EFFECTIVITY OF *Myxobolus koi* SPORES PROTEIN AS IMMUNOSTIMULANT TO PREVENT THE MYXOBOLUSIS IN CARP (*Cyprinus carpio L.*)

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Received 1 /2 /2021 *Accepted 25 /2 /2021*

ABSTRACT

The research was aimed to isolate the whole protein from *Myxobolus koi* spores from common carp (*Cyprinus carpio* L.) by SDS-PAGE method. In addition to, analyze its effects on fish immune response and survival rate as feed supplementation. In the present study, 120 fish with average body weight (10.44 ± 0.22g) and average total body length (8.8 ±0.13cm) were divided into four groups (I,II,III,IV). Fish were treated with (1µg protein/ gram fish and/ or 80 *M. koi* spores/fish). The result showed that there were four bands of whole spore protein with molecule weight (MW) 120 kDa, 86 kDa, 72 kDa and 48 kDa. The result showed total leukocyte counts, total protein and globulin was significant (P<0.05) higher in fish of group II, III and IV as compared with control group. Survival rate increased from 23% to 65% in group II (80 spores/*M. koi*) and III (1µg protein / gram fish+80 spores/ *M. koi*.), respectively. We can conclude that, since the molecular weight of *M. Koi* protein spores is > 10kDa, it could be considered as an immunogenic molecule. In addition to, we our study proved that, *M. Koi* protein spores can used as immunostimulant as increasing leucocyte count and globulin and can decrease common carp (*Cyprinus carpio L.*) death by Myxobolusis.

Key words: SDS-PAGE, *Myxobolus koi*, *Cyprinus carpio L.*, Survival rate, Total protein, Gloulin, Leukocytes.

INTRODUCTION

Freshwater fish such as Nile tilapia and common carp (*Cyprinus carpio*) is the most widely farmed fishes in Egypt (Eissa *et al*., 2009). *Myxobolus koi* is one of *Myxobolus sp*. species which can cause parasitic diseases in fish called

Myxobolusis (Maftuch *et al*., 2017). Which caused difficult fish breathing because there was a nodule or cyst or nodule on the gill filaments (Kismiyati and Mahasri, 2017). Myxozoans are one of the most economically important groups of protozoan parasites causing many serious diseases of their hosts. Myxozoans are one of the most economically important groups of protozoan parasites causing many serious diseases of their hosts (Abdel-Ghaffar *et al*., 2017).

Vaccine is an attempt to cause-specific endurance through vaccination (Yusuf *et al*., 2015). Observations Vaccination is considered as a very effective treatment in overcoming the pathogen problem in cultivation, but the price is very expensive and, on the other hand, may cause stress for the fish (Akhmad, 2018). Immunostimulants is application strengthening the fish immune system to deal with pathogen attack (Labh and Shakya, 2014). Characterization of protein can be done by using the method of Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophorosis (SDS-PAGE) where the results can be known whether protein molecular weight is suitable for use as a vaccine (Kismiyati and Mahasri, 2017). SDS-Page technique is using to know wither molecular weight of protein is suitable for vaccine or not (Mahasri, 2016). *Myxobolus* pada spores protein with molecular weight 70.22 kDa and 22.83 kDa was recorded from fish myxobolusis *Cyprinus carpio* by electrophoresis SDS-PAGE (Asri, 2012). More Insariani *et al.* (2012) put forward the results of the insulating surface glycoproteins *M. koi* using the method of polymerase chain reaction (PCR) obtained a protein with molecular weight of 12 kDa, 25 kDa and 27 kDa. The purpose of this research is to develop spore protein of *Myxobolus koi* as immunostimulant material to prevent the death of carp (*Cyprinus carpio Linn*). Addition of whole spore protein of *Myxobolus koi* on feed of the myxoolusis *Cyprinus carpio* L. increased the growth rate as well as the survival rate (Akhmad, 2018). Leukocytes in blood components is one that serves as a specific body defenses that will neutralize and destroy pathogens through phagocytosis (Mahasri, 2016). The research is aimed to isolate the whole protein from *Myxobolus koi* spores from common carp (*Cyprinus carpio* L.) by

SDS-PAGE method. The objective of this study is to analyze the addition of whole spore protein of *Myxobolus koi* as supplied in feed as the immunostimulant development material to the immune response (leukocyte differentiation) and also survival rate.

MATERIALS AND METHODS

Materials:

Diets.

The basal control diet (diet C) was formulated from practical ingredients to satisfy all known nutrient requirements of Carp fish (NRC, 1993) (Table.1). Test diet (diet T) was a basal diet supplemented with immunostimulant of the whole protein *Myxobolus koi* spores with a dose of 1µg protein/gram fish + Boster® Progol adhesive with a volume of 5ml /kg of feed (Insariani *et al*., 2012). The feed was prepared for each treatment as much as 3% of the total weight of fish using for one feeding (Saad *et al*., 2017). Immunostimulatory mixing with the feed was done by spraying on the stirred feed to make it homogeneous, then it was dried to avoid moisture (Akhmad, 2018). Both of the two experimental diets (diet C and diet T) were prepared and tested for proximat analysis at the Fish Nutrition Department, Central Laboratory for Aquaculture Research.

Fish.

A total number of 120 apparently healthy *Cyprinus carpio* collected from El-Abbassa farm Sharkia Governorate. Fish with average body weight 10.44 ± 0.22 g and of total length 8.8 ±0.13 cm were transported alive to the laboratory at central laboratory of aquaculture. In the laboratory, fish used for treatments were healthy fish marinated first in Methylene blue 3-5 g/m^3 of water for 5 minutes to clear the organism that clings to the body of the fish (Mahasri, 2016). Fish (5 fish per aquarium) were distributed into in glass aquaria (80x40x30 cm) with 30 L capacity and filled with dechlorinated tap water. Continuous aeration was maintained in each aquarium using electric air pumping compressor. During the two weeks acclimatization period, water

changed daily to maintain water quality and to discard the metabolic wastes. Fish were fed once daily (at 12 a.m.) with basal control diet (C diet) at a daily feeding rate 3% of body weight per day. All the aquaria were kept under the same conditions pH (7.2± 0.1), photoperiod (12 hours light/ 14 hours dark) and dissolved oxygen (DO2: 7.5±0.1 mg/l). Temperature was adjusted at 27±1 °C. Abnormal fish were removed from the aquaria as soon as possible and unnecessary handling of fish was also strictly avoided.

Chemicals.

The protein molecular mass marker for SDS-PAGE Precision Plus Dual Color was obtained from Bio-Rad (Hercules, CA, USA), and the SDS molecular weight (MW) Size Standard for SDS-capillary gel electrophoresis (CGE) was obtained from Beckman Coulter Inc. (Fullerton, CA, USA). β-mercaptoethanol were purchased from Bio-Rad. Highly purified water was obtained from the Milli-Q water purification system (EMD Millipore, Billerica, MA, USA). The Dodeca™ Silver Stain kit for gel staining and phosphate buffer saline/PBS were purchased from Bio-Rad.

Methods:

Exterminate design.

Experimental fish were divided into four groups treatments (1µg protein/ gram fish and/ or 80 *M. koi* spores/fish) in triplicate for 14 days.

Group I, control , fish injected with PBS solution (neither protein spore of *M. koi* , neither *M. koi* infested).

Group II. Fish only infested by with 80 *M. koi* spores/fish.

Group III. Fish immunized with whole protein spores (1µg protein / gram fish) and 80 *M. koi* spores/fish.

Group IV. Fish which immunized with whole protein spores m. koi (1µg protein / gram fish) and not 80 *M. koi* spores/fish.

Fish were fed the appropriate experimental diets at 3% of body weight once daily.

The experiments were conducted under the same conditions as in the acclimation period. During the 14 days of the experiment, behavior observations and mortality was observed.

Determination of Survival Rate (SR).

Determination of level of survival rate was conducted to analyze the ability of crude spore protein protection on goldfish. It is expressed in the form of a percentage of the number of koi fish that lives up to the experimental treatment against 30pasca the total number of fish kept. Fish survival is calculated by using the formula :SR (%) = No/ Nt x 100.

SR = survival rate, NT = the number of fish that live at the end of the observation, No = number of fish that live in the beginning of the test.

Gross Examination.

The fish was placed in right lateral recumbency and the left opercular flap removed. Gill clips and skin scrapings were performed using standard methods (Stoskopf, 1993).Additional gill arches were removed in their entirety and placed in 70% ethanol.

Wet Mount And Lugol's Iodine Preparation And Parasite Identification.

Myxobolus koi myxospores were isolated from subclinically Myxobolusis *Cyprinus carpio captured* from infected farm. One of the symptoms for Myxobolus sp. infected fish carp is the apparent parasite cyst in the gills (Maftuch *et al*., 2017). Identification of parasites was done by parasite examination covering the outer parts according to (Mahasri, 2016), Examination of parasite in the outer parts is made by gills scrapings and skin mounting methods (Saad *et al*., 2018). Observations of parasites was carried out using a binoculars microscope and identification of parasites was made using Parasites and Disease of Fish Cultured in the Tropics, a guidebook (Kabata,1985). A separate preparation of myxospores was prepared and stained

with Lugol's iodine for visualization of the iodinophilous vacuole according to (Camus and Griffin, 2010).

Samples Preparation Of *Myxobolus Koi* Spores.

Infected fish with *Myxobolus koi* are washed with aquadest water to be sure that the dirt that clings to the body of the fish is absent, and then nodule myxobolus stuck in gills is taken by using a scalpel and tweezers slowly so that the nodules containing spores are not destroyed. The nodules already taken are then placed in Petri dish congaing PBS. Nodules are cut several pieces to remove spores by using a scalpel then added aquadest, putting in the test tubes and centrifuged at the speed of 2500 rpm for 15 minutes to separate the cells of Myxobolus. The formed superficial layer is then discarded, then added aquadest to the sediment, centrifuged again up to 4000 rpm with speed solidified for 10 minutes. Finally, spores are calculated by haemocytometer, then added as much as 2 ml PBS and stored at 4°C in the freezer.

Isolation of whole proteins spores of Myxobolus koi:

Spores that have been calculated are given PBS to taste then centrifuged at speed 5000 rpm for 10 minutes. For lysis, 500 µl of Buffer Lysis was added, then sonicated in ice (1 minute sonication ½ minute break), carried out repeatedly for 10 times. Then the results, is vortexed (½ minute vortex I minute break) in ice, carried out repeatedly 15 times. The results of a vortex is centrifuged for 5 minutes with a speed of 12000 rpm, the formed supernatant were collected and then carried out the analysis of SDS-PAGE. Determination of the concentration of the whole protein Myxobolus spores used Bio-Rad Protein Assay and read using UV-Visible Spectrophotometer with a wavelength of 600 nm.

Analysis of whole proteins of spores of Myxobolus koi with SDS-PAGE.

The purpose of this activity is to find out the pattern of the molecular weight of each fraction protein. Analysis of the protein is done with a method electrophoresis SDS-PAGE gel separating composition of 12.5% and 5%

stacking gel. This is done by electrophoresis method (Electrophoresis was carried out on Mini-PROTEAN® 3 Cell apparatus (Bio-Rad). Running a gel is created and inserted into the plate glass. After hardened on the top put stacking gel prepared. A total of 10 µg samples that added Laemly buffers with a comparison of 2:1 is done boiling at 100°C for 5 minutes, put in well located in the stacking gel. As a marker used protein with molecular weight in the range 250-20 kDa. Then do a running on the chamber has filled Electrode Buffers 1 x with 100 Volts, 40 mA. The running process is stopped after a blue marker reaches the bottom plate of the gel. Next step, the gel inserted into the washer solution consisting of 25 ml methanol, 3.7 ml acetic acid and 100 ml Aquades. Rocked above the shaker for 30 minutes. Repeated washing is done with a solution that is similar to the reduction of the composition of ethanol and acetic acid addition half of previously for 30 minutes. Subsequent washing with a solution of 10% glutaraldehyde and aquadest for 30 minutes. After this, the washed gel stained with silver nitrate (AgNO3) for 15 minutes, then washing with aquadest twice each for 2 minutes take place. Given the color development solution consisting of formaldehyde 3.7%, zitronsauce 5% and aquadest. After the tape is visible then the reaction is stopped by adding acetic acid 10%. The results of the gel that has a Band-Band protein looks ready documented. The result of SDS-PAGE electrophoresis in the form of bands can be determined by molecular weight by calculating Rf (Retardation Factor) score from each band with the following formula (Rantam, 2003):

Rf = Distance of protein movement from starting point / Distance of color movement from starting point.

Then the Rf score is inserted to the linear regression equation as the following formula: Y = a + b X Where: Y = molecule weight, X = Rf score of sample.

Blood samples.

After 14 days of treatment period, five fish from each treatment (per group) were randomly collected at the end of 14 days at a fixed sampling time.

The fish were anesthetized in 200ml of de-chlorine tap water containing 100 mg/l Tricaine methanesulfonate, (MS-222). Sodium bicarbonate can be used to buffer the solution to a pH range of 6.5-7.5 (Brown and Pavek, 2015). Blood was collected from the caudal artery by a non-heparinized 2-ml syringe for haematological study (blood parameters). The blood sample was allowed to clot and centrifuged at 3000 r.p.m for 15 min, for obtaining non hemolysed serum which used for determination of total protein and albumin

Heamatological studies.

Total leukocytic count and differential leukocytic counts were carried out on stained blood films according to Stoskopf (1993).

Determination of serum total protein Globulin and Albumin.

Serum total protein and albumin were determined using diagnostic kits (Bio-Merieux-France) according to Peters (1968) and Drupt (1974), respectively. Calculation of globulin and albumin/globulin ratio according to Schaperclaus (1992).

Statistical analysis:

Statistical analysis was performed using the one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. All da-ta were represented as the mean of 5 specimens ± SE for each group. The statistical significance was accepted at $p< 0.05$. All Statis-tics were carried out using Statistical analysis systems (SAS) program (SAS, 2009)®.

RESULTS

The Characterization of Myxoolus koi nodules and spors.

Myxobolus koi nodules are pinkish white in color and, irregular round in shape and located in the gills. Spores were elongate and pyriform with a rounded posterior, 15.2 (14.6–16.3) mm long and 8.2 (7.0–8.9) mm wide. Polar capsules were pyriform and elongate, 10.0 (8.9–10.7) mm long and 3.0 (2.6–3.6) mm wide. Polar filaments were coiled perpendicular to the long axis of the

spore making 10 turns (9–11).The morphology of the spores found in the common carp can be seen in Figure 1.

Myxobolus Spores Protein by SDS-PAGE.

The analysis results of *Myxobolus koi* spores protein with SDS-PAGE can be seen in Figure 2. Protein bands were showed as that 4 bands of soluble protein are found with molecular weight 120 kDa, 86 kDa, 72 kDa and 48 kDa (Fig.2).

Survival rate.

Survival rate of common carp *Cyprinus carpio* is calculated on at the end of the expetriment is presented in Fig.3. The results showed that, group I (control) and IV (1µg protein / gram fish) had the highest survival rate (97% and 99%, respectively). While group II (80 spores/fish *koi*. m. ko) and III (1µg protein / gram fish+80 spores/fish *koi*. m.) recorded 23% and 65%, respectively. The result revealed, higher significant difference between group II, III, IV as compared to control group (Fig.3).

Heamatological studies: and differential leukocytic count.

Based on the ion of, Data of calculated leukocytes and differential leucocytes counts common carp can be seen in figures 4-5. Total leukocyte counts (10^3µl) was significant ($P<0.05$) higher in fish of group II, III and IV (80 spores M. koi/fish, spores M. koi/fish+ 1µg protein spores M. koi/gram fish and1µg protein spores M. koi/gram fish, respectively) as compared with control group. As well as, total leukocyte counts of group III and IV was significantly ($P<0.05$) was higher than group II. Also, Lymphocytes, monocytes and eosinophils percentage was significantly ($P<0.05$) higher in fish of group II, III and IV as compared with control group. While, neutrophils percentage was significantly ($P<0.05$) lower in fish of group II, III and IV as compared with control group. Our results showed non significate difference of all leucocytes types between group III and group IV.

Serum total protein, albumin and globulin.

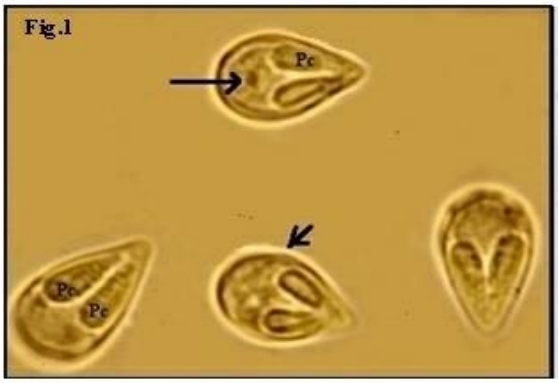
Total proteins and globulin was significantly (P<0.05) higher in fish of group II, III and IV as compared with control group. While, albumin was significantly (P<0.05) lower in fish groups of II, III and IV as compared with control group (Figs.6-8).

Table 1. Proximate composition of Basal diet (C).

Ingredients	Content (%)	Chemical analysis%	
Soya48	49.5		
Rice bran	24.5	Dry matter %	91.7
Corn	15.2	Crud protein 5	35.5
Wheat middling	4.00	Ether extract%	8.9
Soya oil	1.8	Ash%	5.8
Gelatin	1.5	Fier%	6.0
Salt	0.5	GE(Kcal/100g)[4]	389.32
Minerals permix	1.5		
Vitamins permix	1.5		
Total	100		

1- Minerals mix.: Each kg contain manganese 60g, iron 80g, copper 5g, zinc 40g, selenium 0.15 and iodine 0.35g.

2- Vitamins mix. Provide (g, mg or l.U kg diet) Vit. A 5000 *I. U* ,D32.000 I.U, E 100mg, k3 10.0mg,C. 1.000mg. B1 10mg, B2 15.0mg, B6 7.5mg, B12 0.1mg, Biotin 0.2mg, Folicacid 0.4mg, cholin Hcl 1.0g inosit. 3000.0mg, patathemic acid 50.0mg, Nicotinic acid 100mg, P-Aminobenzonic acid 50.0mg.3- Gross energy: Based on 5.65 Kcal/g proteins, 9.45 Kcal/g fat and 4.1 carbohydrate Kcal/g (NRC,1993).



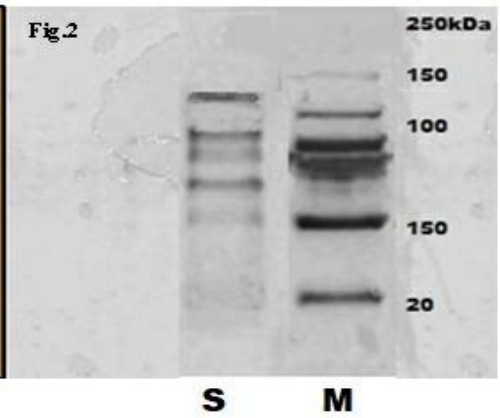


Fig. 1. *Myxoolus koi* infected gills of common carp *Cyprinus carpio.*

Fig. 2. Profile of *Myxobolus koi* Protein Spores by SDS-PAGE for *C. carpio* L. M = marker 250-20 kDa, S1 = samples Protein Spores *Myxobolus koi* with molecular weight (120 kDa, 86 kDa, 72 kDa and 48 kDa).

Fig. 3

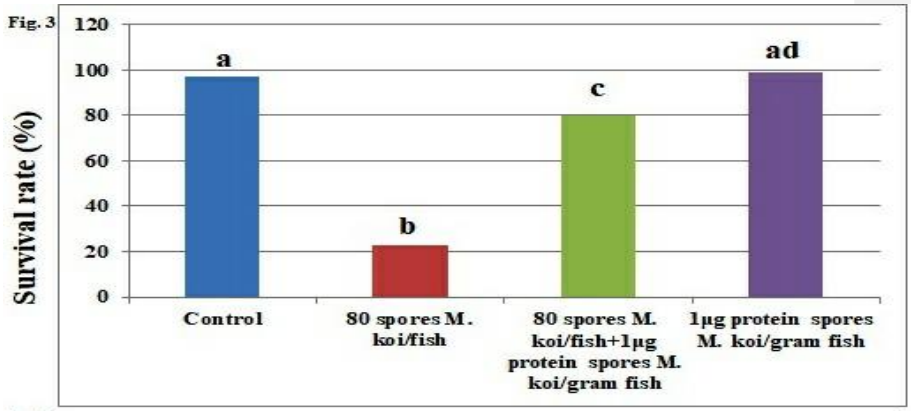


Fig. 4

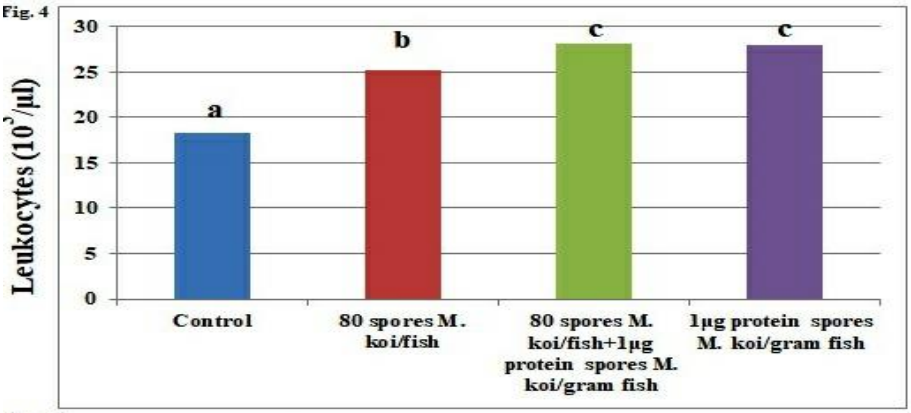


Fig. 5

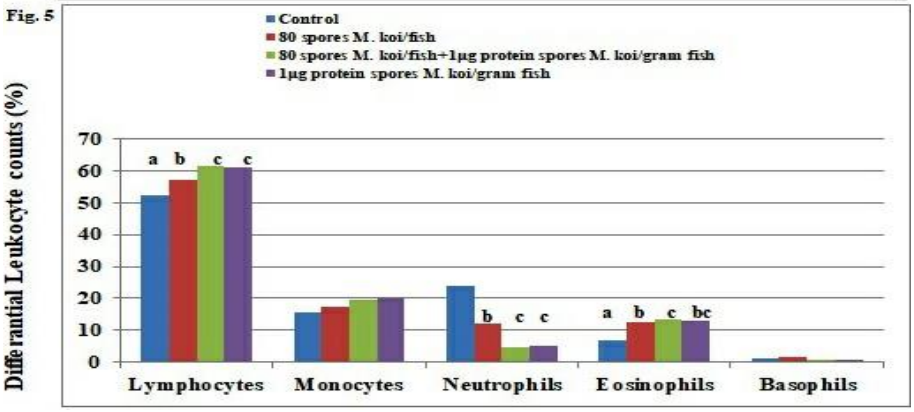
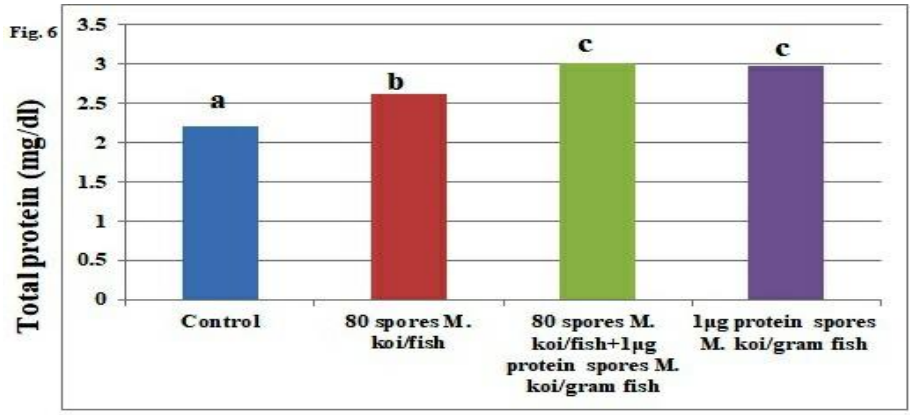
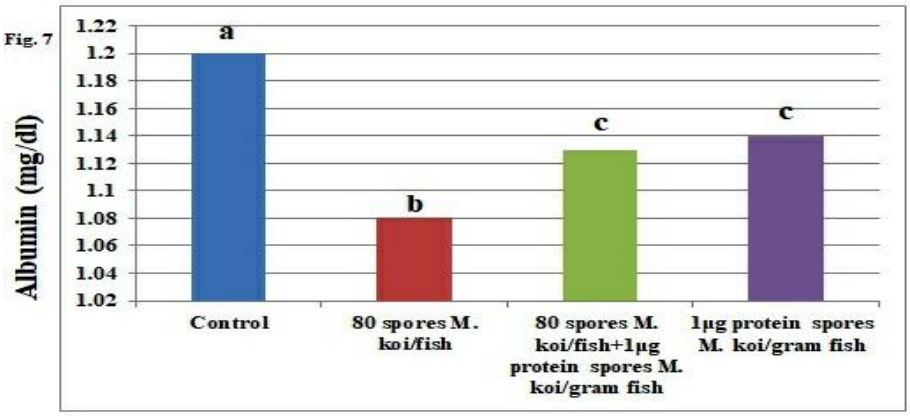


Fig.3. Diagram of survival rate percentage (%) of carp fish *Myxobolus koi* treated with 80 spores *M. koi*/fish and /or 1µg protein spores *M. koi*/gram fish.

Fig.4. Diagram of Leukocyte counts (10^3/µl) of the *Myxobolus koi* of treated with 80 spores *M. koi*/fish and /or 1µg protein spores *M. koi*/gram fish.

Fig.5. Diagram of Differantial Leukocyte counts (%) of the *Myxobolus koi* of treated with 80 spores *M. koi*/fish and /or 1µg protein spores *M. koi*/gram fish.





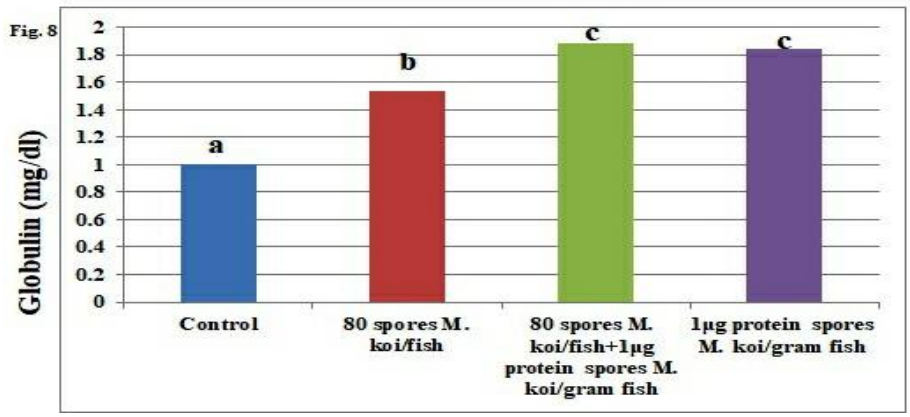


Fig. 6. Diagram of Total protein (mg/dl) of carp fish *Myxobolus koi* treated with 80 spores *M. koi*/fish and /or 1µg protein spores *M. koi*/gram fish.

Fig. 7. Diagram of Albumin (mg/dl) of the *Myxobolus koi* of treated with 80 spores *M. koi*/fish and /or 1µg protein spores *M. koi*/gram fish.

Fig. 8. Diagram of Globulin (mg/dl) of the *Myxobolus koi* of treated with 80 spores *M. koi*/fish and /or 1µg protein spores *M. koi*/gram fish.

Data for figures (4-8) are expressed as the mean ± SE of specimens, N=5 for each group. The small letters indicated statistical significant differences at a *p* value of <0.05 as compared samples from the four groups.

DISCUSSION

Myxobolusis disease causing organism is systematic and recognized from the spore morphology along with the number and location of polar filaments (Camus and Griffin, 2010). In the present study, increased the survival rate from 23% to 65 % of fish (infested *M. koi*) and group (spore protein *M. koi*+ infested *M. koi*), respectively may be attributed to that, spores protein of *M. koi* alleviate the effects of *M. koi* parasite. This result is agreed with Kismiyati and Mahasri (2017) who recorded increased survival rate of carp from 20.0% up to 83.0% in (*M. koi* infested) fish and (*M. koi* infested + spore protein) treated fish, respectively. In addition to , they attributed these results to the protection effect of spore proteins *M. koi* (protective nature) against myxobolusis and decline of the death of goldfish.. Spores protein of *M. koi* can ameliorate the general fish health , this is evidenced by the high survival rate (99%) of (spores protein of *M. koi*) treated fish. Kismiyati and Mahasri (2017) recorded highest survival rate (96%) of (spores protein of *M. koi*) treated fish. Also, they recorded, enhancement of survival rate of carp fish from 10% (*M. koi* infected) up to 86% (*M. koi* infected + spore protein of *M. koi*) treated fish. Akhmad (2018) recorded highest survival rate (99%) in *Cyprinus carpio* L. treated with spore protein of (*Myxobolus koi*) as compared with control group.

The result of the analysis of the whole protein spores *M. koi* used SDS-PAGE , is 4 bands which seem obvious, namely protein with molecular weight (BM) 120 kDa, 86 kDa, 72 kDa and 48 kDa. The results showed that proteins found have a high molecular weight. Other studies conducted by Mahasri (2016) and Yusuf *et al*. (2015) shows the results of the SDS-PAGE electrophoresis analysis of *Myxobolus koi* spores obtained protein bands with molecular weight (150 kDa, 72 kDa and 73 kDa) and (68.1 kDa, 38.5 kDa, 25.6 kDa, 23 kDa, 21.7 kDa dan 18.9 kDa), respectively. Kismiyati and Mahasri (2017) recorded 5 bands of protein with molecular weight (BM) 41.1 kDa, 51

kDa, 89.8 kDa, 121.7 kDa and 230.1 kDa from SDS-PAGE. While, Saad *et al*., (2017) recorded 6-bands protein with molecular weight (68.1kDa, 38.5 kDa, 25.6 kDa, 23 kDa, 21.7 kDa and 18.9 kDa) from SDS-PAGE of whole protein *Myxobolus koi* spores. The results indicated that the separated protein is a good immunogen molecule because it has size of <100 kilodalton (Saad *et al*., 2018). Harlow and Lane (1988) recorded that, an immunogen characteristic molecules having a molecular weight of more than 10kDa. Based on these statements, whole spore protein molecular weight of *M. koi* of the present study obtained by SDS-PAGE is a immunogen molecule. These differences of the molecular weight of SDS-PAGE of whole protein Myxobolus koi spores between the different researches could be due to that, differences of origin, preparation techniques and sampling technique of insulation used (Mahasri, 2016). Ascertain the nature of immunogenic, it needs to do test again with immunoblotting so that potential of immunogenic properties of whole spore proteins of *Myxobolus koi* can be utilized as a candidate of vaccine in tackling myxobolusis.

Fish have a defense system of the body to fight various diseases. Fish immune defenses consists of non-specific (skin, scales and mucus) and specific defenses (Mahasri, 2016). The value of leukocytes in the blood can be used to determine the fish's body's defense system from external disturbances, including pathogens (Syawal *et al*., 2008). For example, change in the total number and types of leukocytes can be used as indicators of the presence of certain infectious diseases in fish (Yusuf *et al*., 2015).

In the present study, total leukocytes counts, Lymphocytes, monocytes and eosinophil showed higher significate value in the groups II, III, IV than the control group. Koi carp fish infected with *Myxobolus* sp. showed high increase in total leukocyte counts as compared with control group (Yanuhar *et al*., 2020). Our results is nearly agreed with Mahasri (2016), who recorded increase percentage of lymphocytes and monocytes in fish vaccinated by (spore protein *M. koi*) than both of fish control and injected fish with (spore protein *M. koi*

+80 spores/fish of M. koi). White blood cells (leukocytes) in fish are part of the fish's body defense system. Factors that affect the number of leukocytes are the condition and health of the fish (Saad *et al*., 2017). Also, Akhmad (2018) recorded increase percentage of lymphocytes and monocytes in fish vaccinated by (spore protein *M. koi*) than both of fish control and injected fish with (spore protein *M. koi* +80 spores/fish of M. koi). While, Yusuf *et al*. (2015) recorded observation of the highest lymphocytes and monocytes in fish treatment (spore protein *M. koi* + 80 *M. koi* spors/tail) and fish (80 *M. koi* spores / tail). Normal lymphocyte percentage of the fish ranges between 60-70% (Affandi and dan Tang, 2002). He attributed his results to the type of the body's defense system response of fish against pathogens (Mahasri, 2016). Lymphocytes functions as antibody producer for immune to interference from disease. (Bastiawan *et. al*., 2001).

In this study, increased the number of monocytes and macrophage may be due to the ingested the *M. koi* and protein spore of *M. koi*. Monocyte with macrophage are defensed against foreign bodies which is the agent of the disease (Bastiawan *et al*.2001 and Ardelli and Woo, 2006). In addition to the phagocyte function of monocyte, making it an antigen presenting cells (APC) to the lymphocytes cells (Kresno, 2001). The primary function of monocyte is phagocytosis and digestion of large particulate matter such as large micro-organisms (Savari *et al*., 2011). It is generally accepted that fish phagocyte after activation are able to generate superoxide anion (O2), which is considered to be toxic for fish bacterial pathogens (Ahmed and Ali, 2013) .

In this study, neutrophils percentage decreased in the three treated groups than the control. Mahasri (2016) attributed this decrease to the increase in lymphocytes and monocytes. Eosinophils is one of the body's defense cells that are dominant in the blood and will increase sharply in number in case of an infection of parasitic diseases (Tizard, 1988). In this study, the highest percentage of eosinophils in of *M. koi* and protein spores *M. koi* treatment could be due to spore infection of *M. koi* increases the number of eosinophils in the blood of fish (Mahasri, 2016). In this study, basophils were observed in

small percentage compared to the other types of leukocytes. Basophils in the fish blood ranged from 0.17-0.19% (Affandi and dan Tang, 2002). The existence of basophils in blood circulation has been observed only in a small number of species of fish. Akhmad (2018) recorded that, lymphocytes and monocytes of fish group (protein spores of *M. koi*) had higher number and neutrophils, eosinophils and basophil was lower than those of control group.

Blood parameters conducted to confirm a diagnosis of a disease, which induce changes in the components of the blood of the fish (Yanuhar *et al*., 2020). The levels of globulin and total protein indirectly reflect the condition of specific humeral immunity (Ali and Ahmed, 2013). The fish body has a mutually supportive defense system to fight against incoming foreign bodies, both specific and non-specific immune systems (Saad *et al*., 2017).

Saad *et al*. (2017) recorded that, immunostimulant (protein spores *M. koi*) added to feed can induce the formation of antibodies in carp fish as seen by increases IgM antibody concentration. Yusuf *et al*. (2015) also recorded increases IgM antibody concentration as a result of immunostimulant (protein spores *M. koi*). In the present the study, the highest concentration of globulin on the (protein spores *M. koi*) treatment may be due to that, spores increased lymphocyte proliferation. This is supported by Kamiso *et al*. (1996), who recorded that, enter of foreign substances into the body potentially increased the proliferation of lymphocytes, as well as increase the number of T cells and B cells and increase the activity of IL-2. This suggestion is evidenced in our study as the higher increase in lymphocytes percentage and globulin of both treatments (protein spores *M. koi*) and (spore protein *M. koi* +80 spores/fish of M. koi). Albumin is an important serum protein for transportation of steroid hormones (Shahsavani *et al*., 2010).

CONCLUSION

We can conclude that, *Myxobolus koi* protein spores is immunostimulant molecule and can decrease common carp (*Cyprinus carpio* L.) death by Myxobolusis.

ACKNOWLEDGMENT

This work was supported by the University of Science and Technology, Zewail City of Science, Technology and Innovation, Egypt.

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فاعلية بروتين جراثيم طفيل ميكسوبولس كوى كمحفز مناعى لمنع الإصابة بمرض الميكسوبوليوسيسس

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الملخـــــص العربـــــي

يهدف هذا البحث لعزل (بروتين جراثيم طفيل ميكسوبولس كوى) بإستخدام الفصل الكهربائى(SDS-PAGE) و دراسة إمكانية إستخدام هذا البروتين كمحفز مناعى و إمكانية قدرة هذا البروتين على تقليل نسبة الوفاة فى السماك الصابة بالطفيل. أجريت هذه الدراسة على عدد ١٢٠ سمكة الكارب سيبرينس كاربيو بمتوسط حجم ٠.٢٢ ± ١٠.٤٤ ومتوسط طول ٠.١٣±٨.٨ وتم تقسيم الأسماك للأربع مجموعات (I,II,III,IV) معالجة ب(١ ميكروجرام /جم من السمكة بروتين جراثيم طفيل ميكسوبولس كوى) و/بدون ٨٠ جراثيم طفيل ميكسوبولس كوى/ للسمكة .

أظهرت النتائج أن المجموعة المعالجة (ببروتين جراثيم طفيل ميكسوبولس كوى) و المجموعة المعالجة (بروتين جراثيم طفيل ميكسوبولس+ ٨٠ جراثيم طفيل ميكسوبولس كوى/ للسمكة) أثبت فاعلية كمحفز مناعى وذلك من خلال زيادة ذات دلالة معنوية (P<٠.٠٥) عدد كرات الدم البيضاء (ليمفوسيط ومنويسط وإزينوفيل) مقارنة بالمجموعة الضابطة والمجموعة المعالجة (٨٠ جراثيم طفيل ميكسوبولس كوى/ للسمكة). كما أظهرت النتائج أن الزيادة فى البروتين الكلى والجلوبيولين دم المجموعة المعالجة (ببروتين جراثيم طفيل ميكسوبولس كوى) والمجموعة المعالجة (بروتين جراثيم طفيل ميكسوبولس+ ٨٠ جراثيم طفيل ميكسوبولس كوى/ للسمكة) زيادة ذات دلالة معنوية (P<٠.٠٥) مقارنة بالمجموعة الضابطة والمجموعة المعالجة (٨٠ سبور طفيل ميكسوبولس كوى/ للسمكة).

أظهرت النتائج إنخفاض نسبة الوفيات من ٦٥ إلى ٢٣ فى المجموعة المعالجة (٨٠ جراثيم طفيل ميكسوبولس كوى/ للسمكة) والمجموعة الضابطة على التوالى. كما إرتفعت نسبة بقاء الأسماك على قيد الحياة زيادة ذات دلالة معنوية (P<٠.٠٥) فى المجموعتان (بروتين جراثيم طفيل ميكسوبولس كوى) و(بروتينجراثيم طفيل ميكسوبولس+ ٨٠ جراثيم طفيل ميكسوبولس كوى/ للسمكة) مقارنة بالمجموعة المعالجة (٨٠ سبور طفيل ميكسوبولس كوى/ للسمكة).

يتضح من هذه النتائج أن إستخدام بروتين جراثيم طفيل ميكسوبولس يصلح كتطعيم للأسماك ضد طفيل ميكسوبولس، كما أن يحسن من الصحة العامة للأسماك.