Dietary Inclusion Levels of White Mulberry (*Morus Alba*) Leaf Meal Effects on Growth Performance and Nutrient Utilization of *Clarias Gariepinus Fingerlings*

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ABSTRACT

African catfish, *Clarias gariepinus*, is among the most economically important freshwater fish species in Nigerian aquaculture. The increasing need for natural and cost-effective feed additives has prompted research into plant-based alternatives that can improve growth and feed utilization in cultured fish. This study evaluated the effects of dietary inclusion levels of *Morus alba* leaf meal on the growth performance and nutrient utilization of *C. gariepinus* fingerlings over a 56-day feeding trial. Five experimental diets were formulated to contain 40% crude protein with *M. alba* leaf meal included at 0.0 (Ma1), 0.5 (Ma2), 1.0 (Ma3), 1.5 (Ma4), and 2.0 (Ma5) g/100 g. A total of 225 fingerlings (mean initial weight: 3.00 ± 0.20 g) were randomly assigned to 15 glass tanks in a completely randomized design with three replicates per treatment. The proximate composition of *M. alba* leaf meal showed crude protein of 16.46%, moisture of 8.24%, ash of 12.12%, crude fibre of 5.30%, lipid of 3.47%, and nitrogen-free extract of 54.41%. Water quality parameters remained within acceptable ranges throughout the trial and did not differ significantly among treatments ($p > 0.05$). Fish fed Ma4 (1.5 g/100 g) recorded the highest weight gain (25.19 ± 2.53 g), specific growth rate (4.05 ± 0.14%/day), feed efficiency ratio (0.76 ± 0.04), and the best feed conversion ratio (1.32 ± 0.03). Growth performance declined in fish fed Ma5 (2.0 g/100 g), suggesting reduced efficacy at higher inclusion levels. Polynomial regression confirmed an optimum dietary inclusion level of approximately 1.6 g/100 g. It was concluded that dietary inclusion of *M. alba* leaf meal at 1.5 g/100 g optimally improved the growth performance and nutrient utilization of *C. gariepinus* fingerlings.

Keywords: African catfish, feed additive, mulberry leaf meal, specific growth rate, feed conversion ratio

INTRODUCTION

African catfish, *Clarias gariepinus* (Burchell, 1822), remains one of the most widely cultured freshwater fish species in sub-Saharan Africa, particularly in Nigeria, where it is highly valued for its fast growth rate, hardiness, and consumer acceptability [1]. The species thrives under diverse environmental conditions and can tolerate low dissolved oxygen levels through its accessory air-breathing organ, making it particularly suited to intensive aquaculture systems [2]. Despite these advantages, fish farmers continue to face challenges associated with poor feed utilization, suboptimal growth, and high production costs under intensive culture conditions [3].

In recent years, the search for natural and functional feed additives has gained momentum as an approach to improving production performance in farmed fish without reliance on synthetic growth promoters or antibiotics [4]. Plant-based feed additives are of particular interest because they are abundant, affordable, and contain a range of bioactive compounds such as flavonoids, polyphenols, alkaloids, saponins, and tannins that can positively influence feed palatability, nutrient digestibility, and metabolic efficiency in fish [5].

White mulberry, *Morus alba* L. (Moraceae), is a plant whose leaves are well known for their use in sericulture as the primary food of the silkworm *Bombyx mori* [6]. However, the leaves of *M. alba* also contain several nutritionally relevant compounds including crude protein, digestible carbohydrates, minerals, and bioactive phytochemicals such as flavonoids, polyphenols, and alkaloids [7]. These compounds have been linked to improvements in antioxidant status, digestive enzyme activity, immune response, and lipid metabolism in various animal species [8]. Studies in livestock have confirmed that mulberry leaves can improve body weight and feed conversion efficiency [9], while in fish, Chen et al. [10] reported improved digestive enzyme activity and growth performance in crucian carp (*Carassius auratus*) supplemented with *M. alba* leaf extract under stress conditions.

Despite these promising reports, information on the use of mulberry leaf meal as a dietary feed additive at low inclusion levels specifically for *Clarias gariepinus* is limited. Most available studies have focused on higher inclusion levels as a protein ingredient rather than on the functional effects of low-level supplementation on growth and nutrient utilization [11]. It is therefore important to determine the inclusion level at which *M. alba* leaf meal provides the greatest benefit to growth performance and feed efficiency in *C. gariepinus* fingerlings. This study was therefore conducted to evaluate the effects of different dietary inclusion levels of *Morus alba* leaf meal on the growth performance and nutrient utilization of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

Study Area

This research was conducted over 56 days at the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State, Nigeria.

Collection and Preparation of Morus alba Leaf Meal

Fresh *Morus alba* leaves were sourced from the Sericulture Unit, Ondo State Agricultural Empowerment Centre (OSAEC), Ondo State, and were identified and authenticated at the Department of Crop, Soil, and Pest Management, Federal University of Technology, Akure. After collection, the leaves were thoroughly rinsed with distilled water and air-dried under shade for seven days. The dried leaves were ground into a fine powder using a Kenwood electric blender (Model BL440, UK). The resulting powder was stored in an airtight container until needed for further use.

Experimental Fish

Healthy *C. gariepinus* fingerlings, averaging 2.50 ± 0.25 g in weight, were obtained from a reputable hatchery in Akure, Ondo State, Nigeria. The fish were acclimatized for two weeks following Afe and Omosowone [12]. During acclimatization, the fish were fed a commercial diet containing 40% crude protein to apparent satiation twice daily between 08:00–09:00 h and 16:00–17:00 h GMT.

Experimental Design

The study followed a completely randomized design (CRD). Immediately after acclimatization, a total of 225 healthy *C. gariepinus* fingerlings with an average weight of 3.00 ± 0.20 g were randomly assigned to 15 glass tanks (70 L; 45 cm × 35 cm × 35 cm) at 15 fish per tank, representing five dietary treatments in triplicate.

Experimental Diet Formulation

Five experimental diets (40% CP) were formulated using different inclusion levels of *M. alba* leaf meal (0.0, 0.5, 1.0, 1.5, and 2.0 g/100 g) following the method of Raissy et al. [13]. The diet containing 0.0 g/100 g served as the control. Other ingredients included fish meal, soybean meal, blood meal, yellow maize, fish oil, vegetable oil, vitamin-mineral premix, starch, methionine, and lysine (Table 1). The ingredients for each diet were thoroughly mixed and pelleted using a Hobart A-200T mixing and pelleting machine (Hobart Manufacturing Ltd., UK) with a 2 mm diameter die. The resulting strands were sun-dried for four days, handcrumbled into fingerling-appropriate sizes, packed into separate airtight polyethylene bags, labeled, and stored at 4°C until feeding.

Table 1: Gross composition of experimental diets (g/100 g) for culturing C. gariepinus fingerlings

Ingredients	**Ma1 (0.0)**	**Ma2 (0.5)**	**Ma3 (1.0)**	**Ma4 (1.5)**	**Ma5 (2.0)**
Fish meal (65% CP)	25.0	25.0	25.0	25.0	25.0
Soybean meal (defatted, mechanically-extracted) (45% CP)	30.0	30.0	30.0	30.0	30.0
Blood Meal (85% CP)	10.5	10.5	10.5	10.5	10.5
Yellow maize (10% CP)	13.0	13.0	13.0	13.0	13.0
Fish oil	5.0	5.0	5.0	5.0	5.0
Vegetable oil (Groundnut oil)	4.0	4.0	4.0	4.0	4.0
Vitamin–mineral premix	2.0	2.0	2.0	2.0	2.0
Starch (Binder)	5.0	5.0	5.0	5.0	5.0
Methionine	0.2	0.2	0.2	0.2	0.2
Lysine	0.3	0.3	0.3	0.3	0.3
Morus alba leaf meal	0.0	0.5	1.0	1.5	2.0

Vitamin-mineral premix in the diet contains: Vit A 4,000,000 I.U, Vit D3 800,000 I.U, Vit E 40,000 mg, Vit K3 1,600 mg, Vit B1 4,000 mg, Vit B2 3,800 mg, Vit B12 3 mcg, nicotinic acid 18,000 mg, pantothenic acid 8,000 mg, folic acid 800 mg, biotin 100 mcg, chloride 120,000 mg, iron 8,000 mg, copper 800 mg, manganese 6,000 mg, zinc 8,000 mg, iodine 400 mg, selenium 40 mcg, Vit C (coated) 60,000 mg, inositol 10,000 mg, cobalt 150 mg, lysine 10,000 mg, methionine 10,000 mg, anti-oxidant 25,000 mg.

Weighing and Feeding of Experimental Fish

Fish were weighed between 06:00 and 09:00 GMT using an electronic weighing balance (Model PB3002) every two weeks throughout the experiment. The fish were fed the experimental diets twice daily at 08:00–09:00 h and 16:00–17:00 h GMT to apparent satiation for 56 days. Unconsumed feed and faeces were removed daily by siphoning before feeding, and the culture water was partially replaced with fresh water. Complete water exchange was carried out twice weekly.

Monitoring of Water Quality

Water quality parameters (temperature, dissolved oxygen, and pH) were monitored twice weekly following Hecht et al. [2]. Temperature was measured using a mercury-in-glass thermometer (Model: Jenway 3150). The pH was determined using a digital pH meter (JENWAY Model 1960). Dissolved oxygen was measured using a Dissolved Oxygen test kit (HANNA Model: HI-9142).

Growth and Nutrient Utilization Parameters

Growth performance data were calculated following Raissy et al. [13] at the end of the experiment:

Weight Gain (WG, g) = Final weight − Initial weight

Specific Growth Rate (SGR, %/day) = (Ln final weight − Ln initial weight) × 100 / Time (days)

Feed Conversion Ratio (FCR) = Feed intake (g) / Weight gain (g)

Feed Efficiency Ratio (FER) = Weight gain (g) / Feed intake (g)

Survival (%) = (Number of fish harvested / Number of fish stocked) × 100

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Proximate Composition

Proximate analysis was carried out on the *M. alba* leaf meal and formulated experimental diets according to the standard methods of the Association of Official Analytical Chemists (AOAC) [14] to determine moisture, ash, crude protein, crude lipid, crude fibre, and nitrogen-free extract (NFE). NFE was calculated as: NFE (%) = 100 − (% moisture + % ash + % fibre + % protein + % lipid).

Statistical Analysis

Data were expressed as mean ± SE and analyzed using one-way Analysis of Variance (ANOVA) [15]. Differences among means were separated using Duncan's New Multiple Range Test [16]. Polynomial regression analysis was used to determine the optimum inclusion level of *M. alba* leaf meal. Statistical significance was accepted at $p < 0.05$.

RESULTS

Proximate Composition of Morus alba Leaf Meal

The proximate composition of *M. alba* leaf meal is presented in Table 2. The crude protein content was 16.46%, while lipid was the least abundant component at 3.47%. Ash content was 12.12%, crude fibre was 5.30%, moisture was 8.24%, and NFE was 54.41%.

Table 2: Proximate composition of Morus alba leaf meal (%)

Parameters (%)	Value (%)
Ash	12.12
Crude Fibre	5.30
Lipids	3.47
Moisture	8.24
Crude Protein	16.46
Nitrogen-Free Extract (NFE)	54.41

Proximate Composition of Experimental Diets

The proximate composition of the experimental diets is summarized in Table 3. Crude protein content ranged from 40.31% in Ma1 to 40.74% in Ma5. Crude fat and crude fibre increased with higher inclusion levels of *M. alba* leaf meal, while NFE decreased progressively across the treatments.

Table 3: Proximate composition of experimental diets (%)

Parameters (%)	Ma1	Ma2	Ma3	Ma4	Ma5
Ash	8.14	8.26	8.31	8.47	8.50
Crude Fibre	2.83	2.89	2.94	2.98	3.03
Lipids	9.11	9.21	9.33	9.53	9.61
Moisture	9.02	9.03	9.05	9.05	9.08
Crude Protein	40.31	40.53	40.68	40.71	40.74
NFE	30.59	30.08	29.69	29.26	29.04

Water Quality Parameters

Water quality parameters monitored throughout the 56-day feeding trial are presented in Table 4. Dissolved oxygen, temperature, and pH values remained within the recommended range for *C. gariepinus* culture throughout the experiment. No significant differences ($p > 0.05$) were observed in any water quality parameter among the five treatment groups.

Table 4: Water quality parameters measured during the experimental period

Parameters	**Ma1**	**Ma2**	**Ma3**	**Ma4**	**Ma5**
DO (mg/L)	6.33±0.37a	6.32±0.36a	6.28±0.34a	6.34±0.40a	6.34±0.34a
Temperature (°C)	27.14±0.37a	27.22±0.39a	27.36±0.40a	27.34±0.40a	27.37±0.41a
pH	7.02±0.04a	7.10±0.03a	7.05±0.03a	7.08±0.04a	7.05±0.21a

Means in the same row with different superscripts are significantly different ($p < 0.05$).

Growth Performance and Nutrient Utilization

Growth performance and nutrient utilization of *C. gariepinus* fingerlings are presented in Table 5. Initial weights were similar across all treatments ($p > 0.05$). Fish fed Ma4 recorded significantly ($p < 0.05$) the highest final weight (28.10 ± 2.54 g), weight gain (25.19 ± 2.53 g), and specific growth rate (4.05 ± 0.14%/day). The best feed conversion ratio (1.32 ± 0.03) and highest feed efficiency ratio (0.76 ± 0.04) were also recorded in Ma4. Growth performance and feed utilization efficiency declined in Ma5 (2.0 g/100 g), where final weight (19.45 ± 1.38 g) and weight gain (16.55 ± 1.38 g) were significantly lower than in Ma4. Survival was not significantly different among treatments ($p > 0.05$). Polynomial regression analysis (Figure 1) confirmed an optimum *M. alba* leaf meal inclusion level of approximately 1.6 g/100 g diet.

Table 5: Growth performance and nutrient utilization of experimental fish

Parameters	**Ma1**	**Ma2**	**Ma3**	**Ma4**	**Ma5**
Initial Weight, IW (g)	2.91±0.00a	2.90±0.01a	2.90±0.02a	2.91±0.01a	2.90±0.02a
Final Weight, FW (g)	12.76±0.44a	16.64±0.97b	20.47±1.30c	28.10±2.54d	19.45±1.38bc
Weight Gain, WG (g)	9.85±0.44a	13.74±0.96b	17.57±1.29c	25.19±2.53d	16.55±1.38bc
SGR (%/day)	2.64±0.09a	3.12±0.09c	3.49±0.10b	4.05±0.14d	3.40±0.16b
Total Feed Intake, TFI (g/fish)	25.03±1.10a	28.45±1.89b	33.21±2.39b	41.31±3.93d	38.88±5.08c
FCR	1.84±0.02d	1.67±0.03c	1.39±0.01ab	1.32±0.03a	1.45±0.04b
FER	0.54±0.03d	0.60±0.04c	0.72±0.02ab	0.76±0.04a	0.69±0.05b
Survival (%)	95.56±3.85a	88.89±3.85a	88.89±3.85a	84.44±7.70a	80.00±11.55a

Means in the same row with different superscripts are significantly different ($p < 0.05$).

SGR = Specific Growth Rate; FCR = Feed Conversion Ratio; FER = Feed Efficiency Ratio.

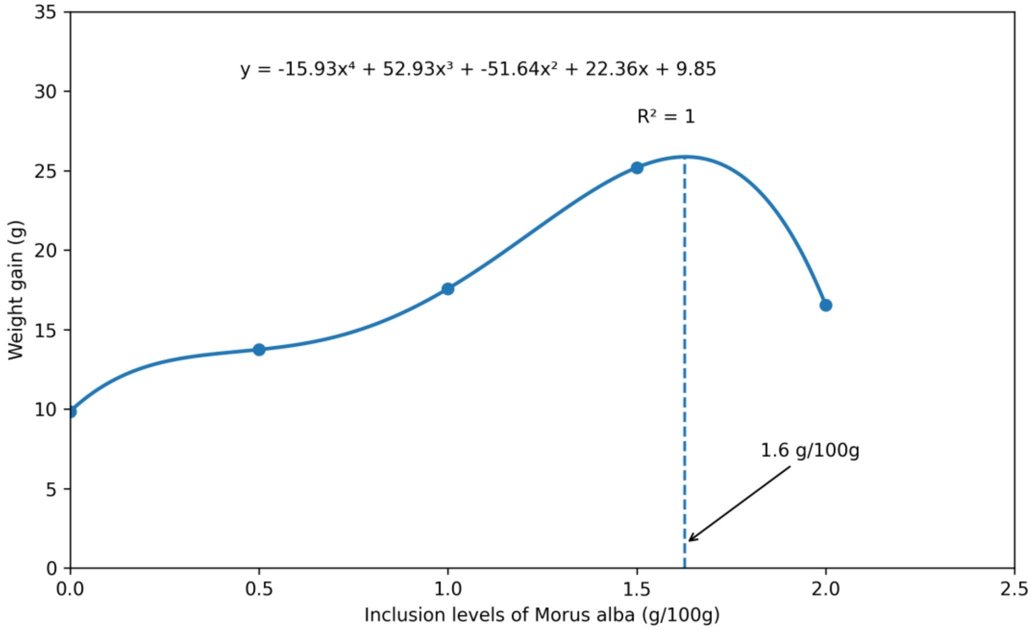

Figure 1: Polynomial regression showing the relationship between dietary Morus alba leaf meal inclusion level and weight gain of Clarias gariepinus fingerlings

DISCUSSION

Proximate Composition of Morus alba Leaf Meal and Experimental Diets

The proximate composition of *Morus alba* leaf meal obtained in this study showed crude protein of 16.46%, crude fat of 3.47%, crude fibre of 5.30%, ash of 12.12%, moisture of 8.24%, and NFE of 54.41%. The crude protein value was slightly lower than the range of 17.3–25.3% reported by Srivastava et al. [7] for different *Morus alba* genotypes. This variation may reflect differences in genotype, plant maturity, environmental conditions at the source, and processing methods [8]. The relatively high ash content indicated that the leaf meal could contribute useful minerals to the diet, while the low moisture content was likely due to the shade-drying method employed during processing, which has been reported to preserve the nutritional quality of mulberry leaves [6]. The high NFE content confirmed that *M. alba* leaves are a good source of digestible carbohydrates, which can serve as a secondary energy source in fish diets.

All experimental diets met the minimum crude protein requirement of 40% for *C. gariepinus* fingerlings. The gradual increase in crude protein, crude fibre, and ash content with increasing *M. alba* leaf meal inclusion levels reflected the proximate composition of the leaf meal itself. Similar observations were reported by Afe and Omosowone [12] and Dadras et al. [5] in studies involving plant-based feed additives. The reduction in NFE with increasing inclusion levels suggests that part of the carbohydrate fraction of the basal diet was displaced by the leaf meal, a trend consistent with findings reported for other plant-based additives [17].

Water Quality Parameters

The water quality parameters recorded during the experiment were within the recommended range for *C. gariepinus* culture throughout the 56-day feeding trial. According to Eyo [1] and Ndakalimwe [3], acceptable ranges for *C. gariepinus* culture are pH 6.5–9.0, temperature 25–32°C, and dissolved oxygen not less than 4.0 mg/L. No significant differences ($p > 0.05$) were observed among the treatment groups in any water quality parameter. The similarity in water quality conditions across all treatments confirmed that the observed differences in fish performance were attributable to dietary treatments rather than environmental variability.

Growth Performance and Nutrient Utilization

The growth performance of *C. gariepinus* fingerlings improved with increasing dietary inclusion of *M. alba* leaf meal up to Ma4 (1.5 g/100 g). Fish fed Ma4 recorded the highest final weight, weight gain, specific growth rate, feed efficiency ratio, and best feed conversion ratio, indicating that this inclusion level supported better growth and more efficient feed utilization than all other treatments including the control. This result is consistent with the findings of Prisingkorn et al. [18], who reported improved growth performance in fancy carp (*Cyprinus carpio*) fed diets supplemented with *Morus alba* fruit extract. The improvement in growth observed in the supplemented groups may be attributed to the bioactive compounds present in mulberry leaves, including flavonoids, polyphenols, alkaloids, and polysaccharides, which have been reported to improve feed palatability, stimulate digestive enzyme activity, and enhance nutrient absorption in fish [4, 5].

The improvement in feed conversion ratio and feed efficiency ratio from Ma1 to Ma4 further confirmed that fish utilized dietary nutrients more efficiently at moderate inclusion levels of *M. alba* leaf meal. Chen et al. [10] similarly reported that *M. alba* supplementation improved digestive enzyme activity and overall growth performance in fish. The progressive increase in total feed intake from Ma1 to Ma4 also suggests that mulberry leaf meal at low to moderate levels improved the palatability and acceptance of the experimental diets by the fish, which further contributed to the better growth observed.

Although growth improved from Ma1 to Ma4, performance declined in Ma5 (2.0 g/100 g). Fish in Ma5 recorded lower weight gain and poorer feed conversion compared with Ma4, despite a relatively high feed intake. This reduction in growth performance at the highest inclusion level may have been due to increased concentrations of antinutritional compounds such as tannins, saponins, and dietary fibre at higher inclusion levels, which could reduce nutrient digestibility and feed utilization [5, 19]. A similar pattern was reported by Afe and Omosowone [12], who observed improved growth at moderate inclusion levels of *Acacia* *auriculiformis* leaf powder in *C. gariepinus* diets but reduced performance at higher levels. The dose-dependent response observed in this study therefore underlines the importance of identifying the optimal dietary inclusion level for plant-based feed additives.

The polynomial regression analysis carried out in this study predicted an optimum inclusion level of approximately 1.6 g/100 g diet, which was very close to the best-performing treatment at Ma4 (1.5 g/100 g). This finding provides a reliable estimate of the dietary inclusion level that would maximize growth performance of *C. gariepinus* fingerlings when *M. alba* leaf meal is used as a feed additive. Survival was not significantly affected by dietary treatment, suggesting that all inclusion levels of *M. alba* leaf meal tested in this study were safe for *C. gariepinus* fingerlings.

CONCLUSION

This study demonstrated that dietary inclusion of *Morus alba* leaf meal significantly improved the growth performance and nutrient utilization of *Clarias gariepinus* fingerlings. Fish fed 1.5 g/100 g *M. alba* leaf meal (Ma4) recorded the highest weight gain, specific growth rate, feed efficiency ratio, and the best feed conversion ratio. Growth performance declined at 2.0 g/100 g, indicating that higher inclusion levels are counterproductive, likely due to the antinutritional effects of increased bioactive compound concentrations. Polynomial regression confirmed an optimum inclusion level of approximately 1.6 g/100 g. Based on these findings, *M. alba* leaf meal is recommended at 1.5 g/100 g diet for optimal growth performance and feed utilization in *C. gariepinus* fingerlings. Further studies should investigate finer inclusion intervals between 1.0 and 2.0 g/100 g and evaluate the effects of *M. alba* leaf meal in grow-out catfish to confirm and extend these findings for commercial production.

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