PJN
ISSN 1680-5194

Pakistan Journal of Nutrition

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 13 (4): 239-242, 2014
ISSN 1680-5194

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 (3.03×10^6 mL, 3.17×10^6 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 Fletouris, 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

Corresponding Author: U.K.I.M. AL-Bayaty, Department of Animal Resources, College of Agriculture, University of Baghdad, Baghdad, Iraq

Table 1: Composition of the basal diet

Ingredients	T1	T2	T3	T4
Fish meal	20	20	20	20
Soybean meal	40	40	40	40
Yellow corn	19	19	19	19
Brown flour	20	20	20	20
Vitamin and Mineral premix*	1	1	1	1
Antibiotic (Tylosin20%)	0 mg	20 mg	60 mg	100 mg

*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
nicotinic 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)

veterinary drugs/Holland), were respectively added (0,20,60, 100 mg/kg body weight of fish) as T1, T2, T3 and T4, respectively. Experimental fish were fed the diet contained 31.04% crud protein at 3% of fish body weight for 28 days (Table 1). Aquaria were supplied with air pumps and 1/4 of their water has been exchanged daily. Temperature and pH of aquaria water were measured during the experiment period. Blood samples were taken from peduncle vein by capillary tubes containing heparin (50 IU per ml blood), in field conditions, immediately after taking the fish out of the aquarium. Laboratory tests were preformed 60 to 80 mins after blood sampling i.e., immediately after delivery of the blood samples to the laboratory. General condition and health status of the fish were examined using routine method of medical-veterinary diagnosis. Modified dices fluid was used to dilute blood to count RBCs by haemocytometer chamber, cover slide, special pipette for dilution fluid and microscope. Blood was filled up to level 0.5, while dilution fluid was filled up to the level 101 of the pipette then mixed in 8 Fig. movement. First few drops were neglected. A drop was dripped on the covered haemocytometer then covered. Five squares were used to count the red blood cells number and four corner square were used to count numbers of red and white cells, respectively (Dacie and Lewis, 1984).

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, 1968). Experiment run under Completely Randomized Design (CRD),data were statistically analyzed and mean significant differences compared at 0.05% probability (Duncan, 1955).

RESULTS AND DISCUSSION

Blood tests has 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 (means±S.E = 25. 13±0.6°C) and the pH range between 7.9-8.2 (means±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 (3.17×10^6/mL) compared to T1 and T2 and was not significant ($p<0.05$) with T3(3.10×10^6/mL). Also treatment T2(3.03×10^6/mL) showed a significant difference comparably with T1(2.83×10^6/mL), but was not significant with T3 (3.10×10^6/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. Therefor, 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 (24.80×10^3/mL) followed by T2 (24.73 x 103/mL), T3 (24.73×10^3/mL) and T1 (24.70×10^3/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 (Aly *et al*., 2008). Statistically Lymphocytes of T4 (69%) 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.

Antibiotic Tylosin 20% acts to inhibit bacterial protein synthesis by inhibiting the 50S ribosome of pathogenic microorganisms. Eradicating this metabolic drain allows more efficient use of nutrients for food production. In addition to provide an opportunity for normal microflora

Table 2: Effect of various levels of supplementary antibiotic (Tylosin20%) on the differential count of white blood cells (WBC)

Items	Before experiment	T1 control	T2	T3	T4
Lymphocytes (%)	68±0.00^b	67±0.00^c	68±0.00^b	68.67±0.33^a	69±0.00^a
Neutrophils (%)	25.33±0.33^a	26.33±0.33^a	26.33±0.33^a	26.33±0.33^a	25.67±0.67^a
Monocytes (%)	2.33±0.33^a	2±0.00^a	2±0.00^a	2±0.00^a	2±0.00^a
Eosinophils (%)	2±0.00^a	1.33±0.33^a	2±0.00^a	1.67±0.33^a	2±0.00^a
Basophils (%)	2±0.00^a	2±0.00^a	2±0.00^a	2±0.00^a	2±0.00^a

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

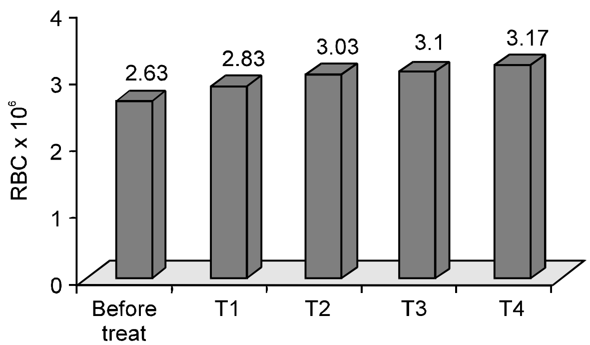

Fig. 1: Effect of different level of supplementary antibiotic (Tylosin 20%) on red blood cell counts of common carp

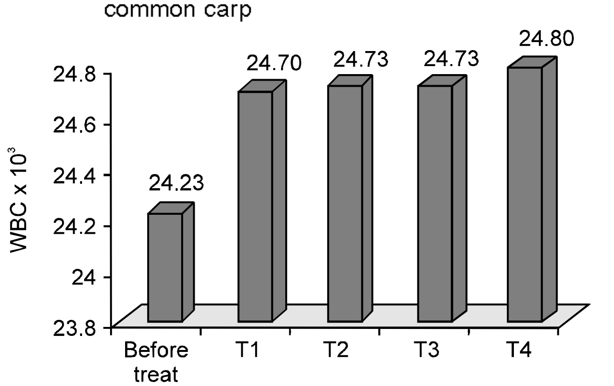

Fig. 2: Effect of different level of supplementary antibiotic (Tylosin 20%) on white blood counts of common carp

to resist the colonization by pathogenic microbes, a phenomenon known as competitive exclusion. Most believe the resident flora suppresses colonization by secreting antimicrobial compounds such as organic acids, by direct stimulation of the immune system and by competing for nutrients and attachment to the mucosal surfaces (Rolfe, 1997; Kelly and King, 2001). The normal microflora stimulate development of intestinal host defenses, including the mucus layer, the epithelial monolayer; and the lamina propria, with its system of immune cells that underlie the epithelium (McCracken and Gaskins, 1999; Kelly and King, 2001). The mucus layer segregates both normal and pathogenic microbes away from the animal tissues and the epithelium provides a barrier to enter into the animal tissues when the mucus layer has been crossed. The underlying 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 Pesti, 1971; Berg and Savage, 1975; Umesaki *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

Aly, S.M., Y.A. Ahmed, A.A. Ghareeb and M.F. Mohamed, 2008. Stuies On *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to Challenge infection. Fish and Shellfish Immunol., 25: 128-136.

Arnold, jill, 2009. Hematology of Fish: WBC and RBC Cell Morphology. Proceeding of the ACVP/ASVCP Concurrent Annual Meetings December 5-9, Monterey, California, USA.

Berg, R.D. and D.C. Savage, 1975. Immune responses of specific pathogen-free and gnotobiotic mice to antigens of indigenousand nonindigenous microorganisms. Infect. Immun., 11: 320-329.

Botsoglou, N.A. and D.J. Fletouris, 2001. Antimicrobial drugs. In: Drug Residues in Foods. Pharmacology, Food Safety and Analysis., Marcel Dekker, Inc., New York, NY, USA, 27-115.

Burton, R.R. and C.W. Guion, 1968. The differential Leucocyte blood count: its precision and individuality in the chicken. Poult. Sci., 47: 1945-1949.

Dacie, J.V. and S.M. Lewis, 1984. Practical Haematology, Churchill Livingston Ed., selecto printing. Co. Ltd. New York, pp: 445.

Duncan, D.B., 1955. Multiple rang and multiple F test. Biometerics, 1: 11-19.

Espinosa, A., 2009. Analysis of antibiotics in fish samples. Anal. Bioanal. Chem., 395: 987-1008.

European Pharmacopoeia, 2004. Tylosin for veterinary use. Directorate for the Quality of Medicines of the Council of Europe, Council of Europe, Strasbourg, 2: 2647-2648.

FAO, 1981. Report of the symposium on new developements in the utilization of heated effluent and of recirculation system for intensive aquaculture. Stavanger, Rome, 29-30 May.

FAO/WHO, 1998. Preparation and use of food based dietary guidelines, World Health Organization, Roma, Italy.

Gordon, H.A. and L. Pesti, 1971. The gnotobiotic animal as atool in the study of host microbial relationships. Bacteriol. Rev., 35: 390-421.

Gordon, H.A., Wostmann, B.S. and E. Bruckner-Kardoss, 1963. Effects of microbial flora on cardiac output and other elements of blood circulation. Proc. Soc. Exp. Biol. Med., 114: 301-304.

Hirsch, R., T. Ternes, K. Haberer and K.L. Kratz, 1999. Occurrence of antibiotics in the aquatic environment. Sci. Total Environ., 225: 109-118.

Kelly, D. and T.P. King, 2001. Luminal bacteria: Regulation of gut function and immunity in Gut Environment of Pigs. A. Piva, K.E. Bach Kudsen and J.E. Lindberg, (eds). Nottingham University Press, Nottingham, UK, pp: 113-131.

McCracken, V.J. and H.R. Gaskins, 1999. Probiotics and the immune system. in Probiotics: Acritical Review. G.W. Tannock, Ed. Horizon Scientific Press, Norfolk, UK., 85-111.

Rolfe, R., 1997. Colonization resistancein Gastrointestinal Microbiology. Vol. 2, Chapman and Hall, New York, pp: 501-536.

Shen, P.F. and L.T. Patterson, 1983. A simplified wright`s stain technique for routine avian blood smear staining. Poult. Sci., 62: 923-924.

Umesaki, Y., H. Setoyama, S. Matsumoto and Y. Okada, 1993. Expansion of alpha beta T-cell receptor-bearing intestinalintraepithelial lymphocytes after microbial colonization in germ-free mice and its independence from thymus. Immunol., 79: 32-37.

USP, 2000c. Macrolides. Veterinary Medicine. The United States Pharmacopeial Convention, Inc. http://www.usp.org/veterinary/monographs/macrolides.

Voigt, L. Gregg and Swist, L. Shannon 2012. Hematology Techniques and Concepts for veterinary technicians. Wiley-Blackwell, Ltd., publication, pp: 12-98.