INFLUENCE OF DIETARY OXYTETRACYCLINE SUPPLEMENTATION ON GROWTH, SURVIVAL AND IMMUNE STATUS OF CYPRINUS CARPIO

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Key words: Cyprinus carpio; oxytetracycline; growth performance; Immunity.

ABSTRACT

A feeding experiment was carried out for 12 weeks to evaluate the effects of oxytetracycline (OTC) on growth performance and immunological parameters of Cyprinus carpio. Eighty fish were assigned randomly to two dietary treatments. The treatments were (I) a basal diet (control) (ii) basal diet+100 mg OTC/kg feed. Each dietary treatment had four replicate aquaria with 10 fish per aquarium. Body weights gain, average daily weight gain, condition factor (K) and immunological parameters of fishes were determined biweekly during the feeding trial. It was observed that at 4, 6, 8, 10 and 12 weeks fishes fed OTC medicated pellets had significantly (P <0.05) heavier body weights than the control fishes. Body weight gains, average daily weight gain and specific growth rate were also significantly (P <0.05) improved by OTC diets throughout the experimental period. Condition index (K) was significantly higher (P < 0.05) after 6, 8, 10 and 12 weeks of OTC administration. The results indicated that oxytetracycline significantly suppressed IgM as well as the level of circulating white cells in Cyprinus carpio. The survival after challenge was significantly affected by oxytetracycline in the present tests. The present study suggests that, at concentrations commonly used in aquaculture, adding of OTC to feed enhanced growth of carp, but on the other hand suppress the immune response and decrease disease resistance.
INTRODUCTION

In animal agriculture, approximately 15.4 million pounds of antibiotics are used each year (CDC, 2004). The benefits of using antibiotics for growth promotion were first reported by Stokstad and Jukes (1950) when chickens exposed to small doses of chlortetracycline grew more rapidly than non-treated chickens. At sub-therapeutic levels, antibiotics are helpful in: (1) improving growth, (2) reducing risk of disease, (3) improving digestion, (4) fattening of domestic animals, and (5) decreasing time and the amount of feed needed to reach slaughter weight (Frost, 1991; Luetzow, 1997).

Several antimicrobial classes are approved for use in food animals including beta-lactams (e.g., penicillin, ampicillin, and cephalosporin), tetracyclines (e.g., oxytetracycline, tetracycline, and chlortetracycline), aminoglycosides (e.g., streptomycin, neomycin, and gentamicin), macrolides (e.g., erythromycin), lincosamides (e.g., lincomycin and pirlimycin), and sulfonamides (e.g., sulfaemethazine) (Mitchell et al., 1998; Hoeben et al., 1998).

Subtherapeutic amounts of tetracyclines are used in certain countries as feed additives for the growth promotion in animal husbandry, e.g. in calves, chickens, turkeys and sheep (Schnappinger and Hillen, 1996; Schwarz et al., 1998).

This study was planned to investigate the effect of feed additive oxytetracycline on growth performance, immune response and susceptibility of Cyprinus carpio to infection with Aeromonas hydrophila.

MATERIALS & METHODS

(a) Fish

Eighty fishes (Cyprinus carpio) were purchased from Central Laboratory for Aquaculture Research (CLAR), Suez Canal University, Faculty of Agriculture. Fish were transported alive to the laboratory in airpumped large clear polyethylene bags. Before transferring fish into glass aquaria they were placed in dechlorinated tap water to which about the same amount of water was added from the trans-porting bags in which the fish was brought from Central Laboratory for Aquaculture Research to bring the temperature and constituents closer to the natural conditions. After 4-5 hours the fishes were stocked in 8 aquaria at the rate 10 fishes per aquaria. The aquaria had a size of 30 x 40 x 60 and a capacity of 60 liters. Aeration of aquaria water was done using air pumps. One air pump with two air stones was used for each aquaria aeration. Fine cloth of net was tied at the top of the tanks to prevent fish from jumping.

The fish did not receive feed for the first two days then fish were fed on commercial pellets with an average protein content of about 30% and
were fed twice a day at rate of 4% of biomass.

(b) Tested compound
Oxytetracyclide is a commercial product available in the market manufactured by Chemical Industries Development (CID)- Giza-A.R.E. Each capsule contains 250 mg oxytetracycline HCL. The dose was calculated to be 100 mg /kg diet (Badran, 1994), then mixed with the feed using eggs as coating agent to prevent leaching of drug. The pellets were prepared biweekly, air dried at room temperature and stored in a refrigerator (4 °C) for daily use.

(c) Experimental design
After acclimatization, the fishes were divided into two major groups. The treatments were (i) a basal diet (control) (ii) basal diet+100 mg OTC /kg feed. Each dietary treatment had four replicate aquaria with 10 fishes per aquaria. The tested fishes were fed pellets medicated with oxytetracycline at a predetermined rate (4% of body weight) every day for 12 weeks. The control group were given the same amount of untreated feed at the same time. Fish in the different experimental groups were monitored and sampled biweekly up until 12 weeks for assessment the growth performance, leucocytic and serum IgM level.

After the immunomodulatory trial for 12 weeks of administering Oxytetracycline through feed, all fish were challenged intraperitoneally with 0.1 ml of 16 hours tryptic soya broth containing $10^8$ CFU /ml virulent strain of A. hydrophila. The fishes were daily observed for any clinical signs and mortalities for 7 days.

(d) Blood and serum sampling
Blood was collected from alive fishes by severing of the caudal peduncle (Orun et al., 2003). Before collecting blood samples, no anesthetic compounds were applied to fish as they may affect blood parameters and tissues (Hoffman, 1977). Part of the blood was heparinized and the rest was allowed to clot at room temperature. Serum was preserved at -20 °C or used immediately for analysis. Blood samples were collected in polystyrene cool bag using anticoagulant containing glass tube while blood count was carried out immediately after sampling.

(e) Morphological parameters:
Fish samples were collected from each treatment and control groups at 2 weeks intervals during the experimental period, then weighted for determining (Average body weight, Body weight gain, Average daily gain (ADG), condition factor (CF) and specific growth rate according to Ricker, (1979) using the following equations:
Total gain (g / fish) = Wt – Wo
Average daily gain (g /fish /day) = Wt – Wo/n
Condition factor (CF) = \( \frac{\text{Weight (g)}}{100 \times \text{Length (cm)}} \)

Specific growth rate (SGR) = \( \frac{(\ln W_t - \ln W_0)}{\text{n}} \)

Wo: Is the initial fish weight (gm) at the start of the experiment.
Wt: Is the final fish weight (gm) at the end of the experiment.
n: Is the duration period of the experiment in day.
Lin: Is the natural logarithm.

**f** Immunological studies:

I- Differential leucocyte count

Blood samples were collected at the beginning of the feeding trial (week 2) and throughout the end of trial (week 12) from the caudal peduncle of both the test and control fishes as described by *Stoskopf (1993)* and *Joshi et al. (2000)*. The blood samples were dispensed into tubes containing sodium heparin anticoagulant. White blood cells (WBC) were counted by Neubauer's improved haemocytometer using Dacies solution as a diluting fluid. 4 large (1sq mm) corner squares of the haemocytometer were counted under the microscope (Olympus) at 1000 X. the total number of WBC was calculated in \( \times 10^3 \) (*Wintrobe, 1967*). For the differential count, a dry fixed blood film by methyl alcohol was stained by Giemsa’s stain, WBC were counted until 200 WBC on blood smears, and the percentage of each WBC type were multiplied by the total WBC count to obtain absolute differential cell counts. This method of manually determining total WBC and differential count has been recommended for fish blood (*Stoskopf, 1993*), because nucleated RBC prevent accurate enumeration using automa-ted analysis (*Huffman et al., 1997*).

II- Determination of serum IgM (mg/ml):

The IgM level was determined by using Turbox immunoglobulin M assay obtained from Orion Corporation Orion Diagnostica, Finland (Catalog no. 67567).

(f) Data analysis

Statistics were calculated with SPSS for windows version 14.0, the means value obtained in the different groups were compared by unpaired student's t-test (*Field, 2000*).

**RESULTS**

Assessment of Growth Performance

The first question we addressed was whether the growth rate of fish fed OTC medicated pellets differ from controls at different time intervals using appropriate t-test. It was observed from figure (1) that after 4, 6, 8, 10 and 12 weeks fishes fed OTC medicated pellets had significantly (p<0.05) heavier body weights than the control fishes.

Body weight gains, average daily weight gain and specific growth rate were also significantly (p<0.05) improved by OTC diets during the feeding period from 4th week and throughout the experimental period. The present results
also revealed significant increase in the condition factor than that of the corresponding control groups after 6, 8, 10 and 12 weeks of OTC administration. The growth performance (Body weight gain, average daily weight gain, specific growth rate and condition factor) of *Cyprinus carpio* fed on pellet medicated with Oxytetracycline showed a significant increase (p< 0.05) compared with the control group by the end of the experimental period (12 weeks) (table 1). **Immunological parameters** The results were analyzed to determine if there is a difference in total leucocytes count and differential leucocytic count means of OTC-treated *Cyprinus carpio* from the controls by using an appropriate t-test. After 4 weeks, there were a significant reduction in total leucocyte count, monocyte and granulocyte count. After 6 weeks there were significant reduction in total leucocyte count, monocyte, granulocyte and lymphocytes were recorded. Also, after 8, 10 and 12 weeks, the results were similar to those noticed after 6 weeks fig (2). With respect to level of serum IgM during the feeding trail, there was significantly decreased level after 4 weeks with a percentage fall of 11.80%. Moreover, the level of Immunoglobulin-M was significantly decreased after 6 weeks and throughout the experimental periods with a percentages fall of 12.58 to 51.67 as compared to corresponding control (fig. 3).
Fig. (1): Effect of dietary OTC supplementation on body weight gain (a) average daily weight gain (ADG) (b) specific growth rate (SGR) (c) and condition factor (d), of *Cyprinus carpio* during feeding experiment.

Table (1): Effect of dietary OTC supplementation on body weight, weight gain (WG) and condition factor (K) of *Cyprinus carpio* by the end of experimental period (12 weeks).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Oxytetracyline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight</td>
<td>23.08 ± 0.12</td>
<td>22.32 ± 0.51</td>
</tr>
<tr>
<td>Final weight</td>
<td>35.63 ± 0.78</td>
<td>47.05 ± 1.54</td>
</tr>
<tr>
<td>Weight gain (WG)</td>
<td>12.22 ± 0.82</td>
<td>24.73 ± 0.26*</td>
</tr>
<tr>
<td>ADG (g / fish / day)</td>
<td>0.15 ± 0.01</td>
<td>0.29 ± 0.01*</td>
</tr>
<tr>
<td>SGR (% / day)</td>
<td>3.03 ± 0.17</td>
<td>5.33 ± 0.08*</td>
</tr>
<tr>
<td>K (%)</td>
<td>2.42 ± 0.02</td>
<td>2.72 ± 0.04*</td>
</tr>
</tbody>
</table>

Weight gain (WG) = average final weight (g) - average initial weight (g). Average daily gain (ADG) = WG (g) / experimental period (days). Specific growth rate (SGR) = (Ln. Final body weight - Ln. Initial body weight) x 100/ experimental period (days).

Data represents the mean value ± S. E. from 10 fish / group.

(*) represents a significant difference between the control and treated group, using Student Unpaired t- test (p< 0.05).
Fig. (2): Effect of dietary OTC supplementation on total leucocytic count monocyte count, of *Cyprinus carpio* during feeding experiment (a) Total leucocytes count (b) Lymphocytes (c) Granulocytes (d) Monocytes.

(*) represents a significant difference between the control and treated groups, using Student Unpaired t-test (*p* < 0.05).
Determination of mortality

The mortality rate after the challenge infection using *A. hydrophila* was 90 and 70% for groups treated with OTC (100mg /kg feed) as well as the control group, respectively (table 3).

Table (3): Mortalities after challenge in *Cyprinus carpio* treated with OTC.

<table>
<thead>
<tr>
<th>Fish groups</th>
<th>No. of fish</th>
<th>Route of injection</th>
<th>Type of Inoculate</th>
<th>Challenge level (cfu/fish)</th>
<th>Died fish during 7 days after injection.</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-ve control</td>
<td>10</td>
<td>I/P</td>
<td>Saline</td>
<td>0.1 ml</td>
<td>_ _ _ _ _ _ _ _</td>
<td>0</td>
</tr>
<tr>
<td>+ve control</td>
<td>10</td>
<td>I/P</td>
<td><em>A. hydrophila</em></td>
<td>$0.1 \times 10^8$</td>
<td>_ 1 2 _ 2 1 _ _</td>
<td>70</td>
</tr>
<tr>
<td>OTC</td>
<td>10</td>
<td>I/P</td>
<td><em>A. hydrophila</em></td>
<td>$0.1 \times 10^8$</td>
<td>_ 2 3 1 1 1 _ _</td>
<td>90</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The growth performance parameters of common carp (*Cyprinus carpio*) fingerlings which fed diets supplemented with OTC as feed additive at a dose of (100 mg / kg) of diet for 12 weeks are shown in figure (1). Body weight gain, average daily weight gain and specific growth rate of *Cyprinus carpio*
fingerlings fed the experimental diets at the start did not differ, indicating that groups were homogeneous. After 4 weeks and throughout the experimental period (12 weeks) the group of fish fed the supplemented diet had significantly (P<0.05) higher Body weight gain, average daily weight gain and specific growth rate when compared with corresponding controls.

These results are in agreement with the results of Rijkers et al. (1980) who found that oxytetracycline increased the growth of carp, *Cyprinus carpio*. Similar results were reported by Ahmad and Matty (1989) who stated that juvenile carp fed on diets supplemented by virginiamycin and terramycin exhibited greater growth than those fed with the control diet.

Similar trend was found, in this respect with Sanchez-Martínez et al. (2008) who studied the effect of supplementing Channel Catfish, (*Ictalurus punctatus*) feeds with oxytetracycline. They observed Oxytetracycline-treated catfish exhibited a significant increase in weight gain, suggesting a growth pro-motion action of this antibiotic agent.

In contrast to the result of the present study, Rawles et al. (1997) reported that dietary supplementation of oxytetracycline did not enhance growth of channel catfish over a period of 8 wk. Also, Toften and Jobling (1997) reported that dietary supplementation of oxytetracycline had a suppressing effect in the growth of Arctic char, *Salvelinus alpinus* after 6 weeks of treatment.

The performance benefits of oxytetracycline have been established for the major livestock species (bovines, swine, chicken), where it improves body weight gain and feed efficiency (Quigley et al. 1997; DiBner and Richards 2005). It is presumed that its effects lie in the reduction of the gastrointestinal tract bacteria (Gaskins et al. 2002; Collier et al. 2003). In chickens, it has been reported that physical changes in the intestine of birds given antibiotics as growth promoters, resulted in improved performance (weight gain) (Miles et al. 2006). The results of the present study indicated that Condition index (K) of *Cyprinus carpio* was significantly higher in oxytetracycline-treated fishes from the 6 weeks and lasted until the end of the trial, indicating that medicated feed with oxytetracycline effectively contributed to enhancing fish performance. Whether this K increase represents an increased fat storage is unknown, but studies in pigs showed that oxytetracycline improves insulin sensitivity of muscle (*B?in-Heick and Heick, 1976*), which has an effect on glucose conversion to total
lipid, and its fixation to muscle (Etherton et al., 1981). These observations may be supported by Sanchez-Martinez et al. (2008) who reported that exposure of healthy channel catfish to oxytetracycline enhanced condition index.

Concerning the non-specific immune modulation in Cyprinus carpio received diets supplemented with OTC. It was clear that low non-specific immunity was developed as manifested by decreasing number of lymphocytes, granulocyte and monocytes compared with controls, in the differential leucocytic count as well as decrease in the total leucocytic count as shown in figure (2). These results agreed with the results obtained by Boon et al. (1995) who studied the effects of oxytetracycline (OTC) on peripheral blood leucocytes Anguilla anguilla L. using measurements of differential white blood cell counts in blood smears, flow cytometry and respiratory burst activity of adherent cells. Results revealed that OTC affected different leucocyte populations. Similar results were reported by Van der Heijden et al. (1996), who worked on European eel and stated that, dietary OTC supplementation modulate the cellular response rather than the humoral response.

Recently, Omorie and Oyebanj (2007) examined the longterm effect of oxytetracycline, on several blood parameters of the Nile tilapia (Oreochromis niloticus) under laboratory conditions. They found a significant reduction in leukocyte. Serum IgM level determined on the fishes treated with OTC-medicated pellets are shown in figure (3). This figure displays significant drop (P<0.05) in IgM levels after 2 weeks and through out the experimental period in comparison with untreated control fishes. These results were in agreement with that of Rijkers et al. (1981) who stated that administration of OTC either by mixing with feed or by intraperitoneal injections decreased serum immunoglobulin levels in carp.

Moreover, OTC as a feed additive was shown to have immunosuppressive effect on Nile tilapia (Badran, 1994). Also, Myers et al. (1995) demonstrated that In vivo exposure to OTC slightly delayed initiation of antibody formation during the primary response. Adding OTC to feed gave mortality rate of 90% at 100mg / kg of feed among Cyprinus carpio challenged by A. hydrophila as compared to control (70%). This could be due to leucopenia and reduction of IgM level in fish fed OTC medicated pellets. This result was confirmed by Grondel et al. (1985) who demonstrated that low concentrations of tetracycline delay leukocytes mitosis, which means that these drugs adversely impact the number of cells available to
guarantee the cellular immune response. It could be concluded that the usage of OTC as a feed additive should be reconsidered because OTC has proved to have immunosuppressive effect and renders the fish more susceptible to infection.

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TD5-3VYTW71-1S&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&view=c&_searchStrId=1034233376&_rerunOrigin=google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=3d35e87872af070b3544c7ddc88d4057 - aff1

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