Original Paper**Effect of Flavomycin and Synbiotic on experimentally-infected common carp with *Pseudomonas aeruginosa***Hamada El Azzy¹, El Sayed, M.¹, Hend megahed², Nahad Kmoura³ and Eman I. Soror⁴¹Bacteriology, ²Biochemistry and ³Clinical Pathology Departments, Animal Health Research Institute (Zagazig branches)⁴Aquatic Animals Diseases and Management Department, Faculty of Veterinary Medicine, Benha University**ARTICLE INFO****Keywords**

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01/10/2020**ABSTRACT**

This study investigated the antibacterial effect of flavomycin and synbiotic against *Pseudomonas aeruginosa* (*P. aeruginosa*) beside the weight gain and haemato-biochemical changes in common carp. Apparently healthy 150 common carp weighing 110 ± 10 g were divided into six equal groups. The 1st group received basal diet without any additives (control group). The 2nd group received basal diet with 8 mg/kg diet flavomycin for 30 days. The 3rd group received basal diet with 3 mg/kg diet synbiotic for 30 days. Fish in 4th, 5th and 6th groups were experimentally infected with *P. aeruginosa*. The 4th group was left untreated. The 5th and 6th groups were treated with 8 mg/kg diet flavomycin or 3 mg/kg diet synbiotic, respectively for 5 days. Swabs were collected to isolate *P. aeruginosa*. Blood samples were collected for haemato-biochemical analysis. Healthy fish received flavomycin or synbiotic revealed significant increase in body weight gain, RBCs, Hb, PCV with non-significant increase in total protein, albumin, globulin, A/G ratio, AST, ALT, urea, creatinine. Infected fish with *P. aeruginosa* showed tail and fin rot, hemorrhagic ulcerative skin, superficial ulcers beside mortality rate up to 32% with significant decrease in body weight, RBCs, HB, PCV %, total protein, albumin, globulin and significant elevation in AST, ALT, ALP, urea and creatinine. Infected fish treated with flavomycin or synbiotic improved the clinical signs, reduced mortality rate to 8% and 12%, respectively, and decreased rate of infection. In conclusion, flavomycin or synbiotic have an effective antibacterial effect against *P. aeruginosa*.

1. INTRODUCTION

Fish culture is an important industry where the production of fish is increasing worldwide. Supplementation of natural components in fish ration improved performance of fish without any harmful effect (El-Ghamry *et al.*, 2002).

Several Bacterial infections are responsible for losses of fish industry through increasing mortality and reducing weight gain. Many bacteria are considered to be saprophytic in nature able to cause diseases when fishes are immune- compromised (Khalil *et al.*, 2001). *Pseudomonas* spp. is considered a widely spread in natural sources of water and associated with septicemia in fish farms (Roberts, 2001).

Fish is susceptible to *P. aeruginosa* with moderate to high losses (Somsiri and Soontornvit, 2002). Main signs appear with *Pseudomonas* infection are hemorrhage in the skin at mouth region, opercula and ventral side of body (Wakabayashi and Egusa, 1972).

Many antibiotics are used to promote growth and health in common carp by improving performance (Parker and Armstrong, 1987), preventing the spread of diseases and increasing body gain (Reilly and Kaferstein, 1997). Flavomycin is a phosphoglycolipid antibiotic that acts by inhibiting bacterial growth (Van Heijenoort, 2001). It

inhibits two groups of bacteria; one is from the group of high activity ammonia production and also gram-negative bacteria (Edwards, *et al.*, 2005). It is used as growth promoters for farmed fish in China (Li *et al.*, 2008).

Synbiotic is defined as mixture of probiotics and prebiotics that positively affects host by improving the implantation and survival of live microbial feed supplements in the digestive system (Hassanpour *et al.*, 2013). Synbiotics have been provided different health benefits as resistance to gut bacterial infection and antibacterial effect and immunomodulatory activities in broilers (Ghasemi *et al.*, 2014).

The aim of this study was to investigate the influence of ration supplemented with flavomycin and synbiotic on controlling *P. aeruginosa* beside its effect on growth performance and certain hematobiochemical parameters in common carp.

2. MATERIAL AND METHODS**2.1. Fish and experimental design**

A total of 150 growing apparently healthy common carp weighing 110 ± 10 g maintained in well-aerated glass aquaria (about 100 liters capacity) filled with dechlorinated tap water of about pH 7.2 and 25 °C temperature. Cleaning

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of water was carried out weekly. A balanced ration, 4% of fish body weight was given. Fish was acclimatized to the laboratory conditions for one week before starting the experiment.

Fish were randomly divided into six equal groups (n= 25). The first group received basal diet without any additives (control group). The second group received basal diet with 8 mg/kg diet flavomycin for 30 successive days (Fairchild et al., 2001). The 3rd group received basal diet with 3 mg/kg diet synbiotic for 30 successive days. Fish in the 4th, 5th and 6th groups were experimentally infected with *P. aeruginosa* (injected intra peritoneal with 0.2 ml of trypticase soya broth containing 3×10^7 cfu/ml *P. aeruginosa* (Eissa et al., 2010). The 4th group infected fish were left untreated. The 5th group fish were infected and treated with 8 mg/kg diet flavomycin for 5 successive days. The 6th group fish were infected and treated with 3 mg/kg diet synbiotic for 5 successive days. Treatment started post clinical signs appearance.

2.2 Materials

2.2.1. Drugs

2.2.1.1. Flavomycin (Pharmastim 4%®)

It is growth promoter produced by Biovet Peshtera (Peshtera, Bulgaria)

2.2.1.2. Synbiotic (PoultryStar sol.®)

It is produced by Biomin –Austria. It was distributed by Dakahlia Poultry Comp. Egypt. It is present in the form of 100 gm sachets contain *Enterococcus Faecium*, *Bifidobacterium Animalis*, *Pediococcus Acidilactici* and *Lactobacillus Salivarius* as probiotics and fructooligosaccharides as prebiotics.

2.2.2. Bacteria:

Pathogenic strain of *Pseudomonas aeruginosa* obtained from Bacteriology Department, Fac. of Vet. Medicine, Zagazig University, was used for experimental infection of fish.

2.2.3. Diet:

The fish were fed on basal diet with the following composition:

Ingredients	% in diet
Soybean meal	40 %
Fish meal	8 %
Ground corn	30 %
Wheat flour	15%
Vegetable oil	3 %
Cod liver oil	1.5%
Dicalcium phosphate	1.50%
Vitamins/ minerals premix	1%

2.3. Swabs collection, culture and identification

Swabs from skin and gills were collected on the first day post treatment period from all treated and untreated common carp. The swabs were streaked on nutrient agar plates and the plates were incubated at 37 °C for 24 hours as described by Cheesborough (1985). The characteristic suspected single colonies were subjected to Gram's staining and then sub-cultured in MacConkey agar and blood agar. *P. aeruginosa* was identified by biochemical test (sugar fermentation test) and biochemical tests were performed following the methods described by MacFadden et al. (2000). Motility test of the isolated *P. aeruginosa* was performed following the method described by Cheesborough (1984).

2.4. Body performance

The fish of each group were weighed at start of the experiment and at last day post supplementation to obtain the body weight gain as indicator for body performance.

2.5. Blood and serum samples

Two blood sample were collected from caudal vein of fish in at the last day post supplementation. The 1st sample was collected in tube contain EDTA as anticoagulant for erythrogram estimation according to Jain (1986), the 2nd sample was collected to obtain serum for estimation of total protein (Dumas et al. 1981), albumin (Drupt, 1974) and globulin was calculated mathematically. Serum was also used for biochemical determination of AST and ALT (Reitman and Frankel 1957), urea (Fawcett and Scott, 1960) and creatinine (Husdan and Rapoport, 1968).

2.6. Statistical analysis:

The obtained data were statistically analyzed by using one-way analysis of variance (ANOVA) according to Petrie and Watson (1999).

3. RESULTS

3.1. Clinical signs

Common carp infected with *P. aeruginosa* showed clinical signs as hemorrhages on body surface, ulcerative skin, tail and fin rot, superficial ulcers with 32% mortality. The treatment with flavomycin and synbiotic reduced the mortality rate to 8% and 12%, respectively (Table 1).

Table 1 Mortality of common carp and re-isolated *P. aeruginosa*.

Groups	Total no	Mortality rate		Re-isolation of <i>P. aeruginosa</i>
		no	%	
Control group	25	0	0	0
Flavomycin	25	0	0	0
Synbiotic	25	0	0	0
Infected	25	8	32	17/17
Infected with Flavomycin	25	2	8	5/23
Infected with Synbiotic	25	3	12	6/22

3.2. Biochemical characteristics of *P. aeruginosa*

P. aeruginosa was identified as Gam-negative short rod bacteria with yellow green colonies. The biochemical tests showed a characteristic pattern with positive oxidase, methyl red, maltose, and catalase with negative indole production.

3.3. Body index

Healthy common carp received flavomycin and synbiotic each alone revealed significant ($p = 0.05$) elevation in body weight gain. *P. aeruginosa* infection in common carp induced significant decrease in body weight gain. Infected fish treated with flavomycin and synbiotic showed significant increase in body weight gain compared to infected group (Table 2).

3.3. Hematological changes

Infected common carp with *P. aeruginosa* showed significant reduction ($p = 0.05$) in RBCs, Hb and PCV compared to control. Fish received flavomycin or synbiotic showed significant elevation in RBCs, Hb and PCV. Common carp infected with *P. aeruginosa* and received flavomycin or synbiotic showed significant increase in

RBCs, Hb and PCV compared to fish infected with *P. aeruginosa* (Table 3).

Table 2 Effect of *P. aeruginosa* and treatment on body weight gains of common carp (n=5).

Groups	Initial body weight	Final body weight	Weight gain
Control group	116.04±0.43 ^a	140.86±1.73 ^b	24.82±1.98 ^b
Flavomycin	115.21±0.37 ^a	146.14±1.98 ^a	30.93±1.02 ^a
Synbiotic	116.16±0.28 ^a	145.48±1.88 ^a	29.32±1.18 ^a
Infected	116.19±0.26 ^a	127.21±1.87 ^d	11.82±1.96 ^c
Infected with Flavomycin	116.15±0.21 ^a	138.23±1.72 ^c	22.88±1.97 ^b
Infected with Synbiotic	115.89±0.23 ^a	137.78±1.86 ^c	21.88±1.92 ^b

Means with different superscripts of the same column indicate significant difference at $P < 0.05$

Table 3 Effect of *P. aeruginosa* and treatment on blood picture of common carp (n=5)

Groups	RBCs count (X10 ⁶ /mm ³)	Hemoglobin content (g/dl)	Packed Volume (%)	cell
Control group	2.34±0.36 ^b	12.81±0.28 ^b	30.27±0.31 ^b	
Flavomycin	4.07±0.29 ^a	14.78±0.27 ^a	33.14±0.37 ^a	
Synbiotic	4.13±0.24 ^a	14.55±0.15 ^a	33.26±0.41 ^a	
Infected	1.60±0.1 ^c	9.73±0.24 ^c	26.10±0.42 ^c	
Infected with Flavomycin	2.21±0.36 ^b	11.89±0.33 ^b	29.56±0.32 ^b	
Infected with Synbiotic	2.16±0.31 ^b	11.56±0.41 ^b	29.44±0.38 ^b	

Means with different superscripts of the same column indicate significant difference at $P < 0.05$

3.4. Biochemical changes

Regarding the protein profile (Table 4), common carp infected with *P. aeruginosa* showed significant ($p = 0.05$) reduction in serum total protein, albumin and globulin. Common carp received flavomycin or synbiotic in tested dose each alone displayed non-significant increase in total protein, albumin, globulin and A/G Ratio compared to control. Common carp infected with *P. aeruginosa* and received flavomycin or synbiotic showed significant increase in total protein, albumin, globulin with non-significant changes in A/G ratio fish infected with *P. aeruginosa* (Table 4).

Regarding the liver and kidney functions (Table 5), common carp infected with *P. aeruginosa* showed significant ($p = 0.05$) elevation in serum AST, ALT, urea and creatinine compared to control. Common carp infected with *P. aeruginosa* and received flavomycin or synbiotic showed significant decrease in serum AST, ALT, uric acid and creatinine compared to fish infected with *P. aeruginosa*. Common carp received flavomycin or synbiotic in tested dose each alone displayed non-significant change in the liver and kidney function compared to control.

4. DISCUSSION

Our observed result revealed that, common carp infected with *P. aeruginosa* showed clinical signs as hemorrhages on body surface, ulcerative skin, tail and fin rot, ulcers with mortality up to 32%. Similar clinical signs and mortality rate were observed by EL Nagar (2010) who stated that *Pseudomonas* species infect various species of fish and cause pseudomonas septicemia. These clinical signs could be attributed to the pathogenic effect of *P. aeruginosa* toxins (Olgerts et al 1982). Similar signs were also observed by Amrevuawho et al. (2014) in *Clarias gariepinus* infected with *P. aeruginosa*. *Pseudomonas aeruginosa* is the important fish pathogen that induced ulcerative syndrome, bacterial hemorrhagic septicemia, tail and fin rot and ascites (Paniagua et al., 1990).

Table 4 Effect of *P. aeruginosa* infection and treatment on protein profile of common carp (n=5)

Groups	T. protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio
Control group	3.89 ±0.28 ^a	1.99 ±0.19 ^b	1.90 ±0.17 ^a	1.05±0.08 ^a
Flavomycin	4.38 ±0.29 ^a	2.27 ±0.11 ^a	2.11±0.10 ^a	1.07±0.06 ^a
Synbiotic	4.33±0.26 ^a	2.24±0.14 ^a	2.09±0.18 ^a	1.06±0.09 ^a
Infected	2.21±0.33 ^b	1.14±0.10 ^c	1.07±0.19 ^b	1.07±0.06 ^a
Infected with Flavomycin	3.68±0.19 ^a	1.89±0.15 ^b	1.79±0.13 ^a	1.06±0.09 ^a
Infected with Synbiotic	3.84±0.20 ^a	1.97±0.19 ^a	1.87±0.21 ^a	1.06±0.07 ^a

Means with different superscripts of the same column indicate significant difference at $P < 0.05$

Table 5 Effect of *P. aeruginosa* infection and treatment on liver and kidney function of common carp (n=5)

Groups	AST (U/L)	ALT (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control group	28.05±0.53 ^b	43.77±0.73 ^b	1.78 ±0.09 ^b	1.21±0.04 ^b
Flavomycin	30.34±0.92 ^b	44.13±0.61 ^b	1.82±0.16 ^b	1.25±0.13 ^b
Synbiotic	30.22±0.81 ^b	44.12±0.68 ^b	1.83±0.18 ^b	1.24±0.11 ^b
Infected	33.51±0.57 ^a	46.88±0.59 ^a	1.98±0.12 ^a	1.39±0.08 ^a
Infected with Flavomycin	29.16±0.77 ^b	43.88±0.28 ^b	1.80±0.12 ^b	1.22±0.06 ^b
Infected with Synbiotic	29.12±0.66 ^b	43.89±0.27 ^b	1.79±0.12 ^b	1.23±0.14 ^b

Means with different superscripts of the same column indicate significant difference at $P < 0.05$

In the present study it has been observed that healthy common carp fish received flavomycin and synbiotic each alone revealed significant elevation in body weight gain. Comparable results were observed by Yong, et al. (2007) in Tilapia fish. Dietary flavomycin induces increase in body weight gain of hybrid Tilapia (Zhigang, et al. 2009). Similar improvement in body weight gain was reported by Kumar, et al. (2018), who found that dietary synbiotic induced improvement in body weight gain in Tilapia and common carp. Moreover, dietary supplementation of synbiotic improved body performance in rainbow trout (Zohre, et al. 2011). The present investigation indicated that *P. aeruginosa* infection in induced significant decrease in body weight gain. Freshwater murrel infected with *P. aeruginosa* showed reduction in weight gain (Koteshwar and Benarjee, 2017). The increase in body weight gain induced by synbiotic could be attributed to the increased activities of the trypsin and chymotrypsin as recorded by Dehaghani et al. (2017).

Treatment infected carp with flavomycin or synbiotic in tested dose induced disappearance of clinical signs, reduced mortality rate to 8% and 12%, respectively and reduction in re-isolation of *P. aeruginosa*. Similar observation was reported by He et al. (2010), who showed that flavomycin reduced clinical signs and reduced mortality in hybrid Tilapia. This result is further confirmed by the findings reported by Gülmez and Güven (2002), who concluded that synbiotic has a therapeutic and protective effect against many intestinal bacteria leading to prevention of many diseases. Same results were reported by Abd El hamid et al. (2009), who stated that dietary synbiotic improved protein picture in rainbow trout.

The present study revealed that, common carp experimentally infected with *P. aeruginosa* showed significant reduction in RBCs count, Hb content and PCV%. Similar hematological changes were recorded by Koteshwar and Benarjee (2017), who concluded that *Pseudomonas* infection induced reduction of RBCs, Hb and PCV in fresh water murrel. The study also indicated that

carp fish received flavomycin or synbiotic showed significant elevation in RBCs, Hb and PCV. Previous researched showed that the inclusion of probiotics with fish stimulates the hemopoiesis in fish (Marzouk *et al.*, 2008). In addition, the PCV% of the blood as well as increased production of erythrocytes and leukocytes were significantly increased in fish fed with a diet with the probiotic (Dahiya *et al.*, 2012). The obtained results agreed with Chuan *et al.* (2010) who reported that carp fish received flavomycin showed elevation in RBCs, Hb and PCV. Treatment of infected carp with flavomycin or synbiotic significantly increased the blood parameters compared to infected fish. It has been demonstrated that synbiotic induced elevation in RBCs, Hb and PCV in common carp (Ivaylo *et al.*, 2018). Experimentally infected fish with *P. aeruginosa* showed significant reduction in serum total protein, albumin and globulin. Our obtained results agreed with those of Magdy *et al.* (2014), who found that *P. aeruginosa* infection induced significant reduction in serum total protein, albumin and globulin in African catfish. This could be attributed to the deleterious effect of *P. aeruginosa* on hepatic function. Receiving flavomycin or synbiotic each alone increased the total protein, albumin, globulin and A/G Ratio. In comparison to infected group, the addition of synbiotic or flavomycin increased the protein indices. The increased protein profile might be attributed to addition of synbiotic that improved intestinal environment which leads to increase of digestion and absorption of nutrients (Mariam *et al.* 2010). Flavomycin improved total protein, albumin and globulin in Tilapia (Yong *et al.* 2007). Also, antibiotic suppress pathogenic microorganisms in intestine leading to improves absorption and assimilation of nutrient (Coles, 1986). These results were supported by Zohre *et al.* (2011), who stated that dietary synbiotic improved protein picture in rainbow trout.

The present study revealed that carp experimentally infected with *P. aeruginosa* had a significant elevation in serum AST, ALT, uric acid and creatinine. Our results come in agreement with those reported by Magdy *et al.* (2014), who demonstrated that *P. aeruginosa* infection in African Catfish show significant increase in AST, ALT, uric acid and creatinine. The increased level of hepatic and liver function could be attributed to the increased lipid peroxidation that caused oxidative damage to the hepatic and kidney cells (Magdy *et al.*, 2014). Fish received flavomycin or synbiotic each alone revealed produced non-significant elevations of serum AST, ALT, urea and creatinine. Liu *et al.* (2009) found that flavomycin increased AST, ALT, urea and creatinine in healthy Tilapia and Cyprinus Carpius. However, Ghasempour (2015) concluded that dietary synbiotic has no effect on ALT and AST, urea and creatinine of common carp. Also, Khalesi *et al.* (2018) found that synbiotic does not affect serum ALT, AST, uric acid and creatinine levels. On the other hand, Hassanein *et al.* (2017) noticed that treated diets with probiotic *S. cerevisiae*, or *L. acidophilus* significantly decreased ALT and AST values compared to control group. Soltan and El-Laithy (2008) found that, ALT and AST levels significantly decreased when Nile tilapia fed diets supplemented with probiotics compared to control group.

5. CONCLUSION

It could be concluded that flavomycin or synbiotic helps in treatment *P. aeruginosa* infection and induced

improvement in hematobiochemical parameters. Therefore, it is recommended to add flavomycin or synbiotic in common carp diet as growth promoters.

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