (mean weight 3.557±0.651 gm) were randomly divided into five experimental groups; Gr. I (Control), Gr. II (2.5gm/kg *M. citrifolia*), Gr. III (5.0 gm/kg *M. citrifolia*), Gr. IV (7.5 gm/kg *M. citrifolia*), and Gr. V (10 gm/kg *M. citrifolia*) each with 30 fish divided into three replicates with 10 fish per replicate. Group I fish were fed basal diet, thus referred to as control group. Groups II, III, IV, and V were fed 2.5 gm/kg, 5.0 gm/kg, 7.5 gm/kg, and 10 gm/kg

Fig. 1. Images of common carp (*Cyrpinus carpio*)

Table 1. Water quality parameters of the water (values are means ± SEM, n = 3 per treatment group).

Water quality parameters	Values
Dissolved Oxygen	7.44±0.049 mg/l
Free Carbon dioxide	10.22±0.053 mg/l
Total alkalinity	92.33±1.452 mg/l
Total Hardness ($CaCO_3$)	126±2.081 mg/l
Water temperature	25.66±0.440°C
pH	7.76±0.145

of *M. citrifolia* fruit extract supplemented diet respectively. The experiment was conducted in glass aquarium with a total capacity of 30L in which 21 L of surface water was used to rearing fish. Water of the aquarium was changed in every alternate day and continuously aerated. The fries were fed @ 3% of their body weight at 10:00 hrs daily (Mandal and Ghosh, 2019). The physico-chemical characteristics of water were estimated as per the standard method (APHA, 1992) and were within the optimum range (table 1). Group weighing of fish in each aquarium was done after two weeks to revise the feed amount. The experiment was continued for a total period of 35 days.

2.2. Experimental diets

The source of *M. citrifolia* fruit extract was the formulated 'Noni capsules' manufactured by the Cosmic Nutracos Solutions Pvt. Ltd (Fig. 2). The *C. carpio* fry of one month old can tolerate the *M. citrifolia* fruit extract dietary supplement up to 30 gm/kg (3%) which was determined as per the guidelines of Organization for Economic Cooperation and Development (OECD) for 14 days before start of the experiment. *M. citrifolia* fruit extract incorporated at 0, 2.5 gm/kg, 5.0 gm/kg, 7.5 gm/kg, and 10 gm/ kg to prepare the basal or control diet, 0.25%, 0.50%, 0.75%, and 1% *M. citrifolia* supplemented diet respectively. The required amount of ingredients (table 2) was mixed together with distilled water to form a dough, which was then steamed and boiled in a pressure cooker. The pellet was created using a pelletizer. The pellet was then dried in sunlight, packed in polythene and preserved in refrigerator until use.

The proximate composition (moisture content, crude protein, crude fat and ash contents) of the diets was determined following the method of the Association of Analytical Communities (AOAC, 1990; NRC, 1993). The moisture content was estimated by a drying oven at 105^{0}C. Micro Kjeldahl apparatus was used to determine the crude protein content by applying the factor of 6.25. Crude fat or Ethar extract was determined using a soxhlet apparatus, using diethyl ether at a boiling point of 40-60^0 C. The ash content of the samples was measured using a muffle furnace at a temperature of 600^{0}C for the period of 6 hours. The proximate composition of the experimental diets and ingredients are presented in the table 2.

Fig. 2. "Noni capsule" manufactured by Cosmic Nutracos Solutions Pvt. Ltd.

2.3. Haematological Parameters

After the end of the 35 days of feeding trial, blood was collected by cutting the caudal peduncle using a sharp knife, after anaesthetized with clove oil. The blood was collected in a watch glass containing EDTA. The total erythrocyte and leukocyte of blood were counted using an Improved Neubauer haemocytometer. Whereas, haemoglobin content of the blood was measured using Sahli's haemoglobinometer (Das *et al*., 2020).

2.4. Proximate composition of fish

After the end of the experiment, the proximate composition of the fish (moisture content, ash content, crude protein, and crude limit) was analyzed as per AOAC (1990).

2.5. Statistical analysis

One-way analysis of variance, followed by Tukey's post hoc test was conducted to compare the means between experimental groups using software (Assad *et al*., 2014).

3. Results and Discussion

There was no mortality of fish in any experimental groups. The result revealed that *M. citrifolia* dietary supplementation significantly ($p<0.05$) improved the growth performance through significantly ($p<0.05$) increasing the feed intake, weight gain, weight gain %, specific growth rate, protein efficiency ratio, and significantly ($p<0.05$) decreasing the feed conversion ratio, compared to the control (Table 3). The present study is in agreement with many previous studies in which *M. citrifolia* fruit extract dietary supplement improves growth performance of Common carp (Das and Ray, 2023), Nile tilapia (Kristiana *et al*., 2020) and in Whiteleg shrimp (Moh *et al*., 2021). *M. citrifolia* dietary supplementation probably augmented the growth performance by enhancing fish's digestive enzyme activity (Moh *et al*., 2021). The anthraquinone of *M. citrifolia* fruit extract reduce the pH of the digestive tract, so that some

Table 2. Experimental diets and ingredients with proximate composition

values are means ± SEM, n=3 per treatment group. Means in a row without a common superscript letter differ (P<0.05) as analyzed by one way ANOVA and the TUKEY test

Ingredients (g/kg)	Basal/ Control diet	0.25% M. citrifolia diet (2.5 gm/kg)	0.50%M. citrifolia diet (5.0 gm/kg)	0.75%M. citrifolia diet (7.5 gm/kg)	1%M. citrifolia diet (10gm/kg)
GNO cake[1]	600	600	600	600	600
Fish meal[2]	200	200	200	200	200
Rice bran[3]	100	100	100	100	100
Wheat flour	90	87.5	85	82.5	80
M. citrifolia[4]	-	2.5	5.0	7.5	10
Vit. & Min. mix	10	10	10	10	10
Proximate composition					
protein	40.7 ± 0.117^{a}	40.2 ± 0.0416^{b}	40.5 ± 0.136^{ab}	40.4 ± 0.04^{ab}	40.5 ± 0.0829^{ab}
fat	8.68 ± 0.0737^{b}	8.66 ± 0.0273^{b}	8.39 ± 0.151^{b}	8.47 ± 0.0498^{b}	9.99 ± 0.0379^{a}
fiber	6.16 ± 0.026^{b}	6.2 ± 0.0603^{b}	6.62 ± 0.0612^{a}	6.16 ± 0.0379^{b}	6.58 ± 0.0702^{a}
ash	8.13 ± 0.0624^{b}	8.21 ± 0.0586^{b}	8.93 ± 0.107^{a}	8.2 ± 0.0603^{b}	8.89 ± 0.0384^{a}
moisture	11.6 ± 0.0265^{a}	10.9 ± 0.0929^{b}	9.47 ± 0.122^{cd}	10.1 ± 0.276^{c}	9.26 ± 0.0491^{d}
NFE	24.7 ± 0.164^{c}	24.3 ± 0.103^{c}	26.1 ± 0.041^{a}	25.6 ± 0.249^{ab}	24.9 ± 0.0874^{bc}
GE(kcal/gm)	372.304	368.151	374.392	372.628	384.564

Composition of vitamin & mineral mixture(premix): Each 1 kg contains Vitamin A 8,00,000 IU, Vitamin D_3 80,000 IU, Vitamin E 0.6g, Nicotinamide 1.2 g, Cobalt 2.2g, Copper 4.7g, Iodine 0.6g, Iron 2.2g, Magnesium 6.5g, Manganese 3.3g, Potassium 0.2g, Sodium 0.04g, and Zinc 10g.

[1] Ground nut oil (GNO) cake contains 55.43% proteins and 14.45% fat; [2] Contains 51.65% proteins and 7.6% fat; [3] Contains 9.25% proteins and 8.3% fat; [4] Contains 2.84% proteins, 2.5% fat, 4.5% ash and 7.1% moisture; [5]NFE (Nitrogen Free Extract)=100-(Protein+Fat+Ash+Crude fiber); [6]GE (Gross Energy): Estimated according to NRC (1993) as 4.64, 9.44 and 4.11 Kcal/gm for protein, fat and carbohydrate respectively.

proteolytic enzyme works optimally, resulting in higher digestion and absorption which ultimately supports the growth rate of fish (Kristiana *et al*., 2020; Esminger *et al*., 1990). In addition, the xeronine and proxeronine of the *M. citrifolia* increase nutrient absorption and play a beneficial role in fish's metabolism (Solomon, 2001). Moreover, *M. citrifolia* is a well-known source of essential amino acids, vitamins (vitamin C or ascorbic acid and provitamin A or ß carotene) and minerals (calcium, phosphorus, potassium, sulfur and selenium) that positively affect the overall metabolism resulting in the higher growth performance in groups II, III, IV, and V compared to the group I (control) (Chan-Blanco *et al*., 2006; Assi *et al*., 2017; Singh *et al*., 2008).

In the whole body composition, moisture and ash content were significantly ($p<0.05$) increased, and crude lipid was significantly ($p<0.05$) diminished in Gr. II, III, IV and V fed *M. citrifolia* dietary supplementation, compared to Gr. I (control) (table 4). There was no significant difference in crude protein among experimental groups. There is no study about the effect of *M. citrifolia* dietary supplement on the whole body composition of fish. However, a plant-based dietary supplement, *Spirulina platensis* increases the moisture and ash content, and decreases the crude lipid content in Gibel carp, *Carassius auratus gibelio* var. CAS III (Cao *et al*., 2018). Similarly, Nandeesha *et al.*, 2001, reported an increase of ash content in Indian Major Carp, Rohu (*Labeo rohita*) and Catla (*Catla catla*) utilizing *S. platensis* as a plant based dietary supplement. *M. citrifolia* rich with minerals like calcium, phosphorus, potassium, sulfur and selenium which are probably accumulated in the fish body results in the higher ash content in group II, III, IV and V than in group I(control). The decrease in crude lipid in *M. citrifolia* dietary supplementation groups was probably due to lowered accumulation of lipids in the fish tissues and higher β-oxidation of fatty acids (Roy and Lall, 2003). Several previous studies also reported a decrease of crude lipid content in fish fillets using plant based dietary supplements (spirulina) (Khanzadeh *et al.*, 2015; Ungsethaphand *et al*., 2010). In an earlier study, Siddhuraju and Becker (Siddhuraju and Becker, 2001) also recorded the similar results in which a plant based proteins

Table 3. Growth performance in fish

values are means ± SEM, n=3 per treatment group. Means in a row without a common superscript letter differ (P<0.05) as analyzed by one way ANOVA and the TUKEY test

	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V
IBW (g)	2.84 ± 0.0462	2.82 ± 0.0136	2.75 ± 0.119	2.76 ± 0.0641	2.86 ± 0.172
FBW (g)	3.2 ± 0.0461	3.5 ± 0.00433	3.17 ± 0.124	3.16 ± 0.0606	3.25 ± 0.167
WG (g)	0.365 ± 0.0024^{c}	0.682 ± 0.0174^{a}	0.422 ± 0.00781^{b}	0.404 ± 0.00581^{bc}	0.382 ± 0.00536^{bc}
FI (g)	1.49 ± 0.023^{b}	1.79 ± 0.0139^{a}	1.5 ± 0.0517^{b}	1.5 ± 0.0799^{b}	1.5 ± 0.0963^{b}
Wt gain %	12.9 ± 0.232^{c}	24.2 ± 0.732^{a}	15.4 ± 0.517^{b}	14.7 ± 0.515^{bc}	12.8 ± 0.413^{c}
SGR	0.347 ± 0.00578^{b}	0.619 ± 0.0174^{a}	0.409 ± 0.0129^{b}	0.39 ± 0.0134^{b}	0.36 ± 0.0253^{b}
FCR	4.07 ± 0.0697^{a}	2.64 ± 0.0885^{b}	3.56 ± 0.0699^{a}	3.72 ± 0.252^{a}	3.92 ± 0.302^{a}
PER	0.614 ± 0.0103^{b}	0.951 ± 0.0317^{a}	0.703 ± 0.0137^{b}	0.679 ± 0.0453^{b}	0.647 ± 0.0509^{b}

IBW: Initial Body Weight; FBW: Final Body Weight; WG: Weight Gain: Final Body Weight – Initial Body Weight; FI: Feed Intake; Wt gain % : Weight gain %: (final body weight-initial body weight)/ initial body weight x 100; SGR: Specific Growth Rate : 100 x (ln final weight-ln initial weight) /days; FCR: Feed Conversion Ratio: Consumed Feed / Final weight-Initial weight); PER: Protein Efficiency Ratio: Weight Gain/ Protein intake

Table 4. Final whole body composition of fish (% wet weight basis). Values are means ± SEM, n = 3 per treatment group. Means in a row without a common superscript letter differ ($P<0.05$) as analyzed by one-way ANOVA and the TUKEY test.

Parameters	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V
Moisture	82.4 ± 0.196^{c}	84.4 ± 0.045^{a}	83.5 ± 0.336^{b}	82.9 ± 0.0375^{bc}	82.7 ± 0.00781^{bc}
Crude Protein	10.5 ± 0.0229^{a}	10.3 ± 0.148^{a}	10.6 ± 0.00737^{a}	10.3 ± 0.0236^{a}	10.5 ± 0.00784^{a}
Crude lipid	2.81 ± 0.0283^{a}	2.38 ± 0.0169^{bc}	2.27 ± 0.0199^{d}	2.34 ± 0.0195^{cd}	2.45 ± 0.0293^{b}
Ash	2.55 ± 0.012^{b}	2.75 ± 0.0434^{a}	2.59 ± 0.0252^{b}	2.78 ± 0.0136^{a}	2.86 ± 0.00924^{a}

Table 5. Haematological parameters of fishes in the experimental groups (values are means ± SEM, n=3 per treatment group. Means in a row without a common superscript letter differ ($P<0.05$) as analyzed by one way ANOVA and the TUKEY test)

	Gr. I	Gr. II	Gr. III	G r. IV	G r. V
RBC count (x 10^6 cells/mm^3)	2.12 ± 0.0145^{b}	2.45 ± 0.0681^{a}	2.25 ± 0.0406^{ab}	2.17 ± 0.041^{b}	2.13 ± 0.0379^{b}
WBC Counts (x 10^3 cells/mm^3)	214 ± 2.77^{b}	260 ± 8.38^{a}	267 ± 1.28^{a}	269 ± 1.77^{a}	272 ± 5.39^{a}
Hb (gm/dl)	7.13 ± 0.176	7.53 ± 0.0667	7.4 ± 0.115	7.4 ± 0.115	7.2 ± 0.115

derived from mucuna (*Mucunapruriens utilis*) lowered the lipid content in common carp. The results of the present study is in consistent with some previous studies, in which the crude protein was not influenced by some plant based dietary supplements like, cineole in rainbow trout (Hoseini *et al*., 2018), and spirulina in common carp (Nandeesha *et al*., 1998). All aquaculture's primary objective is producing quality fillet containing high protein and less fat content (Roohani *et al*., 2018). In the present study, dietary supplementation of *M. citrifolia* decreased the crude lipid content, without significantly affecting the crude protein, demonstrating the production of desirable quality of fish fillet.

The haematological parameters are considered an important tool to evaluate fish health in response to nutritional manipulation (Hoseinifar *et al*., 2019; Arabi *et al*., 2016). In the present study, *M. citrifolia* dietary supplementation significantly ($p<0.05$) improved the haematological parameters of fish by increasing the erythrocyte and leukocyte count in groups II, III, IV and V compared to the group I(control) (Table 5), demonstrating higher fish health. However, the experimental groups had no significant difference in haemoglobin content. The *M. citrifolia* fruit extract increased leukocyte count, demonstrating the immunostimulatory activity. Recently, Das and Ray (2023) also reported the similar result in which *M. citrifolia* dietary supplementation improves the haematological parameters in common carp. Similarly, previous studies with several plant based dietary supplements like myrcine and menthol (Hoseini *et al*., 2019), cineole (Hoseini *et al*., 2018), spirulina (Temouri *et al*., 2013) and chlorella (Raji *et al*., 2018) known to improve the haematological parameters of fish.

4. Conclusion

M. citrifolia fruit extract dietary supplementations positively affect the growth performances, body composition and haematological parameters of the *C. carpio* fry. The optimum dose of *M. citrifolia* fruit extract dietary supplementation is 2.5 gm/kg (0.25%).

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