Effect of Dietary Sorghum Supplemented With Inulin on Ectoparsitic Infection and Protein Gel Electrophoresis of Nile Tilapia *(Oreochromis niloticus)* Fingerlings

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Abstract:

Various sectors of the aquaculture industry can benefit if cultured organisms were conferred with enhanced growth performance, feed efficiency and disease resistance. As such, the cost of medication and production costs could be reduced and consumer perceptions would be improved. The objectives of the present study were to investigate the effects of sorghum and inulin on ectoparasitic infection and protein gel electrophoresis in fingerlings of Nile tilapia (*Oreochromis niloticus*) with initial body weight of 9.2±0.037g. Six diets were formulated by using sorghum as source of carbohydrate incorporated as two levels (15 and 30%) of the diet supplemented with inulin at three doses (2.5, 5, 7.5g). Tilapias fed inulin containing diets (2.5 g) with sorghum (15 and 30%) exhibited significant improvements of immune system of fish and increased resistance of infection. The morbidity rate decreased after cohabitation with parasitic fish in low dose of inulin also number of protein bands increased of protein gel electrophoresis. Inulin in high doses (5 and 7.5g) revealed reverse effect of *Cichlidogyrus tilapae* infection and number of protein bands.

Introduction:

Increase feed supply without jeopardizing natural resources is one of the main challenges for sustainably developing aquaculture. Fish can adapt to feed changes with specific limitations, particularly due to glucose metabolism in carnivorous fishes ***(Polakof et al., 2012a)***

Nile tilapia, *Oreochromis niloticus*, is widely produced globally with annual production reaching 3.7 million metric tons (MT) in 2010 (FAO, 2010) because of their numerous positive characteristics such as tolerance to crowding, high fecundity, fast growth, and consumer demand ***(Shiau, 2002)***. ***Min and Kang (2008)*** showed that Nile tilapia (*Oreochromis niloticus*) has a strong immune system and provides a good capability to tolerate biotic and a biotic stresses.

In aquatic feeds, soluble carbohydrates (CHO) are usually

used as energy sources for aquatic animals. Carbohydrate after absorbed can provide fish with equal amounts of energy as protein. Capacities of fish to utilizing CHO depend on the species ***(Hemre et al., 2002)*** .Dietary carbohydrate inclusion in several fish species appears to produce positive effects on growth and digestibility ***(Li et al., 2013b)***. However, using the appropriate level of carbohydrates in aqua feeds is of great importance, because if the appropriate amount of carbohydrates is not provided, this may have negative effects on nutrient utilization, growth, metabolism and health ***(Li et al., 2012)***.

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of and activity of health-promoting bacteria in the intestinal tract ***(Gibson et al., 1995)***. In aquaculture, prebiotics stimulated growth performances, feed utilization, and positive effects immune system, and disease resistance have been reported ***(Merrifield et al., 2010b*** *and* ***Ringø et al., 2010).*** . Inulin is one of the most studied prebiotic and consists predominantly of polydisperse B-(2-1)-linked fructan and is naturally present in a number of common foods such as garlic, onion, artichoke and asparagus ***(Roberfroid 2007).***

Transmission of ectoparasites is scarce, but four different routes are listed by ***Bakke et al. (1992)****:* (1) contact with living infected fishes; (2) contact with detached parasites on the substrate; (3) contact with infected dead fishes; and (4) contact with detached parasites drifting in the water column. ***Bakke et al. (1992)*** suggested that the ability of detached parasites in the drift to re-infect fishes may have been underestimated. The effect of ambient water temperature on gyrodactylid transmission rates is poorly studied.

Monogenoids are considered to be responsible for the most important parasitic disease in fish farming because they can cause high mortality rates. The presence of these parasites in fish gills can cause hyper secretion of mucus, cell hyperplasia, and even fusion of the filaments of gill lamellae, reducing the host's respiratory capacity ***(Pavanelli et al., 2008)***. In severe infestations, ciliate protozoans can damage fish health and consequently cause economic losses in fish farming systems. Trichodina spp. and I. *multifiliis* occur in the gills and on the body surface of fish and may cause mucus hyper secretion and lesions in the integument and gills ***(Pavanelli et al., 2008)***.

This study was designed to evaluate the effect of dietary sorghum supplemented with inulin on ectoparasitic infection and protein gel electrophoresis of Nile tilapia *Oreochromis niloticus* fingerlings.

Materials and Methods:

Fish:

A total number of 140 adult of *Oreochromis niloticus* were obtained from Fish Research Center, Suez Canal University with a case study of naturally infected with ectoparasites. Naturally infected fish were transported immediately to the laboratory. Their average body weight was 49± 2 g.

Aquaria:

Seven full glass aquaria measuring (75 ×40×35cm) were used for rearing fish. The aquaria were supplied with aerated de-chlorinated fresh water and changed continuously. Continuous aeriation was maintained in each aquarium using an air pump. Thermostatic heaters were used along the experiment to maintain temperature at 25±2°C. The health status was examined throughout the experimental period

Experimental diet:

The experimental diets were formulated by using the available local ingredients. Ingredients were ground into fine powder through a 175-µm mesh before pelleting and an appropriate amount of water was added to produce stiff dough. The dough was pelleted using California pelleting machine with 2mm diameter. All diets were stored in refrigerator until used.

Six diets were formulated by using source of carbohydrate (sorghum) and it was incorporated as two levels (15 and 30%) of the diet. The proximate composition of ingredients and feed formulation of the diets are presented in (Table 1).

Vitamin-mineral premix supplied the following (g Kg-1 mixture); retinyl acetate 0.67; ascorbic acid 120; cholecolciferol 0.1; tocopheryl acetate 34.2; menodione 22; thiamin 5.6; riboflavin 12; pyridoxine 4.5; calcioumpanthothenate 14.1; p-aminobenzoic acid 40; cyanocobalamin 0.03; niacin 30; biotin 0.1; choline chloride 350; folic acid 1.5; inositol 50;canthaxanthin 10;butylated hydroxytoluene 1.5; butylated hydroxyanisol 1.5.; CaHPO4,2H2O 29.5; Ca (H2PO4)2 H2 O 217; NaHCO3 94.5; Na2SeO35H2O 0.011; Kci 100; NaCl 172.4; Ki 0.2; Mgcl2 63.7; MgSO4 34.3; MnSO4 2; FeSO4H2O 10; CuSO4 5H2O 0.4; ZnSO4 10.

Growth energy (MJ kg^{-1} diet) was calculated by using the following calorific values: 23.9, 39.8 and 17.6 kj g^{-1} diet for protein ,either extract and nitrogen free extract , respectively **(NRC.2011**)

Clinical and postmortem examinations:

The fish behavior, movements of the operculum, feeding and any clinical abnormalities like abdominal distention, skin pigmentation, emaciation, skin lesions, wounds, petechial hemorrhages were examined according to ***Hoffman (1999)***.

Parasitological examination:

The fish specimens were examined microscopically for external parasites ***(Lucky 1977).***

External examination

Eyes: For closer examination, the eyes were gently removed using forceps and scissors. They were kept in saline in a Petri dish and examined using a dissecting microscope. The lens, humor, and retina were removed and examined using either the dissecting or the compound microscope for searching of certain species of parasites.

Opercula (gill cover) and gills: The gills and the operculi (gill covers) were removed and examined for macroscopic parasites. Then carefully portion of a gill arch was cut. This was done by grasping the arch by the cartilage with forceps and cutting it out using a scalpel or small scissors. Laying the gill in a drop of water on a clean microscope slide, the cartilage was removed with a scalpel, then gently kept on cover slip over the gill filaments and added water if necessary for detection the microscopic parasites.

Fins: The fins were examined and a portion of the fin was clipped off and placed on a slide with water and a cover slip for closer examination.

Skin surface: Skin was gently scraped a small amount of mucus from the skin behind the pectoral fins and the base of the tail especially where the lesions found. Smears of mucus onto a clean microscope slide with addition of a drop of water and a coverslip. Lesions were noted and a scraping made to examined for samples from any areas of discoloration or where scales are raised or sloughed. This method was used to collect samples from the nasal openings and the oral cavities to detect the protozoal or crustaceans parasites.

Identification of parasites:

The identification of the parasites was undertaken according to the methods adapted by ***Yamaguti (1961) and Hoffman (1999).***

Experimental design:

A total number of 70 fish from naturally infected fish from 82 examined fish were kept in the aquaria which contained treated groups with inulin and sorghum. Each group contained 20 fish (10 treated with fish diets and 10 naturally infected fish with monogenea.

In this trial, a total number of 130 fish were divided to seven groups. Each group of six groups contained 20 fish. Group1 contained inulin 2.5g and sorghum 15%(In 2.5+15), group 2 fed on inulin 2.5g and sorghum 30% (In 2.5+30), group 3 fed on inulin 5g and sorghum 15% (In 5g +15%), group 4 fed on inulin 5g and 30% (In 5+30), group 5 fed on inulin 7.5g and sorghum 15% (In 7.5+15), group 6 fed on inulin 7.5g and sorghum 30% (In 7.5+30). The seventh group contained 10 fish and was kept as control group which contained naturally infected fish with ectoparasites and fed on commercial diets without sorghum and inulin.

The transmission of parasites were carried out by cohabitation (CT).The donor(D) fish that

infected with monogenea. The treated fish (Receptor (R) were marked with passive integrated transponder (PIT) tags to differentiate them. PIT-tags were injected into the tail finof the examined fish.All groups keep under observation to two weeks and recorded the transmission of monogenea and rate of morbidity

Protein analysis of musclature of Nile tilapia:-

SDS-Page method

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine proteolytic changes in control and treated samples ***(Laemmli, 1970).*** This was done with a SE 250 Mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instruments, Ain shams university, cairo) using a 3% acryl amide stacking gel and a 10% acryl amide resolving gel ***(Srinivasan et al., 1997).*** Samples for electrophoresis were prepared by homogenizing 1 g minced raw muscle in 100 ml cold (~5°C) distilled deionizer water with an Ultratorax for 30 s. The homogenate was diluted 1:1 in the sample buffer containing 4% SDS, 0.125 M Tris (pH 6.8), 20% glycerol and 10% b-mercapto ethanol, yielding a sample protein concentration of approximately 1 mg mL-1, assuming a 20% protein content in raw muscle tissue ***(Xiong et al., 2002)***. The samples were then centrifuged for 20 min at room temperature ***(An et al., 1988)***, the supernatants were collected, and protein contents determined by Lowry method ***(Lowry et al., 1951).*** All raw and cooked samples were then boiled in a water bath (100°C) for 3 min and loaded in each gel lane. Unstained SDS-PAGE molecular weight standard, MW range 14.4- 116.0 (Fermentas, SM0431) was diluted 1:2 with loading dye. After the electrophoresis, the gel was stained for 1 hour with Coomassie blue R 250 dye in methanol-acetic acid-water solu- tion (4:1:5, by volume) and destained in the same solution without dye.

Table 1: *Feed formulation and proximate composition of experimental diets.*

Diets	S15	S30
Fish meal	4	6
Poultry –by product meal	8	10
Soybean meal	10	20
Sunflower seed meal	50	26
*Sorghum meal	15	30
Fish oil	3	3
Cotton seed oil	3	3
Microcrystalline cellulose	5	-
Vitamin min. mix[1]	2	2
Chemical composition(%DM basis)		
Dry matter	92.4	92.1
Crude protein	32.74	30.48
Either extract	13.67	12.82
Nitrogen free extract	35.22	40.42
Fiber	7.72	6.47
Ash	10.65	9.81
Growth energy (MJ/kg) diet)[2]	19.45	19.49
Metabolizable (ME/kg diet)[3]	16.23	16.27

*Adding inulin with three doses (2.5, 5.0, 7.5g) to the sorghum meals.

Results:

1- Clinical examination:

A total number of 140 adult of *Oreochromis niloticus* were naturally infected with two species of ectoparasites after morphological and parasitological examinations. The two ectoparasites were identified as (*Cichlidogyrus tilapiae* and Trichodina species). A number of 82 fish from 140 fish contained *Cichlidogyrus tilapiae* and 58 fish from 140 examined fish contained Trichodina species as shown in **table (2)**. Treated group from second experiment infected with 82 fish which contained *C.tilapiae* by cohabitation test. A total number of 70 fish from 82 examined fish were transported to treated groups. Each group contained 20 fish (10 treated with fish diets and 10 naturally infected fish). The transmission of parasites were carried out by cohabitation (CT).The donor(D)fish that infected with *C.tilapiae* were determined by examination of fish before starting the cohabitation. The treated fish (Receptor (R) were marked with passive integrated transponder (PIT) tags to differentiate them. PIT-tags were injected into the dorso-lateral musculature of the examined fish.

After the end of second experiment (45 days of feeding), in this trial, there were seven groups, group1 contained inulin 2.5g and sorghum 15%(In 2.5+15), group 2 fed on inulin 2.5g and sorghum 30% (In 2.5+30), group 3 fed on inulin 5g and sorghum 15% (In 5g +15%), group 4 fed on inulin 5g and 30% (In 5+30), group 5 fed on inulin 7.5g and sorghum 15% (In

7.5+15), group 6 fed on inulin 7.5g and sorghum 30% (In 7.5+30) and the seventh group was kept as control group contained naturally infected fish with ectoparasites and fed on diets without sorghum and inulin.

2- **Clinical picture:**

Postmorton examination: Naturally infected fish showed dark or pale coloration of skin with excessive slime production, detached scales, and small bloody spots at the base of fins. Fish infested with monogenea showed excessive dull bluish grey slim layer on body surface, skin become hyperemic, and loss of scales and presence of ulceration.

Some fish gills appeared congested and pale with excessive slimness secretion, marbling (mosaic) appearance may present and thickening and sticking of the gill tips with grayish coloration in some fish **(Photo, 1).**

Results of clinical signs:

The most characteristic external clinical findings observed on naturally infected fish (*O.niloticus)*were represented as abnormal swimming, flashing and rubbing their external bodies on the sides of the aquaria. Also, suffered from asphyxiat, gathered at the water surface with gulping the atmospheric air. Some fish accumulated towards the air pumps, and become nervous and irritable. Others appeared dull with loss of escape reflex. Fish were shown exhausted with rapid respiration accompanied with over widening of the operculum. Excessive amounts of dull bluish grey mucus layers covering the external body surface as well as scale sloughing. The skin appeared with localized focal bloody spots with small wounds or abrasions. Besides, there were frayed ragged fins with darkening or paleness in some fish.

Parasitological findings:-

Based on the morphological and parasitological examinations, the isolated parasites were identified as monogenean trematodes *Cichlidogyrus tilapiae* and ciliated protozoan Trichodina species.

- ***Cichlidogyrus tilapiae Paperna,* 1960:**

The parasites incriminated to the monogenean disease isolated from gills of *O. niloticus* are related to the phylum Platyhelminthes, class Trematoda, order Monogenea, family Dactylogyridae and genus Dactylogyrus, *Cichlidogyrus tilapiae.*

The gill flukes were characterized with the presence of the prohaptor (anterior end) which was divided into four cephalic lobes, with sticky and adhesive organs (cephalic glands) that serve as attachment to the host during food intake. The mouth was near to the anterior part and was connected to the pharynx via the oral tube. There were four black eye spots. The intestinal limbs were fused together posteriorly and they are oviparous. The parasites appeared dark as they contain dense granular strands of vitellaria occupying the whole body of the worm. The disc-shaped posterior part appeared as

dome-shaped with one large median (central hooks). Seven pairs of small marginal hooklets. (Photo,2 and Photo 3).

-*Trichodina* species:

The isolated protozoan belongs to the class Oligohymenophora, subclass Peritichia, and order Mobilina, family Trichodinidae. These parasites were symmetrically shaped peritrichus ciliated protozoan, with a diameter from 10 up to 30µm. The upper view was round, while the lateral views was either dish or bell- shaped. The top of the body revealed different shapes and the bottom was depressed in a concave lens-shape which acts as an adhesive disc. There was a ring of hollow conical structures with lateral denticles that were inserted into each other. The blades and the straight thorn- like centripetal projections (thorns). There was a striated band with an annular ribbon structure having radial lines hanging from the top of the denticulate ring to the body. The macronucleus was large with a kidney or C- shaped with micronucleus. (Photo, 4 and Photo 5)

Prevalence of infested Nile tilapia:-

The prevalence of external parasitic infections in *Oreochromis niloticus* with monogenea *Cichlidogyrus tilapiae* and ciliated Trichodina species were recorded in Table (2).

After examination of fish, fish with parasite *Cichlidogyrus tilapae* transported to groups of treated fish with inulin and sorghum. There were seven groups; each group contained 10 fish of treated fish act as receptor fish (R) and 10 fish from examined fish with parasite *Cichlidogyrus tilapae* act as donor fish (D). After two weeks of cohabitation between donor and receptor fish, examined fish and recorded of infected fish of treated group after exposed to parasite. Group 1 (inulin 2.5 + sorghum 15%) was the least number ofparasite *C. tilapae* and the rate of morbidity but group 6 (inulin 7.5 + sorghum 30%) was the highest number of infested fish with parasite *C.tilapae* and rate of morbidity.

Effect of sorghum supplement with inulin after infection on electrophoretic pattern of protein of some organs (Musclature):-

Protein electrophoresis is a method for analyzing the proteins in a fluid or an extract. The electrophoresis may be performed with a small volume of sample in a number of alternative ways with or without a supporting medium: SDS polyacrylamide gel electrophoresis (in short: gel electrophoresis, PAGE, or SDS-electrophoresis). Gel electrophoresis is often performed in combination with electroblotting immunoblotting to give additional information about a specific protein. Because of practical limitations, protein electrophoresis is generally not suited as a preparative method.

Thin isoelectric focusing was based upon the migration of a given protein to a fixed point within a stable pH gradient under the influences of an electric

field. The fixed point where protein migration ceased, the isoelectric point (PI), corresponded to the pH at which the protein had a charge.

Changes in Nile tilapia caused by adding sorghum supplement with inulin were followed by SDS-PAGE. Photo 14 showed the effect of inulin on *O.niloticus* electrophoretic pattern. The electrophoretic pattern of samples showed a considerable number of protein bands and thus, all the major proteins generally present in fish. Through electrophoretic analysis, it was seen that the bands, particularly myosin and actin, did not disappear completely, but changed remarkably after treating with inulin. It can be thought that, if some cleavage of MHC and actin into smaller polypeptide chains occurs, the non- appearance and/or decreasing of these bands in our gel would indicate that these are smaller than 5 kDa. It was reported that protein bands with molecular weights lower than 5 kDa are not separated in the SDS-PAGE. In this study, while density of the myosin bands were decreased 34, 60, 57 and 52 % in group 4,5 and 6, the actin bands were decreased 41, 63, 59 and 48 % in group 2and 4.

The muscle proteinograms of Nile tilapia (control sample) exhibited nine fractions. In treated *O. niloticus* fractions number 5, 6, 7 and 8 appeared in all examined fishes, so they are polymorphic bands. The 1st and 9th fractions were not found in groups except group 1and 3.

Photo (1): showing cloudy eyes and haemorrages on the gill cover with bloody spots at the base of fins

Photo (2): Showing unstained monogenean trematode (*Cichlydogyrus tilapiae)* isolated from infected Nile tilapia (*O.niloticus).*

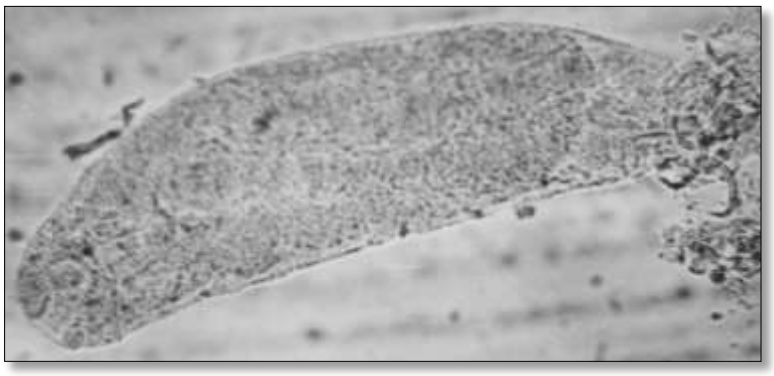

Photo (3): Showing stained monogenean trematode (*Cichlidogyrus tilapiae)*isolated from infected Nile tilapia (*O.niloticus).*

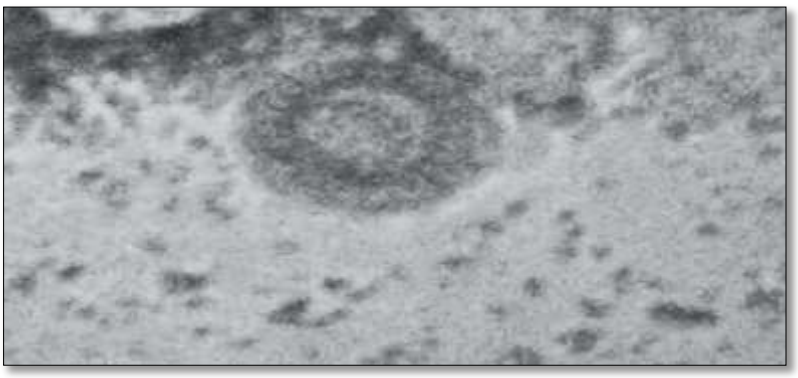

Photo (4): Showing unstained Trichodina species isolated from infected Nile tilapia (*O.niloticus*).

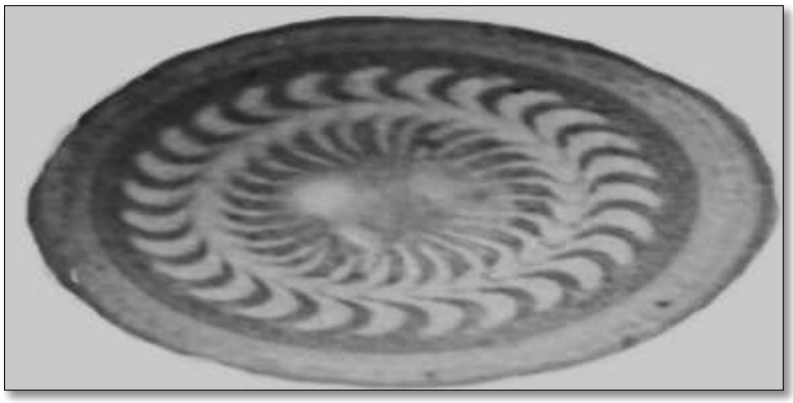

Photo (5): Showing stained Trichodina species isolated from infected Nile tilapia (*O.niloticus*).

Table 2: *Showing the total prevalence of some ectoparasites isolated from Oreochromis niloticus:*

Parasite	No.of examined fish	No. of infested fish	Percentage %
Cichlidogyrus tilapiae	140	82	58.5
Trichodina sp.	140	58	41.4

Table 3: *Effect of sorghum supplemented with inulin on Cichlidogyrus tilapae and morbidity of fish.*

	No. of treated fish (R) non infected	No. donor fish (D) infected	No. of infested fish from R and D	Morbidity (N) after two weeks %
Group 1	10	10	2	10
Group 2	10	10	3	15
Group 3	10	10	3	25
Group 4	10	10	6	50
Group 5	10	10	8	70
Group 6	10	10	9	90
Control group	-	10	9	90
total	60	70		

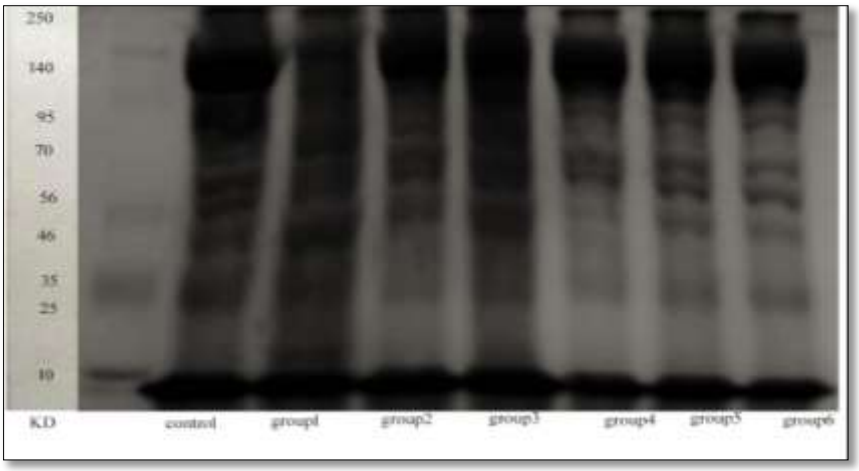

Photo (6): Electrophoretic pattern of control and treated groups of Nile tilapia by gel electrophoresis.

Discussion:

Clinical picture:-

Naturally infected fish showed dark or pale coloration of skin with excessive slime production, detached scales, and small bloody spots at the base of fins. Some fish gills appeared congested and pale with excessive slimness secretion, marbling (mosaic) appearance may present and thickening and sticking of the gill tips with grayish coloration in some fish. These results agree with the finding of ***Eissa et al. (1992) and Eissa et al. (2004)***

Regarding the external parasites, the present study isolated and identified both mongenean trematodes *Cichlidogyrus tilapae* and ciliated protozoan *Trichodinia sp.* based on the morphological and parasitological examination, these findings and description of the external parasite agree with ***Eissa et al. (1993) and Reed et al. (2003)***

In the present study, adding of inulin and sorghum in small doses (inulin 2.5g+sorghum 15%) to diets of Nile tilapia improved the fish health and increased resistance to ectoparasites of donor fish due to enhancement of immune system. *Cichlydogyrus tilapia* infected and attacked treated fish (low doses groups) with few numbers. Adding inulin in large doses gave reverse effect on fish.

This monogenean disease affects mainly the gills of most cultured freshwater fishes. These parasites are active grazers and feed primarily on mucous but ingestion of blood cells leaking from aneurysms in the lamellae. Diseased fish appeared restless, flashing then become dull and inactive.

The use of different chemotherapies is advisable to avoid bacterial infection of fish. Also, using several antibiotics are used to treat bacterial infection, however the recent

techniques have increased drug-resistant bacteria in fish. Whilst vaccination is the method of choice over antibiotic treatments for the control of many fish diseases, vaccines for others are unavailable or, at best, in the early stages of their development. In recent years in the aquaculture industry, increasing consideration has been given to alternative strategies for disease control as adjuncts to vaccination and as a potential route to the reduction in the widespread use of antibiotics. Prebiotics is one group of these alternative strategies that their health promoting effects has been proven by many studies in human and terrestrial animals ***(Cerezuela et al. 2008; Gibson et al., 1995)***

The addition of 0.5% inulin (prebiotic) and*W. cibaria* (probiotic) to the diet of *Pseudoplatystoma* hybrid surubins reduce the number of pathogenic bacteria and stimulate the beneficial intestinal microbiota and may possibly alter their immune defense system ***(Mourino et al., 2012).*** While the role of prebiotics on the growth, nutrition and physiological responses of fish has been widely demonstrated in a number of studies, the involvement of prebiotics in stimulating fish immunity has been documented less frequently ***(Cerezuela et al., 2008 and Buentello et al., 2010)***. ***Ali et al. (2016)*** explored the effects of dietary prebiotics in mucosal immunity of asian seabass (*Lates calcarifer*)**.** Lysozyme catalytically hydrolyzes the bond between N-acetyl muramic acid and N- acetyl glucosamine in the cell wall of bacteria, but alkaline phosphatase has been demonstrated as a potential stress indicator in the epidermal mucus of fish

To the best of our knowledge, there were no literature dealing prebiotics and external parasites in freshwater fish. ***Li & Gatlin (2005)*** studied *Morone chrysops* and M. *saxatilis* hybrids exposed to *Mycobacterium marinum* in regard to their growth when fed with a diet supplemented with the prebiotic GroBioticA. They found that fish supplemented with 2% of commercial prebiotics had a higher survival rate (80%) compared to those supplemented with 1–2% beer yeast and 2% GroBiotic-A (72–73%) after 21 days of treatment with a chronic infection. In contrast ***Cerezuela et al. (2013)*** recorded that the challenge experiment showed that the fish fed inulin or the synbiotic diet had non-significantly lower or significantly higher cumulative mortality, respectively, compared with the control group (non-supplemented diet). These results suggest that inulin and *B. subtilis* modulate the immune response of the gilthead seabream, although the combined administration increases susceptibility to infection by *Photobacteria damselae* subsp.

Concerning the effect of sorghum supplement with inulin after infection on electrophoretic pattern

of protein of fish musclature. The electrophoretic pattern of samples showed a considerable number of protein bands and thus, all the major proteins generally present in fish. Through electrophoretic analysis, it was seen that the bands, particularly myosin and actin, did not disappear completely, but changed remarkably after treating with inulin. These results contrast with ***Burr et al. (2010),*** who said that denaturing gradient gel electrophoresis analysis of the gastrointestinal tract of juvenile red drum microbial community showed no effect of the dietary prebiotics as the microbial community appeared to be dominated by a single organism with very low diversity when compared with other livestock and fish species. Electrophoresis of the microbial community in the biofilters of the independent aquariums showed a diverse microbial community that was not affected by the dietary prebiotics ***(Pond et al., 2006; Ringø et al. 2006; Plante et al. 2007).***

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الملخص العربي

صناعة الأستزراع السمكي سوف تستفيد علي مختلف القطاعات اذا تم تحسين اداء النمو,كفاءة التغذيه وزيادة القدرة علي مقاومة الأمراض. وبذلك يتم خفض تكاليف الأدويه ويتحسن انتاج المزارع السمكيه. تهدف هذه الدراسه الى معرفة تأثير الذره الرفيعه مع الأنيولين علي العدوى بالطفيليات الخارجيه و تحليل البروتين للجل الكهربائي في عدد من إصبعيات أسماك البطي النيلي وكان متوسط اوزانهم (9.2 $\pm$ 0.037جم).

في هذه التجربة يتم اضافة الأنيولين بنسب (2.5 ,5 و 7.5 جم/كجم عليقة)الي علائق الأسماك. تم استخدام 360 سمكه من إصبعيات البلطى النيلي وقسمت الى ست مجموعات فرعيه. المجموعة الأولي تتغذي علي عليقه تحتوي علي الانولين 2.5 جم مع الذره الرفيعة بنسبة 15% و المجموعة الثانية تتغذي علي عليقه تحتوي علي الانيولين 2.5 جم مع الذره الرفيعه بنسبة 30%, المجموعة الثالثة تتغذي علي عليقه تحتوي علي الانيولين 5 جم مع الذره العويجه بنسبة 15% والمجموعة الرابعة تتغذي علي عليقه تحتوي علي الانيولين 5 جم مع الذره الرفيعه بنسبة 30% والمجموعة الخامسة والسادسة تتغذي علي عليقه تحتوي علي الانولين 7.5 جم مع الذره الرفيعه بنسبة 15 و 30% علي التوالي. بعد 45 يوما من التغذية على الذرة الرفيعة بالاضافه مع الإنيولين,تم وضع أسماك مصابة بالسيكليدوجيرس بعد فحصها وتعريفها من الخياشيم والجلد.تم فحص 140 سمكه من البلطي النيلي. وجد 82 سمكه محمله بالطفيل سيكليدوجيرس تيلابي و58 سمكه محمله بالتريكودينا من الهدبيات. تم وضع 10 أسماك مصابة بالسيكليدوجيرس تيلابي الي 10 اسماك معالجة من كل مجموعة من مجموعات التجربة .تم تسجيل عدد الطفيليات والنفوق لمدة اسبوعين. في نهاية التجربة تم اخذ عينات من عضلات الأسماك من كل مجموعة لتحليل البروتين عن طريق الجل الكهربائي. تحتوي هذه التجربه علي 130 من اسماك البلطي مقسمه الي سبع مجموعات. كل مجموعه من الست مجموعات تحتوي علي 20 سمكه (10 أسماك من المحمله بالسيكليدوجيرس و10 أسماك من الأسماك المعالجه بالانيولين) اما المجموعه السابعه تحتوي علي 10 أسماك المحمله بالسيكليدجيرس (المجموعه الضابطه) وتتغذي علي عليقه تجاريه. تم تميز الأسماك المستقبلة (R) بعلامات . ووضعت الاسماك تحت الملاحظه لمدة اسبوعين وسجلت أعداد الطفيليات. من خلال العدوي بالسيكليدوجيرس, الأسماك المصابه تم تمييزه ظاهريا من خلال الحركه البطيئه اثناء العوم وظهور نقط حمراء علي الجلد. أظهرت النتائج بعد اسبوعين قلة عدد الاسماك المصابه في المجموعتين الأولي والثانيه وقلة عدد النفوق بينما باقي المجموعات التي أظهرت زياده في عدد الأسماك المصابه بالسيكليدوجيرس.

تبين من تحليل البروتين للجل الكهربائي للعضلات مجموعات للبروتين عالية في مجموعات 1 و 2 و 3 مقارنة مع الضابطه.أظهرت المجموعات 1 و 2 و 3 المجموعة من البروتين 250 ولكن لم تظهر في المجموعات الأخرى.