

Assessment of a New Local Prebiotic Impacts on the Reproductive Efficiency of African Catfish (*Clarias gariepinus* Burchell, 1822) Brood Stock

Mehrim A.I.^{1*}; M.A. Abdelhamid¹; S.M. Ibrahim² and A.I. Abd El-Wahab¹

1. Animal Production Department, Faculty of Agriculture, Mansoura University, Al-Mansoura, Egypt.

2. Salah Ibrahim Fish Hatchery, Kafr El-Sheikh, Egypt.

*Corresponding Author

ABSTRACT

African catfish, *Clarias gariepinus*, is a highly appreciated species for aquaculture. In recent years, there is a considerable interest in the use of prebiotics as functional feed supplement in fish culture. The present study was conducted to evaluate the effect of a new local dietary prebiotic (T-Protophyt 2000) on reproductive performance of African catfish (*C. gariepinus*) brood stock for 12 week. The dietary treatments (T₁, T₂, T₃ and T₄) containing the prebiotic at the tested levels (0, 1, 2 and 3 g / kg diet, respectively), where eight concrete ponds (each of 4 m³) were used, 2 replicates (ponds)/ treatment. A total of 48 brood stocks of African catfish with an average weight (190 ± 0.50 g) were stocked at six fish per pond at a sex ratio of 1: 2 male: females to avoid the cannibalism among males. Results revealed that the 4th treatment (3 g T-Protophyt 2000 / kg diet) realized significantly (P ≤ 0.05) the best values comparing with other prebiotic levels (T₂ and T₃) or the control treatment (T₁), concerning final body weight, total body weight gain, specific growth rate, feed intake, feed conversion ratio, protein efficiency ratio, blood hematological parameters (Hb, RBCs and WBCs), ovarian length, ovarian specific gravity, absolute fecundity, condition factor, serum progesterone of the female catfish, as well as histological development of both fish gonads (ovaries and testes). So, it could be concluded that the new local prebiotic T-Protophyt 2000 is useful for enhancing fish growth, feed utilization, blood hematological profile, reproduction efficiency (especially for female) and muscular structure, besides histological development of fish gonads (ovaries and testes); therefore, it may be used in African catfish hatcheries, it is useful also from the economic point of view.

Keywords: Catfish – Prebiotic – Reproduction – Fecundity – Histology.

INTRODUCTION

African catfish *Clarias gariepinus* is one of the most important cultured fish in tropical and subtropical regions (Adewolu *et al.*, 2008). This species is known for its high growth rate, resistance to handling and stress, relatively low requirements for water quality, amenability to high stocking densities, excellent meat quality and preference amongst consumers in many African countries (Hecht *et al.*, 1996). Culture of African catfish has received considerable

attention since the early 1970s and 1980s (Micha, 1973 and Clay, 1981). From the biological and socioeconomic points of view, the African catfish is highly suitable for aquaculture, with good prospects for both developing and developed countries (Yamamoto *et al.*, 2000). In aquaculture, seed production is managed by control of fish reproduction in captivity using hormonal and environmental techniques (Zohar and Mylonas, 2001). Some problems are facing the wide spreading of catfish culture, e.g. low seasonal natural

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reproduction. African catfish has been noted that farming is hardly imaginable without availability of fish seed (Chondar, 1980). Since, need for high quality fish seed has necessitated research into various ways of enhancing fertility to meet the growing demand (Adewumi *et al.*, 2005). In addition, latest fish production statistics in Egypt revealed that African catfish production reached about 43292 tonnes (about 3.18 percent of the total aquaculture production in 2011, GAFRD, 2011).

A prebiotic was originally defined as “A nondigestible food ingredient(s) that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” (Gibson and Roberfroid, 1995). Various mechanisms have been proposed to explain the specific action of prebiotics, such as selective stimulation of beneficial microbiota, improvement of immune functions, disease resistance, survival, growth performance and feed efficiency (Manning and Gibson, 2004). Recently, prebiotics have shown promise as preventive and environment-friendly alternatives to antibiotics in aquaculture (Dimitroglou *et al.*, 2010). As natural products pro- and prebiotics have much potential to increase the efficiency and sustainability of aquacultural production (Denev *et al.*, 2009). Many attempts demonstrated positive effects of probiotics and prebiotics in feeds for various fish species, including rainbow trout (*Oncorhynchus mykiss*; Staykov *et al.*, 2009), common carp (*Cyprinus carpio*; Denev, 2008), Indian major carp (*Labeo rohita*; Nayak *et al.*, 2007); Mozambique tilapia (*Oreochromis mossambicus*; Logambal *et al.*, 2000), Nile tilapia (*Oreochromis niloticus*; Pirarat *et al.*, 2006; Abdel-Tawwab *et al.*, 2008 and Abdelhamid *et al.*, 2012), African catfish (*Clarias gariepinus*; Essa *et al.*, 2011), channel catfish (*Ictalurus punctatus*; Welker *et al.*, 2007 and Zhu *et al.*, 2012); European catfish (*Silurus glanis*; Bogut *et al.*, 2006).

Currently, many pro- and prebiotics are successfully used for growth and health management in the sustainable aquaculture industry (Yousefian and Amiri, 2009), but still using of prebiotics is very limited in fishes, compared to that in poultry (Patterson and Burkholder, 2003), as well as the results of feeding prebiotics to fish are inconclusive (Dimitroglou *et al.*, 2010). Furthermore, the prebiotic effect on the reproductive performance has not been extensively investigated in fish. So, the present study was designed to evaluate the effects of a dietary new local prebiotic (T-Protophyt 2000) on the reproductive performance of African catfish *C. gariepinus* brood stock for 12 week.

MATERIALS AND METHODS

Experimental management and treatments

The present study was conducted in the research farm of Dr. Salah Ibrahim's Hatchery, Alriad Center, Kafr El-Sheikh governorate, Egypt, to evaluate the effect of a new local prebiotic (T-Protophyt 2000) on reproductive performance of brood stock African catfish (*C. gariepinus*) for 12 week. Eight concrete ponds (each of 4 m³) were used, two replicates (ponds) / treatment, which were supplied by Nile water. A total of 48 brood stock African catfish with an average weight (190 ± 0.50 g) were stocked at six fish per pond at a sex ratio of 1: 2 males: females to avoid the cannibalism among males. The dietary treatments (T₁, T₂, T₃ and T₄) containing the prebiotic at the tested levels (0, 1, 2 and 3 g / kg, respectively). A commercial floating feed (manufactured by Hendrix, Egypt factory, contained 32% crude protein, 4.51% crude fat, 4.48% crude fiber, and 4160 kcal / kg gross energy, and consisted of fish meal 72%, soybean meal 44%, wheat bran, corn gluten 60%, monocalcium phosphate, lime stone, soya oil, L-lysine and vitamins and minerals mixture) was offered to fish at two meals daily (one third at 10 a.m. and 2 thirds at 10 p.m.) at 3% of the fish biomass. Corn oil was used at 2% of the diet to distribute the graded levels of the tested

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prebiotic (T-Protphyt 2000) on the experimental diet. T-Protphyt 2000 prebiotic is a new local product with a patent No. 23593 at 14/1/2004 under the name "Formula from a group of enzymes, dried fungi, and zinc salts". It consists of 15% zinc salts, 10% inorganic phosphorus, 5% dried fermentation products of *Aspergillus oryzae* growth, and starch as carrier up to one kilogram. Each gram of this product contains 100 unit of phytase, 75 unit of protease, 25 unit of lipase, and 15 unit of amylase.

Fish growth performance and feed utilizations

At the start and at the end of the experiment, fish samples were collected and kept frozen till the proximate analysis of the whole fish body according to AOAC (2000). Their gross energy contents were calculated according to NRC (1993), being 5.65, 9.45 and 4.22 kcal / g protein, fat, and carbohydrate, respectively. Also, at the end of the experiment, blood samples were collected from the fish caudal peduncle of the different groups. Fish growth performance and feed utilizations parameters were estimated at the end of the experimental period according to Abdelhamid (2009).

Blood samples

Blood samples were collected at the end of the experiment, fish in each aquarium were weighed and 5 fish were taken randomly for blood sampling. Blood samples were received in plastic tubes. Blood serum was isolated by centrifugation for 20 minutes at 4000 rpm. Serum samples were kept in deep freezer (-20 °C) till the biochemical analysis.

Hematological parameters

Red blood cells count (RBCs $\times 10^6 / \text{mm}^3$) and white blood cells (WBCs $\times 10^3 / \text{mm}^3$) were counted by using an A₀ Bright-Line Häemocytometer (Neubauer improved, Precicolor HBG, Germany). Hemoglobin was determined by using commercial colorimetric kit (Diamond Diagnostic, Egypt).

Serum biochemical parameters

Blood serum biochemical constituents were determined calorimetrically using commercial kits produced by Diagnostic System Laboratories, INC, USA. Serum cholesterol determination was carried out according to the method described by Trinder (1969). Also, blood serum determinations for the sex steroid hormones, progesterone and testosterone were done using commercial ELISA test kits catalog No., BC-1113 (BioCheck, Inc), and BC-1115 (BioCheck, Inc), respectively according to Tietz (1995).

Fish reproductive parameters

The brood stock fish (females and males) were killed at the end of the experiment and soon the abdominal cavity was opened to remove gonads which were weighed individually, and their length as well as size and density were measured and calculated. Gonadosomatic index (GSI) was calculated as $\text{GSI} = \text{gonads weight} \times 100 / \text{fish weight}$ (Tseng and Chan, 1982). Total egg weight and number per female as well as individual egg weight and diameter were measured too. Eggs number was counted using 1 mm insulin syringe, then related to ovary weight and body weight of fish. Absolute fecundity (AF, number of eggs / female) and relative fecundity (RF, number of eggs / g female weight) were calculated according to Bhujel (2000). Fish measurements were taken for the nearest 0.1 g and 0.01cm to calculate the condition (K) factor according to Ricker (1975).

Histological and histometric examinations

At the end of the experiment, fish were sacrificed and the target organs (gonads) and dorsal muscles were sampled. Samples were fixed in 10% neutralized formalin solution followed by washing with tap water, then dehydrated by different grades of alcohol (70, 85, 96 and 99%). Samples were cleared by xylene and embedded in paraffin wax. The wax blocks were sectioned to six micron. The

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sections were stained by hematoxyline (H) and eosin (E) and then subjected to a histological examination for gonads according to Roberts (2001) and histometric examination for dorsal muscles according to Radu-Rusu *et al.* (2009).

Statistical analysis

The obtained data were statistically analyzed using SAS (2001) procedures for personal computer, where ratio and percent data were arcsine transformed prior to analysis. When F-test was positive, least significant difference ($P \leq 0.05$) was calculated for the comparisons among means, which were made by using Duncan multiple ranges' test (Duncan, 1955).

RESULTS AND DISCUSSION

1- Growth performance and feed utilizations

Prebiotic compound is referred to as a non-digestible feed ingredient that beneficially affects the host by stimulating the growth and improving its intestinal balance (Ringø *et al.*, 2010). The gradual increases of the prebiotic levels led to significant ($P \leq 0.05$) increases (proportional to the increase of prebiotic level) in the final body weight, total weight gain (TWG) and specific growth rate (SGR, %/d). Therefore, T₄ (3 g/kg feed) was the best treatment, followed by T₃, T₂ and lastly the control T₁. The same trend was recorded for the feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER), Where the best treatment was T₄ and the worst was T₁ (Table 1). Since the first use of prebiotics in aquaculture, a growing number of studies have demonstrated their ability to increase the growth rate and feed utilization of farmed fish, hybrid tilapia (Genç *et al.*, 2007 a&b), Crucian carp (Xu *et al.* 2009), African catfish (Mahious and Ollevier 2005 and Essa *et al.*, 2011), rainbow trout (Salamatdoustnobar *et al.*, 2011), European sea bass (Torrecillas *et al.*, 2007).

As the present findings regarding the positive effects of tested prebiotic on the growth

performance and feed efficiency of African catfish, Essa *et al.* (2011) reported that adding high level of dried yeast, 2%, recorded highest final body weight, average daily gain, total production and profit per cage of African catfish (*C. gariepinus*). Also, they added that FCR was lower in the treated group than the control one, where yeast is a source of nucleic acids and β -1,3-glucans which have been recognized to effectively enhance immune functions of African catfish (Yoshida *et al.*, 1995). Additionally, Uys and Hecht (1987) concluded that *C. gariepinus* is physiologically equipped to utilize infrequent and irregular meals effectively, since *C. gariepinus* has no intrinsic digestive enzyme cycle, but it has a relatively rapid digestive enzyme secretory response, as well as the positive effects of prebiotic on the growth performance and feed utilization of fish may be associated with its source of nutrients and enzymatic contribution to digestion (Sakata, 1990). Furthermore, the beneficial influence of tested prebiotic on growth was possibly due to an alteration of the intestinal microflora. In addition, the fish gut microbiota played a role in host health, and the establishment of a normal gut flora could be regarded as complementary to the establishment of digestive enzymes (Ringø and Gatesoupe, 1998).

Conversely, high levels of prebiotics may yield harmful influences on the performance and health status of fish (Olsen *et al.*, 2001). In contrast to positive effects of tested prebiotic, studies by Genç *et al.* (2006) on African catfish (*C. gariepinus*) that treated with different levels of dietary mannan oligosaccharide prebiotic (1, 2 and 3%) for 80 days, also Peterson *et al.* (2010) did not observe improvement regarding weight gain in juvenile channel catfish (*Ictalurus punctatus*) by adding Bio-MOS[®] prebiotic. Recently, Hernández *et al.* (2012) also reported that the use of commercial prebiotics (FLAVOXIN[®] and UNIWALL MOS 50[®]) at 2g / kg diet for each has a positive effect on survival of silver catfish (*Rhamdia quelen*),

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Table 1: Effect of the tested prebiotic on the growth and feed utilization of African catfish

Treatment	Initial weight (g)	Final weight (g)	TWG (g)	SGR (%/d)	FI (g)	FCR	PER
T ₁	190.0	333.7 ^b	143.7 ^b	0.61 ^b	510.0 ^b	3.56 ^a	0.84 ^b
	±0.00	±7.10	±5.02	±0.02	±0.40	±0.17	±0.04
T ₂	192.5	352.5 ^b	160.0 ^b	0.66 ^b	512.4 ^b	3.21 ^{ab}	0.94 ^b
	±0.50	±5.00	±3.18	±0.01	±4.35	±0.12	±0.03
T ₃	193.0	377.9 ^a	184.9 ^a	0.73 ^a	528.5 ^a	2.86 ^{bc}	1.05 ^a
	±0.00	±2.90	±2.05	±0.01	±1.65	±0.05	±0.02
T ₄	192.0	396.2 ^a	204.2 ^a	0.79 ^a	540.2 ^a	2.65 ^c	1.13 ^a
	±0.00	±4.60	±3.25	±0.01	±6.50	±0.03	±0.01

Means in the same column with different superscripts are significantly ($P \leq 0.05$) different.

T₁, T₂, T₃ and T₄ are the dietary treatments containing the prebiotic at the prospective levels (0, 1, 2 and 3 g / kg, respectively).

without modifying growth parameters. Generally, the intake of prebiotic is primarily dependent on the types of ingredients used in diet formulation and will therefore vary widely among species and diets. Considerations in supplementing prebiotics in fish diets have been arisen to some extent. The type of prebiotic to supplement specific animal characteristics (species, age, stage of production) and type of diet are important considerations (Burr *et al.*, 2005).

2- Blood hematological parameters

Dietary yeast may improve growth performance and hematological picture in fish (Kobeisy and Hussein, 1995). Blood hematological parameters of female African catfish affected by tested prebiotic were illustrated in Table 2, where the positive effects of dietary prebiotic on hematological parameters (Hb, RBCs and

WBCs) increased significantly ($P \leq 0.05$) by increasing levels of tested prebiotic, since the treatment No. 4 was the best among all treatments. The increased count of WBCs may be caused by protein resorption (Merck, 1986). In this context, erythrocytic counts and hemoglobin content, total serum protein, A/G ratio and phagocytic activity in *O. niloticus* fed the diets containing *Micrococcus* species were higher than the control group (Osman *et al.*, 2010), also leucocytes play an important role in non-specific or innate immunity and their count can be considered as an indicator of the health status of fish (Roberts, 1978).

In agreement with the present hematological findings, Nile tilapia fed diets containing 1.0 – 5.0 g yeast / kg exhibited higher RBCs, Hb, and Ht values, where these results

Table 2: Effect of the tested prebiotic on the blood hematological measurements of the female African catfish

Treatment	Hb (g/dl)	RBCs (X10 ⁶ / mm ³)	WBCs (X10 ³ / mm ³)
T ₁	7.68 ^{bc} ±0.17	2.22 ^{bc} ±0.05	873.8 ^b ±12.48
T ₂	7.10 ^c ±0.50	2.20 ^c ±0.07	697.5 ^c ±22.50
T ₃	8.35 ^{ab} ±0.09	2.44 ^{ab} ±0.11	877.5 ^b ±19.31
T ₄	8.75 ^a ±0.10	2.56 ^a ±0.04	937.5 ^a ±13.15

Means in the same column with different superscripts are significantly ($P \leq 0.05$) different.

T₁, T₂, T₃ and T₄ are the dietary treatments containing the prebiotic at the prospective levels (0, 1, 2 and 3 g / kg, respectively).

suggest an improvement of fish health when fed a yeast supplement (Abdel-Tawwab *et al.*, 2008). Recently, Ebrahimi *et al.* (2012) reported that haematological (Ht and WBC) parameters and plasma total protein concentration were significantly higher ($P < 0.05$) in *Cyprinus carpio* fed diets containing 1.5 and 2.5 g/kg of commercial prebiotic, Immunogen in relation to the control. Additionally, haematocrit values and the proportion of lymphocytes were significantly higher in the 2% oligofructose prebiotic group than in the control group (Hoseinifar *et al.*, 2011).

The present result contrasts the results that have been reported by Welker *et al.* (2007), which showed that hematological parameters (e.g., RBC, Hct and Hb) were not affected in channel catfish (*Ictalurus punctatus*) fed 0.2% mannanoligosaccharide (MOS) prebiotic, and Sado *et al.* (2008) who reported that 0.2–1% MOS had no effect on hematological parameters of tilapia (*O. niloticus*).

3- Reproductive efficiency

3.1- Female

Data in Tables 3 and 4 show the reproductive performance parameters of the African catfish as affected by the graded levels of the dietary inclusion of the tested prebiotic. There were significant ($P \leq 0.05$) effects on either of egg diameter, ovarian specific gravity and ovarian length (Table 3), absolute fecundity and K-factor (Table 4), where T₄ (3g prebiotic / kg diet) was the best treatment regarding to

these parameters as compared with other treatments. However, no significant ($P > 0.05$) effects of the prebiotic inclusion on both of egg number per gram, ovary weight (Table 3), relative fecundity, gonadosomatic index (GSI %) and progesterone (Table 4) among all treatments. In this respect, **Bart and Dunham (1996)** reported that the largest egg mass produced the lowest ($P \leq 0.05$) fertilization rate. Yet, **Abasali and Mohamad (2011)** indicated that GSI, fry production and relative fecundity were significantly ($P < 0.01$) higher in platy fish (*Xiphophorus maculatus*) fed high level (1.5%) of prebiotic Immunogen as compared to the control group and other experimental groups. One of the most important parameters in the fish farming industry is measuring fish length and then fish grading. Today, quality along with quantity is considered in developed countries and producers used condition factor (k) for fish quality evaluation (**Balcazar *et al.*, 2004**).

In the present study, improved reproductive performance of female African catfish may be potential related with tested prebiotic (T-Protphyt 2000) components, where its ingredients, dried fermentation products of *Aspergillus oryzae* growth, enhance nutrition by synthesizing essential salts (zinc salts, inorganic phosphorus) and enzymes (phytase, protease, amylase and lipase). Also, related with the improved physiological responses in the female African catfish by tested prebiotic, which were detected as the hematological parameters (Table 2).

Table 3: Effect of the tested prebiotic on some ovarian measurements of the female African catfish

Treatment	Egg diameter (mm)	Egg number /g	Ovarian specific gravity (g/cm ³)	Ovarian length (cm)	Ovarian weight (g)
T ₁	0.81 ^{bc} ± 0.03	1100 ± 20.4	82.00 ^a ± 7.79	7.75 ^c ± 0.48	82.35 ± 15.02
T ₂	1.08 ^{ab} ± 0.09	1225 ± 110.9	68.25 ^{ab} ± 11.24	9.50 ^{ab} ± 0.29	76.25 ± 2.39
T ₃	0.67 ^c ± 0.19	1041 ± 39.7	52.50 ^b ± 4.79	8.38 ^{bc} ± 0.69	59.98 ± 1.78
T ₄	1.20 ^a ± 0.09	1163 ± 68.8	83.75 ^a ± 2.39	10.13 ^a ± 0.31	84.85 ± 2.56

Means in the same column with different superscripts are significantly ($P \leq 0.05$) different.

T₁, T₂, T₃ and T₄ are the dietary treatments containing the prebiotic at the prospective levels (0, 1, 2 and 3 g / kg, respectively).

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Table 4: Effect of the tested prebiotic on the reproductive efficiency, condition factor, gonado-somatic index and serum progesterone of the female African catfish

Treatment	Absolute Fecundity	Relative Fecundity	K-factor	GSI (%)	Progesterone (ng/ml)
T ₁	89818 ^a ± 15072	272.4 ± 44.25	0.77 ^b ± 0.03	24.97 ± 4.43	5.33 ± 0.23
T ₂	93000 ^a ± 7153	288.5 ± 22.56	0.74 ^b ± 0.02	23.64 ± 0.65	7.48 ± 1.98
T ₃	62568 ^b ± 3781	196.8 ± 7.27	0.74 ^b ± 0.02	18.91 ± 0.15	7.59 ± 0.29
T ₄	98165 ^a ± 3538	242.1 ± 18.31	0.87 ^a ± 0.03	20.76 ± 0.51	10.08 ± 2.33

Means in the same column with different superscripts are significantly ($P \leq 0.05$) different.

T₁, T₂, T₃ and T₄ are the dietary treatments containing the prebiotic at the prospective levels (0, 1, 2 and 3 g / kg, respectively).

3.2- Male

Data in Table 5 show no significant differences among all treatments on the testes measurements, GSI, condition factor, serum cholesterol and testosterone of the male African catfish. With the increased level of dietary prebiotic inulin, the mean values of cholesterol concentration increased in great sturgeon (*Huso huso*, Ahmdifar *et al.*, 2011). However, in contrast to these findings, previous studies have reported that dietary inulin had no effect on serum cholesterol levels of beluga fish (Akrami, 2007) or rainbow trout (Akrami *et al.*, 2007). Also, findings with those reported by Assadi (2009) showed that 0.5, 1, 1.5 and 2 kg per ton of the *Saccharomyces cerevisiae* yeast periodic supplement increased the length in the rainbow trout, where the fish length is the best indicator of the production farm efficiency and therefore, in this study, it was investigated.

Generally, the improved health condition in the African catfish is probably due

to the tested prebiotic components. A number of similar outcomes were recorded in previous attempts (Santarém *et al.*, 1997 and Li and Gatlin, 2004). Moreover, the beneficial influence of tested prebiotic (T-Protphyt 2000) on reproductive performance of African catfish was may be due to alteration of the fish intestinal microflora and improving the beneficial bacteria growth by T-Protphyt 2000 ingredients, since dried fermentation products of *Aspergillus oryzae* growth enhance nutrition by synthesizing essential salts (zinc salts, inorganic phosphorus) and enzymes (phytase, protease, amylase and lipase). Where, zinc is required for healthy reproductive, immune function, tissue repair and renewal (Gatlin, and Phillips, 1989), also it is required for normal testicular development (Merck, 1986), as well as, reduced reproductive performance has been observed in both males and females fed zinc deficient diets (Underwood and Suttle, 1999). In addition, Abasali and Mohamad (2011) demonstrated that

Table 5: Effect of the tested prebiotic on the testes measurements, gonado-somatic index, condition factor, serum cholesterol and testosterone of the male African catfish

Treatment	Length (cm)	Weight (g)	GSI (%)	K-factor	Cholesterol (mg/dl)	Testosterone (ng/ml)
T ₁	4.25 ± 0.75	3.23 ± 0.35	0.96 ± 0.10	0.63 ± 0.02	132.50 ± 7.50	2.31 ± 0.88
T ₂	4.25 ± 0.25	3.25 ± 0.45	0.96 ± 0.20	0.68 ± 0.06	124.00 ± 12.0	2.60 ± 0.44
T ₃	4.50 ± 0.50	3.30 ± 1.00	1.00 ± 0.15	0.64 ± 0.01	116.00 ± 17.0	3.14 ± 0.05
T ₄	5.50 ± 0.50	3.40 ± 0.40	1.03 ± 0.14	0.69 ± 0.03	99.25 ± 2.75	3.83 ± 0.96

T₁, T₂, T₃ and T₄ are the dietary treatments containing the prebiotic at the prospective levels (0, 1, 2 and 3 g / kg, respectively).

platy fish (*Xiphophorus maculatus*) fed dietary prebiotic Immunogen at high level (1.5%) favorably influenced GSI, fry production, relative fecundity and fry survival as compared to the control group and other experimental groups.

4- Histological examination of the gonads

4.1- Ovary

The histological examination in ovaries of African catfish *C. gariepinus* brood stock fed the basal ration (control, T₁) showed normal structure of ovarian lamellae, which contain oocytes at various stages of oogenesis (Fig. 1a). Yet, Figure (1b) showed oocytes in primary stage. However, fish fed dietary prebiotic T-Protphyt-2000 at level of 1g/kg (T₂) showed oocytes in late vitellogenic stage (Fig. 1c). From other hand, fish fed prebiotic at level 2g/kg diet (T₃) showed oocytes in yolk vesicles (YV) and yolk globules (YG) stages (Fig. 1d). Meanwhile, fish fed prebiotic at level 3g/kg (T₄) showed normal structure of ovarian lamellae, in migratory nucleus (MN) stage (Fig. 1e). The histological characteristics of ovaries of African catfish brood stock revealed the presence of different development stages of oocytes, which were accordingly with those reported by Wallace (1985) and West (1990). Whereas, major developmental events can be divided into six phases: oogenesis, primary oocyte growth, cortical alveolar stage, vitellogenesis, maturation and ovulation (Tyler and Sumpter, 1996). Similar structures were reported by Hussein (1984).

So, in the present study, results of African catfish fed T-Protphyt-2000 prebiotic at levels of 1g/kg (T₂), 2g/kg (T₃) and 3g/kg (T₄) revealed the superiority in development stages of oocytes compared with the control treatment (T₁). This superiority of dietary prebiotic may be due to its role to enhance host enzyme secretion which, increase the digestive efficacy of the complex proteins and lipids included in the diet; thus, increasing feed digestion and absorption by the host (Ringø *et al.*, 2010). Besides, its role in

improving ovarian measurements (Table 3), absolute fecundity and physiological responses, and serum progesterone level (Table 4) than the control treatment (T₁). Additionally, there were positive correlations between the presence of proteins and fatty acids in the brood stock diet and reproductive-related factors such as better oocyte development and maturation, higher rate of vitellogenesis and larger egg size (Ling *et al.*, 2006). Moreover, Abasali and Mohamad (2011) reported that the dietary prebiotic Immunogen could enhance the reproductive performance of platy fish (*Xiphophorus maculatus*) brood stock during reproductive stages.

4.2- Testes

Testes of African catfish brood stock fed the basal ration only (T₁, as a control) showed normal structure of seminiferous tubules with little spermatocytes (Fig. 2a). However, dietary prebiotic Protphyt-2000 at level of 1 g / kg (T₂) caused histological maturation of testis in treated fish, that showed normal structure of seminiferous tubules filed with spermatocytes (scy) and spermatids (st) (Fig. 2b), which cleared by microscopic high magnification (Fig. 2c). However, fish fed 2 g prebiotic / kg diet (T₃) showed normal structure of seminiferous tubules filed with spermatozoa (spz) (Fig. 2d), whereas microscopic high magnification revealed mature spermatozoa within the lumen of seminiferous tubules (Fig. 2e). Meanwhile, African catfish fed prebiotic at level of 3 g / kg diet (T₄) showed normal structure of seminiferous tubules and lumen filed with spermatids (st) and spermatozoa (spz) (Fig. 2f). So, it could interpretate that all of these histological development in testis of experimental fish are due to dietary supplementation of prebiotic and also are confirmable with those of the higher male GSI (%) and higher concentration of testosterone hormone of fish fed dietary prebiotic as compared with the control group (Table 5).

Several stages of spermatogenesis (spermatocytes, spermatid and spermatozoa) in

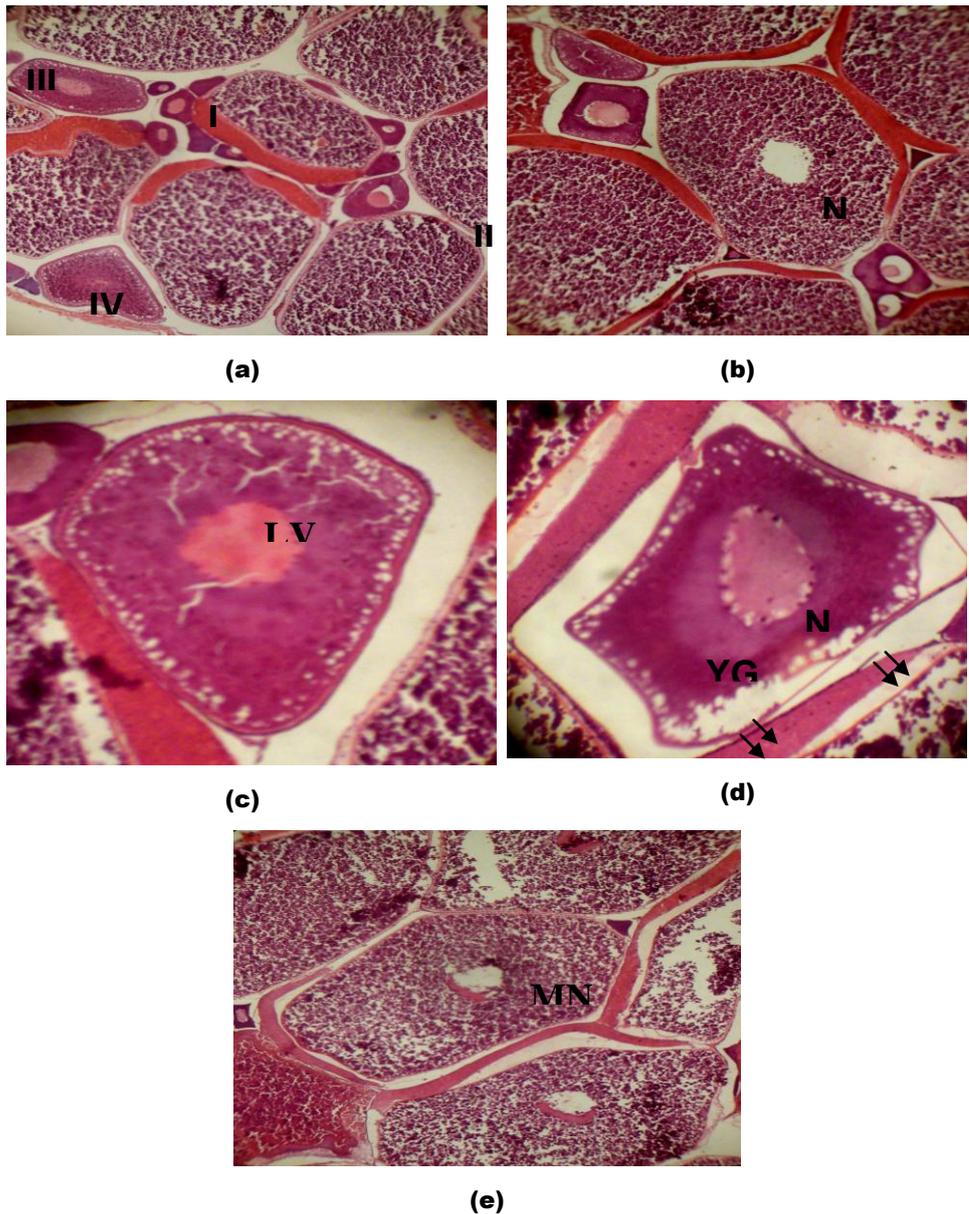


Fig. 1: Transverse section in ovary of African catfish brood stock, (a) T_1 (control): showing normal structure of ovarian lamellae, which contains oocytes at various stages of oogenesis; (b) the same treatment showing primary oocytes stage, N: nucleus (X 60, H&E stains); (c) T_2 : showing oocytes in late vitellogenic (LV) stage (X 160, H&E stains); (d) T_3 : showing oocytes in yolk vesicles (arrows) and yolk globules (YG) stages (X 160, H&E stains); (e) T_4 : showing migratory nucleus (MN) stage (X 160, H&E stains). T_1 , T_2 , T_3 and T_4 are the dietary treatments containing the prebiotic at the prospective levels (0, 1, 2 and 3 g / kg, respectively).

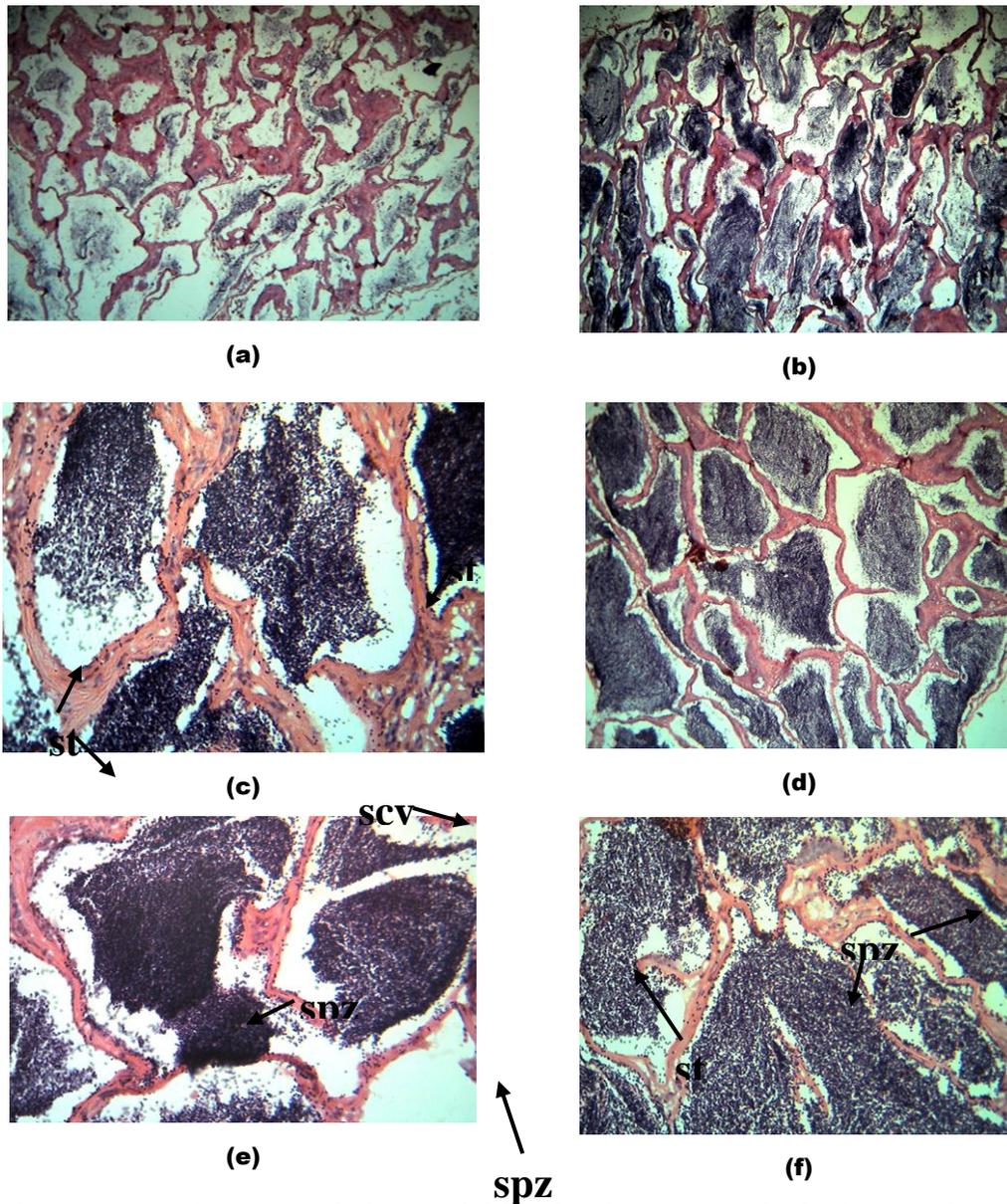


Fig. 2: Transverse section in testes of African catfish brood stock (a) T_1 (control): showing normal structure of seminiferous tubules with little spermatocytes (scy) (X 60, H&E stains); (b) T_2 : showing normal structure of seminiferous tubules filed with spermatocytes (scy) (X 60, H&E stains); (c) High magnification of (Fig. 2b) showing spermatocytes (scy) and spermatids (st) (X 400, H&E stains); (d) T_3 : showing normal structure of seminiferous tubules filed with spermatozoa (spz) (X 100, H&E stains); (e) High magnification of (Fig. 2d) showing spermatozoa (spz) (X 400, H&E stains); (f): T_4 : showing spermatids (st) and spermatozoa (spz) (X 400, H&E stains). T_1 , T_2 , T_3 and T_4 are the dietary treatments containing the prebiotic at the prospective levels (0, 1, 2 and 3 g / kg, respectively).

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the present study are similar with those reported by Msiska (2002). In addition, secondary spermatocytes were illustrated by darkly staining chromatin as in other teleost fish. Meanwhile, spermatozoa were concentrated in the lumen (Tyler and Sumpter, 1996). Generally, the obtained results in the present study revealed that the prebiotic incorporated diets helped to increase the reproductive performance and gonads maturation of the experimental fish. This is in agreement with the reported results of Ghosh *et al.* (2007) that reproductive performance was enhanced in the fish fed dietary probiotic. These could be attributed to the balanced production of essential nutrients (in particular essential fatty acids) by intestinal probiotic bacteria (Irianto and Austin, 2002).

5- Histometric examination of fish dorsal muscles

There were insignificant ($P \geq 0.05$) increases of smallest diameter (μm), largest diameter (μm), mean diameter (μm), smallest / largest ratio and the percentage of interstitial connective tissue/ mm^2 of fish fed dietary prebiotic T-protphyt-2000 (T_2 , T_3 and T_4) compared with the control group (T_1). However, intensity of muscular bundles/ mm^2 and the percentage of muscular bundles area/ mm^2 of dorsal muscles of fish fed the prebiotic were decreased insignificantly ($P \geq 0.05$) compared with the control (T_1) (Table 6). In this respect, it

is of interest to note that, fish treated with dietary prebiotic T-protphyt-2000 realized the best growth performance and feed utilization (Table 1) of fish compared with the control treatment.

The present findings agree with those reported by Abdelhamid *et al.* (2004), they found that the *O. niloticus* group fed diet containing 1 kg Betafin[®]/ton and 600 ml Biopolym[®]/ton was the best treatment among all treatments concerning the muscular bundles and total surface area occupied by the muscular bundles/ mm^2 , which was related with the high growth performance, feed and nutrients utilization and characteristics of fish production. Moreover, Mehrim (2009) reported that supplementation of commercial probiotic Biogen[®] at level of 0.3% to fish diets under high stocking densities led to improvement of most histometric characteristics of the dorsal muscles of fish compared with the control group (T_1).

CONCLUSION

From the above results it can be concluded the, useful using of this new local prebiotic T-Protphyt 2000 for improvement of growth performance, feed utilization, physiological responses, gonads histological development and reproductive performance especially for female African catfish *C. gariepinus* brood stock, which showed a

Table 6: Effect of the tested prebiotic on histometric characteristics of fish dorsal muscles

Treatment	T_1	T_2	T_3	T_4
Smallest diameter (μm)	19.0 \pm 1.00	25.0 \pm 2.24	23.0 \pm 2.00	21.0 \pm 1.00
Largest diameter (μm)	26.0 \pm 2.45	29.0 \pm 1.00	30.0 \pm 1.58	27.0 \pm 2.00
Mean diameter (μm)	22.5 \pm 1.58	27.0 \pm 1.46	26.5 \pm 1.70	24.0 \pm 1.22
Smallest/Largest ratio	0.75 \pm 0.06	0.86 \pm 0.06	0.77 \pm 0.04	0.77 \pm 0.07
Intensity of muscular bundles/ mm^2	884.8 \pm 113.2	679.0 \pm 92.6	679.0 \pm 92.6	792.2 \pm 178.5
% of muscular bundles area*/ mm^2	82.75 \pm 10.5	81.26 \pm 8.35	57.66 \pm 8.67	78.73 \pm 6.05
% of connective tissue**/ mm^2	17.25 \pm 10.5	18.74 \pm 8.35	42.34 \pm 8.67	21.27 \pm 6.05

* % of muscular bundles area / $\text{mm}^2 = [3.14 \times (\text{mean diameter}/2)^2] \times \text{Intensity of muscular bundles}/\text{mm}^2 \times 100$, whereas: the muscular bundles were considered as approximately circularity shape.

** % of connective tissue / $\text{mm}^2 = (1 - \text{muscular bundles area, mm}^2) \times 100$.

T_1 , T_2 , T_3 and T_4 are the dietary treatments containing the prebiotic at the prospective levels (0, 1, 2 and 3g/Kg, respectively).

promising new development and could solve the problem of massive use of antibiotics in aquaculture industry, besides it may realize economic benefits for catfish hatcheries. Consequently, further studies are needed about this prebiotic and its combination with probiotic for improvement the reproductive performance of African catfish or other fish species in this stage or different maturation stages under different management and dietary conditions

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تقييم تأثير بريبيوتيك محلي جديد على الكفاءة التناسلية لأمهات أسماك القرموط الأفريقي

أحمد إسماعيل محرم¹، عبد الحميد محمد عبد الحميد¹، صلاح محمد إبراهيم² وأحمد إبراهيم عبد الوهاب¹

1. قسم إنتاج الحيوان – كلية الزراعة – جامعة المنصورة – المنصورة – مصر.

2. مفرخ صلاح إبراهيم السمكي – كفر الشيخ – مصر.

تعد أسماك القرموط الأفريقي من الأنواع عالية القيمة في الاستزراع المائي. في السنوات الحالية زاد الاهتمام بشكل كبير باستخدام البريبيوتيك كإضافات علفية في الاستزراع السمكي. أجريت الدراسة الحالية لتقييم تأثير البريبيوتيك الغذائي المحلي الجديد T-Protphyt 2000 على الكفاءة التناسلية لأمهات أسماك القرموط الأفريقي لمدة 12 أسبوعاً. المعاملات الغذائية (الأولي، الثانية، الثالثة والرابعة) تحتوي على البريبيوتيك بالمستويات المختبرة (صفر، 1، 2 و 3 جم بريبيوتيك / كجم عليقة على التوالي)، حيث استخدمت ثمان أحواض أسمنتية (كل منها 4 متر مكعب)، في مكررتين (حوضيين) / معاملة. إجمالي 48 من أمهات أسماك القرموط الأفريقي بمتوسط وزن (190 ± 0.50 جم)، خزنت بمعدل 6 أسماك لكل حوض بنسبة جنسية 1 ذكر: 2 أنثى لمنع الافتراض بين الذكور. أوضحت النتائج أن المعاملة الرابعة (3 جم بريبيوتيك / كجم عليقة) حققت أفضل القيم معنوياً بين كل مستويات البريبيوتيك (المعاملة الثانية، الثالثة) أو المعاملة الضابطة (المعاملة الأولى) فيما يتعلق بالوزن النهائي للجسم، الزيادة الوزنية الكلية، معدل النمو النوعي، استهلاك الغذاء، كفاءة التحويل الغذائي، كفاءة الاستفادة من البروتين، قياسات الدم الهيماتولوجية (الهيموجلوبين، خلايا الدم الحمراء والبيضاء)، طول المبيض، الكثافة النوعية للمبيض، الخصوبة المطلقة، معامل الحالة الجسمي، هرمون بروجستيرون السيرم لإناث أسماك القرموط، بجانب التطورات النسيجية لكل من مناسل الأسماك (المبايض، الخصي). ومن ثم يمكن التوصية باستخدام المفيد للبريبيوتيك المحلي الجديد T-Protphyt 2000 لتحسين نمو الأسماك، الاستفادة من الغذاء، صورة الدم الهيماتولوجية، الكفاءة التناسلية (خاصة للإناث) وتركيب العضلات بجانب التطور النسيجي لمناسل الأسماك، لذا ربما يكون استخدام هذا البريبيوتيك في مفرخات أسماك القرموط الأفريقي مفيد أيضاً من وجهة النظر الاقتصادية.