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Original research

Effect of adding garlic, probiotics and moringa on growth performance, food utilization and haematological parameters of grass carp fingerlings Ctenopharyngodon idellus (Valenciennes, 1844)

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Abstract:

The grass carb fingerlings (initial weight: 10+1.24 gm) were caught from earthen ponds and maintained in 12 glass aquaria (35 cm x 70 cm x 40 cm) in 4 treatments (3 replicates per treatment) control, Garlic (Ga) TGa, Probiotics (TPr) and Moringa (TMo) with stocking densities (10 fish/aquarium). The fish were fed 5 % of body weight (50% commercial pellet diet, 50% Azolla plant) and Garlic (Ga), Probiotics (Pr), Moringa (Mo) were used as food additives (1%/Kg diet) in the experimental groups. The diet TGa, Tpr and TMo gave the highest weight gain of (11.92±0.17, 12.09+0.22 and 12.00±0.11g) whereas, the lowest (10.36g) was in control diet after 90 days. The highest Specific growth rate (SGR) (1.31+0.01, 1.32+0.02 and 1.31±0.01) was recorded in treatment TGa, Tpr and TMo, respectively which was significantly different from control (1.18). The fish groups fed diet TGa, Tpr and TMo showed significantly ($P<0.05$) higher PPv, and PER followed in a significant ($P<0.05$) decreasing order by the Control diet. The group of carp fed on control diet had the lowest lipase (269.27+3.41), and Amylase (306.87+2.09b) while group TPr had the highest value (275.14±3.46) and (491.29±3.84) which was significantly higher ($p<0.05$) than the lipase and Amylase of the carp on the control diet.

Keywords: Azolla, Garlic, Probiotics, Moringa, lipase, Amylase

1- Introduction

Fish production is growing up rapidly across the globe now a day because it has been identified to be a high quality protein animal source, it has been found that intensive fish culture is required to meet the protein requirement as wild catch has been found insufficient.

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One of the problems militating against the development of this industry is the high cost of good quality fish feed. It is, therefore, necessary to focus now on replacing high-cost animal ingredients feed with high-quality natural plants. It may be beneficial for fish to diversify the sources of vegetable protein used as an additive to the feed (Hashem et al., 2017). And because plant protein sources contain antioxidants, antibacterials and antiviruses, as well as active biological compounds that are biodegradable, they are considered environmentally friendly (Sitarasu, 2010). Therefore, it can be said that all of these compounds activate the immune responses of fish and increase the demand for feed, as they activate enzymes. Digestive system when feeding fish on it (Sitarasu, 2010). The search for food additives to fish feed is still a necessary and important work for researchers in the field of aquaculture (Cho and Lee, 2012).

Recently, governmental policy makers and consumers criticized seriously the use of antibiotic growth promotors in animal and fish feeds for the potential harmful effects and development of microbial resistance to these products on human health (Williams and Losa, 2001Botsoglu and Fletouris, 2001; McCartney, 2002). Natural feed additives such as medicinal plants, Probiotic and prebiotic recently are used in poultry and fish diets to enhance the the immune response and performance of birds and fish.

In aquaculture fish combination of feed additives in fish diets objects to improve quality of flesh by improve fish performance and its immunity. Aquaculture researchers still searching for new feed additives (Cho and Lee, 2012). Garlic (*Allium sativum*) is from family Liliaceae used as traditional medicine and spice. it comprises various valuable combinations such as phosphorus, calcium, carbohydrates, silicates which positively affecting on the skeletal and vascular system, sulfur salts which have positive effects on cholesterolemia, skeletal system and control liver, iodine salts which positively affecting on the circulatory system, plus contains a lot of vitamins such as vitamin B complex ,A and C as well as linoleic acid (Dragan et al., 2008). Garlic is an antioxidant, antimicrobial (Kumar and Berwal, 1998) and antihypertensive agent (Konjufca et al., 1997). An important component of garlic is lysine which has an anthelmintic effect (Iqbal et al., 2001). Garlic contains bioactive compounds such as steroidal saponins, flavonoids, oil-soluble analogs, and water-soluble nutrients (Putnik et al., 2019) that have an effect on metabolism and the immune response system (Esmaeili, et al., 2017; Pourzand et al.., 2016; Saljoughian et al., 2018; Sharma, et al 2010; Talpur & Ikhwanuddin, 2012). Intensification of aquaculture fishes increases their exposure to pathogens, which affects the growth performance of fish and outbreaks of diseases, to prevent these diseases, antimicrobial drugs and antibiotics are given, which leads to the development of unwilling strain of bacteria, and thus decreases competence of antibiotics for fish (Lee, *et al* 2016) so in the Union countries of Europ since 2006, antibiotics banned (Weedman, 2009).Consequently , works are being made to use optimistic and eco-friendly methods for disease control and improve fish health, Thus, there is a newer concept to control the disease by use probiotics in aquacultur and increase feed efficiency. Probiotics are explained as alive microbial aid that has a useful effect on the host by rising growth performance and improving its nutritional, these agreeing to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).

Immunostimulants are factors that improve the immune system to ready the organism against attackers (Watanuki, et al 2006). Later, a lot of research work on complementing herbs and plants in fish feed motivates the immune system and improves growth (Srichaiyo et al., 2020). These plants and herbs are inexpensive and readily available (Reyes-Becerril, et al 2019). From the benefits of medicinal plants are improving immunity, antibacterial, and stimulating

growth (Van Hai, 2015). Moringa oleferia is from the moringa ceae family and is usually known as Sohanjna or drumstick tree. It is a deciduous tree, the average size is 10-12 meters. Moringa is found in sub-Himalayan tracts including India, Pakistan, Asia Minor, Arabia and Africa (Shahzad, et al 2013). Its leaves are plentiful in vitamins, mostly vitamin C, B (Ferreira, et al 2008) flavonoids and phenolic acids (Vergara et al , 2017) and minerals like calcium, zinc, iron, magnesium and copper (Gopalakrishnan, et al 2016), moringa comprise phytochemicals, inclusive teridpenos, flavonoids, tannins, anthocyanins and proanthocyanidins, it has antioxidant and immunostimulant possessions and antigenotoxic actions, also Moringa is rich in protein and essential amino acids, such as methionine, lysine, tryptophan, and cysteine. (Ogunsina *et al*., 2011; Hamed & El-Sayed, 2019).

The aim of this research is to study the effect of adding dietary garlic, probiotics and moringa on growth performance, food utilization and haematological parameters of grass carp fingerlings Ctenopharyngodon idellus (Valenciennes, 1844)

2- Materials and Methods

Study Area and Period

This study took place in El-shalal fish hatchery of the General Directorate of Water Resources and Irrigation in Aswan, during the period from December 2020 to January 2021.

Fish and Laboratory Condition

The grass carb fingerlings were caught approximately in the same size (initial weight: 10 grams) from earthen ponds and maintained in the laboratory in 12 glass aquaria (35 cm x 70 cm x 40 cm) containing 98L of filtered Nile water in 4 treatments (3 replicates per treatment) control, Garlic (Ga) TGa, Probiotics (TPr) and Moringa (TMo), with stocking densities (10 fish/aquarium).

Diet

The fish diet provided was a commercial floating pellets diet from Aller Aqua Egypt company containing (30% protein, 6% lipids, fibers 4.5%, total energy not less than 4100-kcal / Kg) as shown in table (1) and Azolla plant which was cultivated. Garlic (Ga), Probiotics (Pr) and Moringa (Mo) were used as food additives.

Table (1): Composition (%) of the experimental diets provided to Grass Carp.

Ingredients	Percent
Soybean	46%
Fish meal	60%
Corn gluten	60%
Protein	30%
Fiber	4,5 %
Total energy	4100 kilocalories / Kg diet

Water Quality

These aquaria were provided with aeration and running water. To keep water in good quality, the aquarium was cleaned and the water was changed weekly. Through the experiment, the water quality was maintained as shown in Table (2). To calculate the growth parameters of fish based on biometry of grass carb, At the beginning and the end of the experiment fish samples were taken for initial and final body weight.

Table (2): Water quality parameters (mean ± SD) after 90 days of feeding grass carb on experimental diets containing different diets additives.

	DO (mg/L)	PH	TC°
C	5.70±0.31[a]	7.31±0.13[a]	25.50±0.24[a]
TGa	5.40±0.26[a]	7.19±0.31[a]	26.07±0.29[a]
Tpr	5.85±0.17[a]	7.32±0.42[a]	25.41±0.12[a]
TMo	5.83±0.29[a]	7.28±0.28[a]	25.25±0.42[a]

Values within columns with the same superscript are not significantly different (P > 0.05).

Feed Analyze

The feed ingredients used were analyzed chemically, where crude fat was determined by the Soxhlet apparatus. Also, the protein was estemated by computing nitrogen (N×6.25) using the Kjeldahl method and ash by combustion at 550°C.

Feeding Rate

The fish were fed 5 % of body weight (50% commercial pellets diet, 50% Azolla plant) and Garlic (Ga), Probiotics (Pr), Moringa (Mo) were used as food additives in experimental groups with percentages 1 %/Kg diet.

Sampling and Analytical Methods

Blood samples were collected by inserting a syringe needle along the dorsal midline of fish and drawing blood from the vein, then the sample was sent to the laboratory on ice. Blood was placed in non-heparinized tubes and left to clot at 4°C for 15 min., Afterwards, tubes were centrifuged at 3000 rpm using an Eppendorf centrifuge for 10 min to obtain serumThe quantitative determination of serum glucose was carried out using commercially available diagnostic Experimental Protocols kits Pars Azmoon, Iran (1 500 0178), at 546 nm and 37°C by the glucose oxidase method according to Trinder. Serum total protein levels were determined using Pars Azmoon, Iran (1 500 028) kit, with bovine serum albumin serving as standard at 546 nm and 37°C.

Analytical Methods Body Composition

At the end of the experiment, some fish samples were selected randomly for taken tissue samples, rapidly frozen and stored at -20°C for composition analysis.

Statistical Analysis

Data were analyzed (one-way) by ANOVA to determine statistically significant differences, and the LSD0.05 test was applied when there were significant differences.The following calculations have been conducted:

Weight gain (g) = Mean final weight (g) − Mean initial weight (g)

Specific growth rate (SGR) (% day−1) = (Ln final weight (g) −

Ln initial weight (g)/(Period in days) ×100
Feed conversion ratio (FCR) = Feed intake (g)/Live weight gain (g)
Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g)
Survival rate % = (No. of harvested fish/No. of initial stocked fish) × 100

Results and Discussion

China shares the world's highest production of aquatic organisms. Grass carp is considered one of the most important types of fresh water spread in it. China's production of grass carp exceeds 5.5 million tons (FAO, 2019). In Egypt, grass carp is one of the organisms used in biological control of weeds in canals and agricultural drains.

The results of the growth performance of grass carp fishes are shown in (Tables 2, 4, 5). It is clear from the results that there is a significant difference between control and all treatments.

Table (3): Growth response and feed utilization (mean ± SD) of grass carb, after 90 days of feeding on experimental diets containing different diets additives.

	C	TGa	TPr	TMo
Survival	100%	100%	100%	100%
IBW	10+1.24	10+1.24	10+1.24	10+1.24
FBW	20.36±0.03^{b}	21.92±0.17^{a}	22.09±0.22^{a}	22.01±0.11^{a}
PPV	29.83±0.72^{b}	35.20±0.40^{a}	36.19±1.14^{a}	35.71±0.98^{a}
PER	1.36±0.00^{b}	1.55±0.03^{a}	1.58±0.03^{a}	1.55±0.01^{a}
SGR	1.18±0.01^{b}	1.31±0.01^{a}	1.32±0.02^{a}	1.31±0.01^{a}
FCR	1.75±0.01^{a}	1.54±0.03^{a}	1.51±0.03^{a}	1.54±0.01^{a}
FI	18.16±0.04^{a}	18.37±0.16^{a}	18.25±0.04^{a}	18.43±0.09^{a}
Gain	10.36±0.03^{b}	11.92±0.17^{a}	12.09±0.22^{a}	12.00±0.11^{a}

Values within columns with the same superscript are not significantly different (P > 0.05).

There were significant differences when using various feed additives in the growth rates in all treatments.The use of medicinal herbs as nutritional supplements, with their antioxidant content, protects aquatic organisms from many disease problems (Awad and Awaad 2017; Rajabiesterabadi *et al*. 2020). In the current study, garlic, probiotics and moringa were used as food additives to enhance the growth performance and utilization of food for grass carp fish, which eat a meal containing 50% of the Azolla plant as an alternative to feed.

The study conducted by Awad and Awaad, (2017) confirmed that the garlic plant Allium sativum is used all over the world and is characterized by having an effective medicinal effect as it is anti-oxidant, anti-cancer, anti-microbial, activates the liver, and strengthens the immune response. Etyemez Büyükdeveci *et al*., (2018); Talpur and Ikhwanuddin, (2012) shown that the adding of garlic increases the survival rate as well as improves the growth performance of different species of fish.

Francis, *et al*., (2001) confirmed that Moringa is the most valuable supply of crude protein, fiber and fat. Previous research studies also confirmed the effects of Moringa in stimulating growth and improving food utilization (Ozovehe, 2013; Ahmed *et al*., 2014).

Falowo *et al*., (2018), defined Moringa as “the miracle tree” it is a fast-growing species found in tropical and subtropical environments. Several studies have shown good positive effects

of adding Moringa to fish feed, including improving growth performance in tilapia (Elabd *et al*., 2019) and (Hussain *et al*., 2018a). Kaleo *et al*., (2019); and Mansour *et al*. (2020) noted that it improves digestion and utilization of feed for catfish. Moringa also increases the immune response and improves disease resistance (Monir *et al*., 2020).

The results of the present study are also approval with (Wang and Xu 2006) in common carp, (Eissa *et al*. 2021) in shrimp Koroma, *Penaeus japonicus* and (Kumar *et al*., 2006) in Indian carp as indicated by (Ziai-Nejad *et al*., 2006; Yu *et al*., 2008). The effect of positive probiotic supplementation on growth performance in shrimp, as well as in tilapia, (El-Haroun *et al*., 2006) and on poultry performance (Wang and Gu, 2010). This was also similar to the results (Pu *et al*. 2017; Yousefi *et al*. 2020) who used garlic as a food supplement for fish, which significantly enhanced growth.

The diet TGa, Tpr and TMo gave the highest weight gain of (11.92, 12.09 and 12.00g) respectively, while, the lowest (10.36g) were reported in control group Table (3) after 90 days.

To compare the growth performance on a daily basis, the value of the specific growth rate is calculated. The important and influential role of protein in the feed, the actual indicator of which is the high growth rate. The highest SGR 1.31, 1.32 and 1.31 was recorded in treatment TGa, Tpr and TMo respectively was significantly different from control 1.18 after 90 days of experiment which. As presented in Table (3) results told that the fish groups fed diet TGa, Tpr and TMo showed significantly ($P<0.05$) higher PPv, and PER followed in a significant ($P<0.05$) decreasing order by the Control diet.

During the experiment period, the quantities of feed were investigated as per fish requirements and given to fish. Fish that were fed on TMo had got The maximum amount of food (18.43g) followed by TGa (18.37g), TPr (18.25g), and control (18.16g) respectively.

It is noted from the results of the statistical analysis that the amount of feed given to fish was significantly higher than the TGa,, Tpr and TMo than control.

The results on FCR in different treatment groups have been depicted in Table (3). The lowest FCR was found with Tpr then Tga and TMo which was significantly different from control, The best FCR was for TPr, as can be seen from the mean values of the coefficients (1.51), followed by TGa and TMo (1.54), which were significantly higher as compared to control during 0-90 days of feeding experiment.

Water quality was the main factor in maintaining the survival rate of fish at 100% in the experimental ponds during the experimental period.The approximate composition of the whole body did not show significant differences with the different feed additives.

Table (4): Body composition (mean ± SD) of grass carb, after 90 days of feeding on experimental diets containing different diets additives.

	C	TGa	TPr	TMo
CM	79.05+0.29 [a]	79.13+0.24 [a]	79.00+0.16 [a]	79.39+0.19 [a]
CP	16.27+0.22 [a]	16.50+0.20 [a]	16.51+0.10 [a]	16.62+0.23 [a]
CF	2.03+0.04 [a]	1.97+0.19 [a]	2.01+0.10 [a]	1.92+0.11 [a]
Ash	2.56+0.68 [a]	2.41+0.58 [a]	2.48+0.05 [a]	2.07+0.30 [a]

Values within columns with the same superscript are not significantly different ($P > 0.05$).

Haemato-biochemical parameters (WBC, RBC counts and Hb concentration), mean globulin, blood glucose, albumin and total plasma protein levels of the grass carp fish, according

to their diets, are presented in Table (5). The hematological parameters of catfish were shown in a study conducted by (Ezekiel *et al*., 2016), and (Ayoola *et al*., 2013) that the addition of Moringa leaves as a nutritional supplement in the feed increases the efficiency of blood readings such as hemoglobin (Hb), white blood cell count (WBC) and packed cell volume (PCV).

Table (5): Changes in Haemato-biochemical parameters of grass carb (M±SE) fed diets supplemented with different diets additives. for 90 days.

	C	TGa	TPr	TMo
TP g/dl	3.78±0.13^{a}	3.79±0.07^{a}	3.75±0.08^{a}	3.69±0.08^{a}
Cholesterol mg/dl	287.63±0.57^{a}	226.67±2.46^{b}	232.53±2.76^{b}	235.54±3.41^{b}
Trigly	224.00±1.70^{a}	221.53±2.52^{a}	221.28±2.32^{a}	223.29±2.62^{a}
Amaylease	306.87±2.09^{b}	476.33±4.35^{a}	491.29±3.84^{a}	487.54±3.27^{a}
lipase	691.27±3.41^{a}	273.21±4.52^{a}	275.14±3.46^{a}	274.31±2.68^{a}
Albumin g/dl	1.32±0.12^{a}	1.36±0.08^{a}	1.13±0.01^{a}	1.22±0.05^{a}
Globulin g/dl	2.46±0.12^{a}	2.43±0.04^{a}	2.62±0.08^{a}	2.48±0.09^{a}
WBC	6.86±0.09^{b}	7.87±0.10^{a}	7.88±0.09^{a}	7.86±0.06a
RBC	1.86±0.01^{a}	1.82±0.06^{a}	1.89±0.04^{a}	1.71±0.07^{a}
MCV	168.81±1.17^{a}	174.05±4.47^{a}	167.12±2.99^{a}	184.38±8.21^{a}
MCHC	27.83±0.36^{a}	27.56±0.27^{a}	27.81±0.16^{a}	27.55±0.29^{a}
MCH	46.97±0.27^{a}	48.15±1.64^{a}	46.47±0.64^{a}	47.75±1.88^{a}
HCT	31.45±0.07^{a}	31.56±0.23^{a}	31.51±0.09^{a}	31.48±0.13^{a}
HB	8.75±0.09^{a}	8.73±0.05 ^{a}	8.76±0.07^{a}	8.67±0.06^{a}
Glucose	98.31±2.73^{a}	98.27±2.18^{a}	97.96±1.93^{a}	97.83±2.38^{a}

Values within columns with the same superscript are not significantly different (P < 0.05).

Fazio (2019) decided that the number of white and red blood cells is an important indicator of the health, physiological and immune response of fish. In this study, dietary supplementation with both Moringa, garlic and probiotics increased the number of white blood cells compared to control. This result is consistent with what was found by (Nya and Austin 2009) that adding garlic powder to rainbow trout fish feed led to an increase in the number of white blood cells, while it had no effect. on the number of red blood cells.

In the current study the group of carp fed on control diet had the lowest lipase (269.27), and Amylase (306.87) while group TPr had the highest value (275.14) and (491.29) which was significantly higher ($p<0.05$) than the lipase and Amylase of the carp on the control diet.

Similarly, blood cholesterol was markedly elevated in the carp on the control diet, while fish on other diets had the lowest blood cholesterol level.

Conclusion

The use of medicinal herbs such as garlic, probiotics and moringa as nutritional supplements, with their antioxidant content, protects aquatic organisms from many disease problems. And increases the survival rate as well as improves the growth performance, improves digestion and utilization of feed, increases the efficiency of blood readings of and improves of immune response of grass carp fingerlings *Ctenopharyngodon idellus*.

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