Effect of Supplementing Different Dietary Levels of Antibiotic (Tylosin 20%) on the Blood Picture of Common Carp (Cyprinus carpio L.)

U K I M. AL-Bayaty and S A S. AL-Shawi
Department of Animal Resources, College of Agriculture, University of Baghdad, Baghdad, Iraq

Abstract: For the study of the Effect of supplementing different dietary levels of antibiotic (Tylosin 20%) on the blood picture of common carp (Cyprinus carpio L.) an experiment was carried out. Fish were fed with a laboratorial manufactured diet with protein content of 31.04%, energy 1437 calories/kg, and an antibiotic Tylosin 20% added at different levels 0, 20, 60, 100 mg/kg B.W statistically red blood cells count (RBC) did not show great differences among treatments In spite of significant differences (p<0.05) between T2 and T4 treatment (3.03 x 10^6 mL, 3.17 x 10^6 mL), respectively white blood cells(WBC)did not record any significant differences among all treatments. While the count of differential white blood cells record significant differences(p<0.05) for the treatment T3 and T4 (68.67 and 0.69%) respectively in the lymphocytes, whereas there were not any significant differences among all experimental treatments in neutrophils, monocytes, eosinophils and basophils cells.

Key words: Tylosin, RBC, WBC, antibiotic, common carp

INTRODUCTION
Antibiotics are a group of natural or synthetic compounds that destroy bacteria (bactericidal) or inhibit their growth (bacteriostatic). They are sufficiently nontoxic to the host, and they are used as chemotherapeutic agents in the treatment of infectious diseases of humans, animals and plants (Espinosa, 2009). Tylosin is an antibiotic of the macrolide class developed for veterinary use. It is made naturally by the bacterium Streptomyces fradiae and acts to inhibit bacterial protein synthesis by inhibiting the 50S ribosome, a cellular structure only certain bacteria have and use to make internal proteins (Botsoglou and Filetours, 2001). Tylosin is a mixture of four macrolide antibiotics. The main component of the mixture is Tylosin A (>80%), Tylosin B (Desmycosin), tylosin C (Macrocin) and tylosin D (Relomycin). All four components contribute to the potency of tylosin, which should not be less than 900 IU/mg, calculated with reference to the dried substance (European Pharmacopoeia, 2004). Tylosin is also used to treat bovine respiratory and swine dysentery diseases. In some countries, tylosin is also registered for use as a growth promoter for a variety of terrestrial such as poultry, pigs and cattle in addition to aquatic animals which are grown for human consumption (Hirsch et al., 1999). Tylosin is licensed for use as a broad spectrum antibiotic for injectable or oral use in treatment of respiratory tract and skin infections in livestock. Spectrum of activity similar to that of Erythromycin but more active than Erythromycin against certain Mycoplasmas (USP, 2000). A complete blood count is an important diagnostic tool, with laboratory protocols and reference ranges well established in both human medicine and in veterinary medicine of domestic animals (Arnold, 2009) Blood tests been studied because they are important physiological studies, both theoretical and practical because they are the basis for understanding the situation of disease and normal life of fish, especially when using different levels of the antibiotic tylosin 20% in the diets, that may leave marks on the physiology of fish and reflects directly on the blood parameters. The most common cell encountered in the blood is the erythrocyte, overall, there are approximately 1,000 erythrocyte for every leukocyte. Red blood cells (Erythrocyte) in fish contain a nucleus and are oval-shaped and get their characteristic color of hemoglobin, which consists of protein (Globin) and Heme pigment (red yellowed) contains iron. The leukocytes (White Blood Cells), are the major participants in both the inflammatory and immune response mechanisms. There are five types of leukocytes. Neutrophil, Monocyte, Lymphocytes, Basophiles and Eosinophil (Voigt and Swist, 2012).

MATERIALS AND METHODS
Fish were transported from local fish farm located at southern part of Baghdad, Iraq. Fish were set randomly in 8 glass aquaria (capacity 72L) filled with dechlorinated tap-water at 4 individuals per aquarium and acclimated to experimental diet (Table 1) for two weeks before started the trail. Initial mean weight of fish was 23.62±0.125 gm. Antibiotic tylosin 20% brought from local market (manufactured by Alfasan company for
Table 1: Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Brown flour</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Vitamin and Mineral premix*</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Antibiotic (Tylosin20%)</td>
<td>0 mg</td>
<td>20 mg</td>
<td>60 mg</td>
<td>100 mg</td>
</tr>
</tbody>
</table>

*Premix of Minerals and vitamins produced by Supravit Jordan (each g containing:
- Vitamin A: 7000 IU
- Vitamin D3: 1300 IU
- Vitamin E: 0.8 mg
- vitamin K3: 1.75 mg
- vitamin B1: 0.45 mg
- vitamin B2: 0.45 mg
- Vitamin B6: 0.22 mg
- vitamin B12: 0.007 mg
- niacin acid: 5.2 mg
- 0.045 mg folic acid
- manganese sulfate: 0.0035 mg
- zinc sulfate: 0.001 mg
- iron sulfate: 0.001 mg copper sulfate: 0.003 mg)

RESULTS AND DISCUSSION

Blood tests have been studied because they are important physiological studies, both theoretically and practical because they are the basis for understanding the situation of disease and normal life of fish, especially when using different levels of the antibiotic tylosin 20% in the diets, which may leave marks on the physiology of fish and reflects directly on the blood parameters water temperatures ranged between 24.5-25.75°C (mean±S.E = 25.13±0.6°C) and the pH range between 7.9-8.2 (mean±S.E = 8.05±0.15) during the experiment. Their values were within normal levels of common carp living (FAO, 1981). Statistically RBCs count showed a significant differences (p<0.05) in T4 (3.17 x 10^6/mL) compared to T1 and T2 and was not significant (p<0.05) with T3 (3.10 x 10^6/mL). Also treatment T2 (3.03 x 10^6/mL) showed a significant difference comparably with T1 (2.83 x 10^6/mL), but was not significant with T3 (3.10 x 10^6/mL) (Fig. 1).

As a result of experimental fish being fed on diet containing tylosin 20%, which works to reduce harmful bacteria increase vitamins and minerals utilization and absorption from food intake especially iron. Because of the synergistic role of the tylosin 20% with a mixture of vitamins and minerals additive to the diet may help to increase the absorption of iron from the intestines and preserve its dissolved shape in water. Iron is essential matter in the formation of red blood cells and hemoglobin. Therefore, an increase the absorption of iron can affect the increase of hemoglobin concentration and red blood cell count (FAO/WHO, 1998). The leukocytes (White Blood Cells), are the major participants in both the inflammatory and immune response mechanisms. Statistically analysis showed no significant differences (p<0.05) among all experimental treatments. The highest value was recorded in T4 (24.80 x 10^3/mL) followed by T2 (24.73 x 10^3/mL), T3 (24.73 x 10^3/mL) and T1 (24.70 x 10^3/mL) (Fig. 2). This may due to an increased portability of digestion help to perpetuate the microbial balance in the gut and increase the metabolism of food and increase the growth and immune response (Al et al., 2008). Statistically Lymphocytes of T4 (66%) showed high significant differences (p<0.05) from T1 and T2 and before experiment. T4 was not differ with T3 (68.67%). Also Lymphocytes of T2 (68%) differ significantly (p<0.05) from T1 (67%). Statistical analysis of Neutrophils, Monocytes, Eosinophils and Basophils showed no significant differences (p<0.05) between all experimental treatments Table 2.

Differential White Blood Cells Count done by smears a blood on the glass slides and after dry blood (about 10 min) pigmentation slides with a mixture of pigment Wright-Giemsa according to the method Shen and Patterson (1983), counted types of white blood cells using optical microscopy and the power zoom 100 x (Burton and Guion, 1969). Experiment run under Completely Randomized Design (CRD), data were statistically analyzed and mean significant differences compared at 0.05% probability (Duncan, 1955).
Table 2: Effect of various levels of supplementary antibiotic (Tylosin 20%) on the differential count of white blood cells (WBC)

<table>
<thead>
<tr>
<th>Items</th>
<th>Before experiment</th>
<th>T1 control</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes (%)</td>
<td>68±0.00*</td>
<td>67±0.00</td>
<td>68±0.00*</td>
<td>68±0.00*</td>
<td>68±0.00*</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>25.3±0.33*</td>
<td>26.3±0.33*</td>
<td>26.3±0.33*</td>
<td>26.3±0.33*</td>
<td>26.3±0.33*</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2.3±0.00*</td>
<td>2±0.00*</td>
<td>2±0.00*</td>
<td>2±0.00*</td>
<td>2±0.00*</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2±0.00*</td>
<td>1.3±0.00*</td>
<td>2±0.00*</td>
<td>1.6±0.33*</td>
<td>2±0.00*</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>2±0.00*</td>
<td>2±0.00*</td>
<td>2±0.00*</td>
<td>2±0.00*</td>
<td>2±0.00*</td>
</tr>
</tbody>
</table>

*Means with the same letters in the same row were not significantly different (p<0.05)

Network of immune cells provides antibodies, cytotoxic and helper T cells and phagocytic cells. These immune cells combat not only pathogenic bacteria and their toxins but also the overgrowth of inappropriate attachment by the normal microflora. Evidence here is from studies of germ-free animals, which exhibit delayed lymphocyte and other immune cell development in the lamina propria and far fewer Immunoglobulin A producing cells when compared to conventionally reared animals (Gordon and Pest, 1971; Berg and Savage, 1975; Umesh et al., 1999). IgA is an antibody that plays a critical role in mucosal immunity (McCracken and Gaskins, 1999). Indeed, the majority of evidence supports the notion that the intestinal immune system develops in parallel with the development of the normal microflora. Therefore it should be noted, while the microflora induced development of the intestinal immune system may be the key to the long-term health of the animal (Gordon et al., 1963).

REFERENCES


