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Research Article

Therapeutic Efficacy of Maca *(Lepidium meyenii* Walp) Root as Growth and Pro-Fertility Agent on the Reproductive Indices of *Clarias gariepinus* Broodstock

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Abstract
Nutritional composition and dietary effects of medicinal plants on aquatic animals have been ascertained through the improvement of the sexual performance of the fish leading to the production of improved egg quality and enhancing the fertility of sperm. This research was assessed to determine the therapeutic efficacy of Maca Root Meal (MRM) on the growth and pro-fertility agent of *C. gariepinus* broodstock. Eighty *C. gariepinus* (400±0.01g) were used for the experiment. They were fed 5 compounded diets at different inclusion levels (0.0, 12.5, 25, 37.5 and 50%) and denoted as MRM_1 – MRM_5 respectively of 40% crude protein at 3% body biomass with 8 fish per treatment, replicated twice for 56 days. Measurements were taken on growth performance and reproductive indices at the end of feeding trials. The results revealed that body weight gain, feed conversion ratio and production productive index were significantly higher ($P<0.05$) in MRM 4 compared to the control and other treated groups. The reproductive indices such as milt volume, motility duration, sperm counts, the weight of ovary, relative fecundity, hatchability and percentage fertilization were significantly higher ($P<0.05$) in the treated groups compared with the control. MRM5 recorded higher hatchability and percentage fertilization of 81.78 and 84.27% compared to the control of 62.11 and 71.53% respectively. Conclusively, the study revealed the therapeutic potential of maca root meal as growth and pro-fertility agent and that the inclusion of 50% maca root meal in the diet of *C. gariepinus* broodstock could increase weight gain and better reproductive indices in male and female *C. gariepinus* broodstock.

Keywords: *Clarias gariepinus, Feed utilization, Growth performance, Maca root, Pro-fertility agent*

Introduction

Clarias gariepinus is one of the main fish produced in Nigeria for commercial purpose due to its characteristics such as tolerance to wide environmental condition, fast growth rate, resistance to diseases, feeding habits and high feed conversion ratio (Adesulu and Sydenham, 2007). The Nigeria high population reported to be above 140 million require 12.5kg per capita consumption of more protein. Unfortunately, they are costly and not within the reach of common man, hence fish is comparatively less in cost but also rich in protein could be very important alternative. Fry production has been a major problem in fish farming and the demand is higher than the supply. This necessitates r the production of good quality broodstock for the artificial propagation of fish. Fish with high-quality milt and eggs will enhance the productivity of fish seeds to meet the world protein needs (Adedeji *et al.*, 2006). Synthetic drugs like growth promoter and fertility enhancer have been discouraged by scientists because of their negative effects on fish, the environment, and consumers. Medicinal plants have been given

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awareness as alternatives to synthetic drugs because most plants are rich in antioxidant, antimicrobial, antifungal, growth promoter, and fertility enhancer that has little or no side effects on the organisms and human (Olusola and Olorunfemi, 2017; Dada and Ajilore, 2009).

Maca (*Lepidium meyeni*i) belongs to the family Brassicae and it has been used in developed countries as a nutrition and fertility enhancer. Maca is very rich in protein, amino acids, and minerals that helps in the production of estrogens and testosterone. Leon, (1964) has reported that maca possess the medicinal functions of fertility enhancer; improved sexual performance, anti-proliferative effects and growth promoter. However, information on the utilization of *L. meyenii* root on *C. gariepinus* as fertility enhancer and improve sexual performance has not been adequately documented. Therefore, this study was carried out to assess the efficacy of *L. meyenii* root on the growth and reproductive performance in male and female *Clarias gariepinus* broodstock.

Materials and Methods

Experimental Site: This study was carried out in the Research and Teaching Farm, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria. The experiment lasted for eight weeks (56 days) between February - April 2022.

Plant Source, Identification and Preparation: Maca roots were obtained separately from Tula in Kaltungo Local Government Area of Gombe State, Nigeria. They were identified and authenticated at the Department of Biological Sciences (Botany Programme) of Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria. Maca root obtained was washed separately with distilled water and sun-dried at 25℃ for two weeks after which it was ground into a fine powder and stored until required.

Determination of Phytochemical Analysis: Phytochemical constituents such as saponins, phenols¸ tannins, flavonoids, glucosinolates, steroids, anthraquinones and glucosinolates were done as described by Fakoya *et al.*, (2020)

Source of Experimental Fish: Eighty male and female *C. gariepinus* (mean weight 400±0.01g) were procured from Olokoabata Farm in Okitipupa, Ondo State, Nigeria. The broodstock was acclimatized for two weeks in a fibre holding tank at the Research and Teaching Farm, Olusegun Agagu University of Science and Technology, Okitipupa. During this period, they were fed commercial diets (blue crown, 6 mm) of 40% crude protein twice daily at 3% body weight.

Experimental Diets and Set up: The ground maca root at different inclusion levels of 0.0, 12.5, 25, 37.5 and 50 were added to feed ingredients such as fishmeal, soybean, groundnut cake, rice bran, maize, wheat offal, Di-calcium phosphate, salt, vitamin premix and vegetable oil to formulate 40% crude protein diet. Each diet mixture treated separately was extruded through a 6 mm mincer pelleting machine to form a noodle-like suitable size for the adult *C. gariepinus*. The pelleted diets were sun-dried, packed in labelled polythene bags and stored until needed (Table 1). Water was sourced from the borehole in the farm and 9 fiber-holding tanks were filled with water to a depth of 1.2 m each. The eighty *C. gariepinus* were stocked into the 9 fibre holding tanks containing eight (8) *C. gariepinus* (4 males and 4 females) per treatment tank. The fish were fed at 3% body biomass at 08:00hours – 09:00hours and 17:00hours – 18:00hours for 56 days. Each treatment was batch weighed fortnightly, their average weights were recorded and the daily feed intake for each treatment was readjusted accordingly.

Biological Evaluation: The growth performance and nutrient utilization indices were calculated as described by Olaifa and Bello, (2011).

Weight gain = Final body weight – initial body weight

$$\text{Weight gain (\%)} = \frac{(\text{Final body weight} - \text{initial body weight})}{\text{Initial body weight}} \times 100$$

Increase in standard length (CM) = L2 – L1

Where L2 = Final standard length; L1 = Initial standard length

$$\text{Specific growth rate (SGR)} = \frac{100\ (\log \text{final body weight} - \log \text{initial body weight})}{\text{Time (days)}}$$

Feed conversion ratio (FCR) =
$$\frac{\text{Dry weight of feed fed (g)}}{\text{Fish weight gain (g)}}$$

Survival rate (%) =
$$\frac{\text{Initial Number of Fish Stocked – Mortality}}{\text{Initial Number of Fish stocked}} \times 100$$

Condition factor (k) = 100W/L3
Where: W= weight of fish (g); L=standard length (cm)

Nitrogen metabolism = $\frac{(0.549)\ (a+b)h}{2}$
Where a = Initial mean weight of fish; b= final mean weight of fish; h= experimental periods in days.

Evaluation of Reproductive Indices: At the end of the feeding experiment, 2 each of males and females were randomly picked per dietary treatment for reproductive parameters, including milt volume, motility duration, sperm counts, egg size estimation, relative fecundity, percentage fertilization, percentage hatchability. Others such as the gonado-somatic index (GSI), the weight of ovary and belly diameter were calculated as described by Olusola *et al*., 2021; Dada, 2012; Rainis *et al.*, 2005; Mims, 1991

Artificial Propagation: Two (2) male and female spawners from each treatment were taken and used for artificial propagation techniques- hormone administration, stripping, fertilization and incubation as described by Olusola *et al*., (2022).

Statistical Analysis: One-way Analysis of variance (ANOVA) and descriptive statistics was performed using SPSS 22 for the window software package at ($P<0.05$) significance level. Significant means were subjected to multiple comparisons at α 0.05 level.

Results and Discussion

Phytochemical analysis of maca (*L. meyenii*) root: The *L. meyenii* root was evaluated for the presence of flavonoids, tannins, glucosinolates, saponins, alkaloids, anthraquinones and triterpenes and steroids. The different phytochemical components revealed the level of metabolites concentration which were recorded in Table 2 as detected in small quantity (+), moderate quantity (++) and absent (-).

Growth performance and nutrient utilization of *C. gariepinus* fed different graded levels of *L. meyenii* for 8 weeks: The results of this study show a general increase in the body weight of *C. gariepinus* during 8 weeks of feeding trials. The value of weight gain, specific growth rate, feed conversion ratio, nitrogen metabolism and production productive index was higher in the treated groups compared with the control and giving significant differences ($P<0.05$) among the treatments (Table 3).

The reproductive performance of male *C. gariepinus* fed dietary supplementation of *L. meyenii* for 8 weeks: The weight of testis was highest in MRM 5 (6.59g) and the lowest in MRM 3 (4.02g), the volume of milt was comparatively higher in the control (2.39 ml) and least in MRM 3 (1.19 ml). The motility duration was highest in MRM 5 (51 s) and lowest in MRM 2 (33 s) while the highest sperm counts were recorded in MRM 5 (664,000) as against (406,000) in the control (406,000) (Table 4a)

The reproductive performance of female *C. gariepinus* fed dietary supplementation of *L. meyenii* for 8 weeks: The female reproductive indices such as weight of ovary, belly diameter, gonado- somatic index, relative fecundity, hatchability and percentage fertilization were better in the treated groups compared to the control and there were significant differences ($P<0.05$) among the dietary groups (Table 4b).

The results of the phytochemical constituents show the presence of tannins, glucosinolates, saponins, alkaloids, triterpenes and steroids while flavonoids and anthraquinones were not. Alkaloids and glucosinolates recorded moderate amounts of phytochemical constituents of *L. meyenii* root. This study supports the report of Gonzales *et al*., (2005) who observed the presence of alkaloids, steroids, tannins, saponins and glucosinolates in *L. meyenii* root.

The result of growth performance and nutrient utilization of *C. gariepinus* fed different graded levels of maca root meal-based diet revealed a

general increase in the growth of the fish when compared with the value obtained before the experiment. There was a significantly higher weight gain among the treatment dietary groups, the treated groups recorded better weight gain when compared to the control. The value of percentage weight gain, specific growth rate, nitrogen metabolism and production productive index was higher in the treated groups compared with the control and there were significant differences ($P<0.05$) among the dietary groups. Also, it was observed that as the inclusion levels of maca root meal increased the value of weight gain, percentage weight gain, specific growth rate, nitrogen metabolism and production productive index increased among the dietary groups. This study supports the work of Olusola *et al.*, (2022) who reported better value of weight gain, specific growth rate and percentage weight gain in *C. gariepinus* fed different graded levels of *Carpolobia lutea* when compared to the control. The report of Shittu *et al.*, (2022) aligned with this present study and reported better weight gain and feed conversion ratio in the treated groups when compared to the control. The higher value recorded in the treated groups might be a result of phytoconstituents (saponins and flavonoids) found in the plant. A better feed conversion ratio was observed in the treated groups when compared to the control and this study supports the observation of Bello *et al.*, (2012) who found better feed conversion ratio in *C. gariepinus* fed different inclusion levels of walnut leaves and onion bulb when compared to the control. The MRM 4 recorded a better value of weight gain, specific growth rate, nitrogen metabolism and production productive index compared with the other treated groups and the control.

The result of male reproductive indices indicated that weight of testis, milt volume, motility duration, and sperm counts were higher in the treated groups compared to the control and they were significantly different ($P<0.05$) among the dietary groups. However, the MRM 5 recorded a better value of the weight of the testis, milt volume, motility duration, and sperm counts when compared to the other treated groups and the control. This study was in line with the work of Olusola *et al*., (2021) who observed better values of the weight of testis, sperm counts, milt volume and motility duration in *C. gariepinus* fed different inclusion levels of *Datura stramonium* (thorn apple) compared to the control. Also, this study supports the report of Adeparusi *et al*., (2010) that reported better milt volume, motility duration and sperm counts in *C. gariepinus* fed *Kigelia africana* when compared to the control. The study accords with the observation of Gonzales *et al*., (2001) that obtained better value on spermatogenesis, sperm count and sperm motility of male rats fed *L. meyenii* compared to the control.

The result of female reproductive indices shows that the weight of the ovary, number of eggs, and weight of eggs were higher in the control compared to the treated groups and were significant different ($P<0.05$) among the dietary groups. The value of belly diameter, size of eggs, gonado somatic index, relative fecundity, hatchability and percentage fertilization were higher in the treated groups compared to the control and they were significant differences ($P<0.05$) among the dietary groups. This study was aligned with the report of Dada, (2012); Dada and Ajilore (2009) that found better egg size, hatchability, percentage fertilization, relative fecundity, gonado somatic index in *C. gariepinus* fed *Garcinia kola* seed meal and ethanol extracts of *Garcinia Kola* respectively. However, the MRM 5 recorded a better performance in hatchability, percentage fertilization, and size of eggs when compared to the other treated groups and the control. The better value of reproductive indices recorded in the maca root-based supplemented diets might be a result of the higher content of antioxidants found in the plant. Most plants that are rich in antioxidants tend to increase sperm count and motility and enhance the production of estrogen and testosterone (Li *et al*., 2017). Macamides are specific alkamides of Maca that are known for their antioxidant effects (Zhao *et al.*, 2005; Chain *et al.*, 2014). The presence of alkaloids in maca and maca amides was reported to influence sexual performance (Li *et al*., 2017).

Conclusion

Results of this study indicated that growth performance and reproductive indices on the maca root supplemented dietary treatments were better than the control. The growth performance was observed to increase with the increased inclusion

of maca root meal-based diets. It is observed that maca root meal shows great potential as a growth promoter and pro-fertility agent to *C. gariepinus* broodstock and the inclusion of a rate of 37.5 - 50% will enhance fish productivity in organic fish farming.

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Table 1: Gross and proximate composition of experimental diets (g/100g)

INGREDIENTS	**CONTROL (0%)**	**MRM 2 (12.5%)**	**MRM 3 (25.0%)**	**MRM 4 (37.5%)**	**MRM 5 (50%)**
Soya bean	26.86	26.86	26.86	26.86	26.86
Groundnut cake	26.86	26.86	26.86	26.86	26.86
Fish meal	13.43	13.43	13.43	13.43	13.43
Yellow maize	8.62	8.62	8.62	8.62	8.62
Wheat offal	8.62	8.62	8.62	8.62	8.62
Rice bran	8.62	7.54	6.46	5.39	4.31
Starch	1.00	1.00	1.00	1.00	1.00
DCP	1.00	1.00	1.00	1.00	1.00
**Vit - min premix	2.00	2.00	2.00	2.00	2.00
Vegetable oil	1.00	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00	1.00
Methionine	1.00	1.00	1.00	1.00	1.00
Maca root meal	------	1.08	2.16	3.23	4.31
***Proximate composition (% DM)					
Moisture	6.08	5.98	5.92	5.98	5.89
Crude protein	40.01	40.00	40.02	40.01	40.02
Crude lipid	13.55	12.78	12.95	12.88	12.78
Ash	12.41	14.88	14.45	14.78	14.99
NFE	27.95	26.36	26.66	26.35	26.32
Gross Energy (KJ/g)	19.60	19.02	19.15	19.06	19.02

** Vit- min premix for vitamin and minerals premix. Each 2.kg of premix contain; 12.5 million international unit (MIU); D_3, 2.5 MIU; E, 40g; K_3 2g; B1,5.5g; BB6,5g; Niacin 55g; Calcium Pantothenate 11.5g; Chlorine chloride 500g; Folic acid, Biotin,0.08g; Manganese, 120g; Iron, 100g; Zinc, 80g, Copper,8.5g; Iodine, 1.5g; Cobalt,0.3g; Selenium, 0.12g; Anti-oxidant, 120g. ** Determined using standard methods [AOAC 2005). All samples were analyzed in triplicates. NFE= Nitrogen Free Extract = 100 - (Crude protein + Crude fibre + Lipid content + Moisture content + Ash), gross energy (KJ/g) = (protein content x 23.6) + (lipid content x 39.5) + (carbohydrate content x 17.2). *** Proximate composition of experimental diet using standard methods of AOAC, (2005), MRM = Maca Root Meal

Table 2: Determination of Phytochemical of *maca* (L. meyenii) root

Phytoconstituents	***C. procera***
Alkaloids	++
Saponins	+
Anthraquinones	-
Tannins	+
Flavonoids	-
Triterpenes and Steroids	+
Glycosinolates	++

+ = detected in low amount ++ = detected in moderate amount - = absent.

Table 3: Growth performance and nutrient utilization of *C. gariepinus* fed different graded levels of *L. meyenii* for 8 weeks

	Control	**MRM 2**	**MRM 3**	**MRM 4**	**MRM 5**
IBW (g)	400.00±0.00^{a}	400.00±0.01^{a}	400.00±0.00^{a}	400.00±0.00^{a}	400.00±0.01^{a}
FBW (g)	730.00±0.01^{a}	750.00±0.02^{b}	770.00±0.05^{d}	790.00±0.07^{e}	760.00±0.03^{c}
WG (g)	330.00±0.05^{a}	350.00±0.09^{b}	370.00±1.00^{d}	390.00±0.97^{e}	360.00±0.65^{c}
PWG (%)	82.50±0.02^{a}	87.50±0.61^{b}	92.00±0.67^{d}	97.50±0.98^{e}	90.00±0.01^{c}
SGR (g)	0.47± 0.00^{a}	0.49±0.03^{b}	0.51±0.02^{c}	0.53±0.04^{d}	0.50±0.00^{bc}
FCR	0.95±0.71^{d}	0.90±0.02^{c}	0.85±0.01^{b}	0.80±0.01^{a}	0.87±0.04^{b}
SR (%)	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
NM (g)	17370.36±5.07^{a}	17677.81± 3.98^{b}	17985.24±8.00^{d}	18292.68±10.00^{e}	19831.52±16.00^{c}
PPI	589.29± 1.20^{a}	625.00±1.80^{b}	660.72±0.99^{d}	696.43±0.78^{e}	642.86±0.86^{c}

Key: IBW= Initial Body Weight, FBW = Final Body Weight, WG = Weight Gain, PWG = Percentage Weight Gain, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, SR = Survival Rate, NM = Nitrogen Metabolism, PPI = Production Productive Index. The above values are mean triplicate data, mean values in each row with similar superscript are not significantly different (P > 0.05), MRM = Maca Root Meal

Table 4a: Reproductive performances of male *C. gariepinus* fed different graded levels of *L. meyenii* for 8 weeks

	Control	**MRM 2**	**MRM 3**	**MRM 4**	**MRM 5**
Weight of male (g)	730.00±0.01^{a}	750.00±0.02^{b}	770.00±0.05^{d}	790.00±0.07^{e}	760.00±0.03^{c}
Weight of testis (g)	6.10±0.73^{b}	4.36±0.16^{a}	4.02±0.08^{a}	5.26±0.67^{ab}	6.59±0.12^{b}
Milt volume (ml)	2.39±0.16^{a}	1.32±0.70^{a}	1.19±0.11^{a}	2.17±0.82^{a}	2.26±0.66^{a}
Motility duration (s)	36.50±1.50^{ab}	33.00±1.00^{a}	39.00±1.00^{b}	44.00±2.00^{c}	51.00±1.00^{d}
Sperm counts	406,000.00±4000^{a}	435,000±10000^{a}	419,000±1000^{a}	548,000±3000^{b}	664,000.00±16000^{c}

Key: The above values are mean triplicate data, mean values in each row with similar superscript are not significantly different (P > 0.05), MRM = Maca Root Meal

Table 4b: Reproductive performances of female *C. gariepinus* fed different graded levels of *L. meyenii* for 8 weeks

	Control	MRM 2	MRM 3	MRM 4	MRM 5
Weight of female(g)	730.00±0.01^{a}	750.00±0.02^{b}	770.00±0.05^{d}	790.00±0.07^{e}	760.00±0.03^{c}
Belly diameter (cm)	10.75±0.25^{a}	9.50±2.00^{a}	10.25±2.00^{a}	12.50±2.00^{a}	11.25±2.00^{a}
Weight of ovary (g)	153.70±5.00^{d}	88.75±1.58^{a}	109.48±1.00^{b}	195.71±2.00^{e}	118.44±1.85^{c}
Size of eggs (cm)	1.13±0.13^{a}	1.13±1.01^{a}	1.00±0.00^{a}	1.00±0.01^{a}	1.45±0.05^{b}
Number of eggs	142,55200±15.01^{e}	116,596.00±7.17^{c}	71,532±0.98^{a}	128,178.00±10.12^{d}	113,079.00±2.67^{b}
Weight of eggs (g)	147.57±10.91^{d}	120.70±8.42^{b}	74.05±1.67^{a}	132.69±9.08^{c}	117.06±6.89^{b}
GSI	19.40±0.44^{a}	13.95±0.03^{a}	17.56±1.02^{a}	31.81±4.14^{b}	17.56±0.35^{a}
Relative fecundity	179.89±1.70^{b}	183.41±3.75^{b}	114.64±4.59^{a}	208.09±24.96^{b}	167.76±6.21^{b}
Latency period (hr)	10.10±0.10^{a}	11.65±0.35^{b}	10.65±0.35^{ab}	10.65±0.35^{ab}	10.50±0.50^{ab}
Hatching time (hr)	26.00±0.00^{a}	36.00±0.02^{b}	26.00±0.01^{a}	36.00±0.04^{b}	24.00±0.02^{a}
Hatchability (%)	62.11±0.04^{a}	78.67±0.86^{cd}	70.39±0.06^{a}	73.39±0.15^{ab}	81.78±0.08^{d}
Fertilization (%)	71.53±2.00^{a}	82.19±1.50^{bc}	75.30±1.00^{ab}	80.85±2.00^{bc}	84.27±1.50^{c}

Key: The above values are mean triplicate data, mean values in each row with similar superscript are not significantly different (P > 0.05), MRM = Maca Root Meal