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Serum proteins and leucocytes differential count in the common carp (*Cyprinus carpio* L.) infested with ectoparasites

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Abstract- A total of 48 Pond reared common carp *C. carpio* ranged between 2.5-11.8 cm in total length were obtained from University of Basrah's fish farm during May-June 2011. It was investigated for parasites, and it was infested with four different groups of ectoparasites, including Ciliophora (*Ichthyophthirius multifiliis* and *Trichodina* sp.), Monogenoidea (*Dactylogyrus* spp. and *Gyrodactylus* sp.), Trematoda (*Ascocotyle coleostoma* metacercariae) and Crustacea (*Dermoergasilus* sp. and *Lernaea* sp. copepodid larval stage). Differential count of leucocytes, total protein level, albumin and globulin in blood serum were determined in healthy (non-infested) and infested fish. No significant differences ($p>0.05$) have been reported between infested and non-infested fish in all blood parameters except in monocyte counts of the third group (infested with two species of Ciliophora) and sixth group (double infestation between *A. coleostoma* and *Dermoergasilus* sp.). This study showed that common carp can withstand different light and mild infestation of ectoparasites. We can considered common carp among a fish category having very good resistibility towards ectoparasites.

Keywords: Immunity, Differential leucocytes, ectoparasite, fish pond, *Cyprinus carpio,* Iraq.

Introduction

The expansions of aquaculture have led to a growing interest in understanding fish disease and enhanced the interest in the defense mechanisms against these infestations. Water quality affects the health status of fish and limits the physiological performance of cultured fish. The presence of acute stressors produce an alarm reaction in fish, with an increase of disease frequency, the latter is due to impaired of immune function (Cecchini and Saroglia, 2002). For example, chronic exposure to pollutants can lead to decreased resistance to viral, bacterial and parasitic diseases (Shahi *et al*., 2009). Therefore survival in the aquatic environment requires an immune system that can combat the constant challenge of waterborne pathogens (Galindo-Villieas and Hosokawa, 2004).

In bony fish, blood chemistry and physiology represent important parameters to evaluate the general physiological condition of the body, used specially as stress indicators (Docan *et al*., 2012). Hematology can be a useful tool for monitoring health status of fish (Tavare-Dias *et al*., 2007; Ponsen *et al*., 2009; Shahsavani *et al*., 2010) and aid in the diagnosis of disease in fish (Haniffa and AbdulKader Myden, 2011).

In clinical chemistry, Total serum protein represents the most important indicator of the nutritional condition of the organism and of fish health condition (Yang and Chen, 2003).

The total protein concentration in fish serum differ, depending on a series of factors such as food diet, species, season, degree of sexual maturity, water temperature (Patriche *et al*., 2009; 2011).

The levels of globulin and total protein indirectly reflect the condition of specific humeral immunity (Stosik *et al*., 2001; Maqsood *et al*., 2009). Albumin is an important serum protein for transportation of steroid hormones (Shahsavani *et al*., 2010). Also, the importance of albumin has been described in respect to fish pathology, Albumin: Globulins ratio (A/G) is widely used as an index of physiological state (Nakagawa, 1978).

Blood cells constituents are utilizable as biological indicator in the study of fish physiology (Yaki, 1957); however, the number or appearance of fish leucocytes is one of the most elementary ways to assess the immune system (Tavares-Dias *et al*., 2008). In vertebrates, white blood cells are a primary line of immunological defense (Tierney *et al*., 2004). Quantity and quality of leucocyte cells are generally used to determine immune reactions and diseases (Tierney *et al*., 2004).

Moreover, changes in leucocytes also occur when fish are stressed and environmental quality is altered (Ponsen *et al*., 2009). Both innate and adaptive immune response is mounted by fish to control parasite infection (Alvarez-Pellitero, 2008). The innate (non-specific) immune system includes: the phagocytic cells (granulocytes (neutrophil) and monocytes/macrophages) and non-specific cytotoxic cell (Magnadottir, 2006).

Few local studies concerning the effect of parasites and disease agents on blood parameters of fish (Al-Daraji, 1995 ; Khamees, 1996; Al-Awadi, 1997; Jori, 1998; Al-Salim and Jori, 2002a,b; Ali, 2001; Al-Salim and Ali, 2003a,b; Al-Tamimi, 2001; Al-Tamimi *et al*., 2001; Al-Jadooa, 2002; Jori, 2006; Jori and Mohamed, 2008 and Al-Salim *et al*., 2009).

Increasing impact of some parasites on fish health and its economic relevance in aquaculture and fisheries in Iraq has enhanced the need for studies on the fish-parasite relationship, However, the aim of this work is to find out the direction of quantitative indices of blood including differential count of leucocytes and total protein level, albumin and globulin in cultured common carp *Cyprinus carpio* infested with different groups of ectoparasites.

Materials and Methods

Source of fish:

A total of 48 Pond reared common carp *C. carpio* were obtained from Basrah University fish farm during May-June 2011. These fish were ranged between 6-15.6 g in weight and between 2.5-11.8 cm in total length. The temperature, dissolved oxygen and salinity of the fish ponds were measured on the day of collecting fish.

The blood was taken immediately after capture using micro capillary tubes from caudal vein by cutting the caudal peduncle. Blood smears were prepared too.

Parasites identification:

After blood sampling, each fish was inspected to detect any parasite. Following blood sampling, fish were examined externally such as skin, fins using dissecting microscope. Fish was dissected longitudinally and removed each internal viscera according to Amlacher (1970). Wet Smear taken from different organs and examined under compound microscope (40-1000x). All parasites found was removed from the host, counted, fixed and identified. Parasite identified based on Lom and Dykova (1992) for Ciliophora, Gussev (1985) for Monogenoidea, Yamaguti (1971) for Trematoda and Kabata (1979) and Ho and Do (1982) for parasitic Copepoda.

All Monogenoids of present study have been identified to generic level based on the comparative morphology of the anchor/bar complexes, due to impossible to determine the specific level of them based on temporary smear and unstained specimens (Kritsky *et al.*, 2013), So the prepared permanent stained specimens were so difficult when we dealt with many hematological works which carried out in the same time.

Analytical methods:

Blood samples were loaded into standard capillary tubes, spun in a microhematocrit centrifuge at 10000 rpm for 2 minutes. The separated serum was used to analyzing total serum protein concentration and serum albumin concentration. Serum total protein was estimated by Biuret method using a total protein kit (BIOLABO.FR). Albumin was estimated by bromocresol green binding method using albumin kit (BIOLABO.FR). Globulin was calculated by subtracting albumin values from total plasma protein. A/G ratio was calculated by dividing albumin values by globulin values (Coles, 1986).

Blood smears were immediately made with a drop of blood and allowed to air dry. Slides were stained using Giemsa stain. Slides were examined using an Olympus oil-immersion light microscope with 1000x magnification. These slides were used to determine the different count of leucocytes. A random area of the slide was chosen to begin counting lymphocytes, granulocytes and monocytes. The slide was moved in one direction until 100-200 total leucocytes were counted. Data were recorded as percent lymphocytes, granulocytes and monocytes. The white blood cells were identified according to Yuki (1957); Ellis (2001); Tierney *et al.* (2004); Tavares-Dias and Barcellos (2005) and Rowan (2007). Statistical analysis: Results were presented as mean with standard error of mean (SE). The results were also analyzed using f-test. The level of significant was $p<0.05$.

Results

Pond water temperature ranged between 24-28°C, salinity 0.75-1.3 g/l and oxygen concentration 5.9-9 mg/l. The fish specimens were divided into seven groups according to the presence or absent of parasite and the kinds of ectoparasite as clarified in Table (1). Blood analysis was conducted on non-infested fish and six naturally infested groups. The results for these two groups were statistically compared. The changes in the total serum protein, albumin and globulin have been indicated in Table (2). No significant differences (p>0.05) have been reported between infested and non-infested fish. A non-significant (p>0.05) increase in total protein and globulin was shown in infested fish which infested by *Dactylogyrus* spp. (group 2) and double infested with *I. multifiliis* and *Dactylogyrus* spp. or *A. coleostoma* and *Dactylogyrus* spp. (group 5). While a non-significant (P>0.05) decrease in total protein was noticed in infested fish (groups 3, 4, 6 and 7) comparing with the non-infested fish.

White blood cells were distinguished by their morphology and staining characteristics. In general, lymphocytes were the smallest in area but had the largest nucleus-to-cytoplasm ratio. Granulocytes were characterized by their multi-lobed nucleus. Monocytes are larger than other circulatory blood cells. The lymphocytes were the most common leucocytes followed by granulocytes then monocytes. On the other hand neutrophils were the most abundant granulocytes. Table (3) shows the percentage of different leucocytes (lymphocytes, granulocytes and monocytes) in healthy and infested fish. No significant differences (p>0.05) was shown in the percentage of lymphocytes and granulocytes between infested fish and the control, while significant increasing of monocyte percentage values occurred in some infested fish (groups 3 and 6).

Lymphocytes percentage show a non-significant (P>0.05) decrease in infested fish (groups 2, 3, 4, 5, 6 and 7) comparing with the control. A non-significant decrease was shown in the most infested groups (groups 2, 3, 4, 5, 6 and 7). Granulocytes percentage show a non-significant (p>0.05) increase in the most infested fish (groups 2, 4, 6 and 7) compared with the control. A significant (P<0.05) increase in monocytes percentage was noticed in some infested fish (groups 3 and 6), while a non-significant (p>0.05) decrease in monocyte was noticed in most infested fish (groups 2, 4, 5 and 7) comparing with the control fish.

Table 1. Number and total length (cm) of fish individuals to each parasite (s) infested group.

Group No.	Parasite group	Host No.	Intensity	Fish Length (cm)
1	Control (no infestation)	8	-	6.0-9.7 (8.0)
2	*Dactylogyrus* spp.	5	1-32	7.8-11.5 (9.1)
3	Ciliophora	3	1-5	7.0-9.0 (8.0)
4	*Ascocotyle coleostoma* (metacercaria)	7	1-4	6.5-8.0 (7.5)
5	*Dactylogyrus* spp.+*A. coleostoma* (met.) or *Dactylogyrus* spp. +*Ichthyophthirius multifilis*	8	2-13	8.0-10.0 (8.9)
6	*Dermoergasilus* sp.+*A. coleostoma* (met.)	5	4-10	7.0-8.0 (7.2)
7	Multi infestation with 3[1] , 4[2] or 5[3] parasites species	5	6-18	7.5-9.0 (8.3)

[1]consist from *Dactylogyrus* spp. + *A. coleostoma* + *Dermoergasilus* sp.

[2]consist from *Dactylogyrus* spp. +*A. coleostoma* + *Dermoergasilus* sp. + *Gyrodactylus* sp.

[3]consist from *Dactylogyrus* spp. + *A. coleostoma* + *I. multifiliis* + *Trichodina* sp. + *Lernaea* sp. copepodid 4th larval stage.

Table 2. Total protein (g/dl), albumin (g/dl), globulin (g/dl) concentration and A/G ratio in the blood plasma of infested and non-infested groups of common carp.

group	Intensity	Total protein			Albumin			Globulin			A /G		
		N	mean	SE	N	mean	SE	N	mean	SE	N	mean	SE
1	-	4	2.625	0.74	4	0.76	0.13	4	1.859	0.71	4	0.60	0.27
2	1-32	3	2.933	0.92	3	0.76	0.17	3	2.179	0.76	3	0.37	0.05
3	1-5	4	1.750	0.33	4	0.39	0.11	4	1.364	0.39	4	0.36	0.11
4	1-4	3	1.253	0.62	2	1.28	0.13	2	1.545	0.45	2	1.54	0.45
5	2-13	4	2.5	0.50	4	0.44	0.17	4	1.812	0.27	4	0.608	0.34
6	4-10	2	2.71	0.85	2	0.35	0.15	2	1.336	1.17	2	0.263	0.10
7	6-18	2	1.930	0.57	2	0.73	0.02	2	1.205	0.55	2	0.750	0.33

Table 3. Mean percentage and standard error mean (SE) of differential count of leucocytes (lymphocyte, granulocyte and monocyte) in infested and non-infested groups of common carp.

group	Intensity	Lymphocyte			Granulocyte			Monocyte		
		Number of fish	% Mean	SE	Number of fish	% mean	SE	Number of fish	Mean	SE
1	-	6	63.82	3.44	6	34.87	3.39	6	1.02	0.36
2	1-32	3	47.00	10.50	3	49.67	11.83	3	1.37	0.35
3	1-5	3	58.67	5.84	3	38.00	7.09	3	3.87*	0.98
4	1-4	6	49.67	5.75	6	48.33	5.49	6	1.22	0.38
5	2-13	6	63.35	5.11	6	34.10	5.10	6	1.68	0.57
6	4-10	4	56.25	10.75	4	41.75	10.78	4	2.18*	0.33
7	6-18	5	60.52	7.37	5	38.00	6.65	5	1.68	0.68

*means significant differences was found with the control (group1).

Discussion

In the present study, four main different groups of ectoparasites were recognized Ciliophora, Monogenea, Trematoda and Crustacea, which appeared in seven different levels of infestation in cultured common carp, with *Dactylogyrus* spp. being the most abundant parasites found. The genus *Dactylogyrus* includes common parasites on freshwater fishes, mainly cyprinids, in Iraq with so far 77 valid species were recorded which included 31 species from the common carp from different parts of the country (Mhaisen, 2013). It is well-known that defense mechanisms in fish play an important role in all stages of parasites infestation (Tort *et al.*, 2003). This study demonstrates that common carp can withstand infestation of light intensity of ectoparasites (1-32) without any significant differences in serum total protein, albumin and globulin. A/G ratio still in its normal values in infested fish. The differential count of leucocytes did not significantly differ between healthy and infested fish except of monocyte in fish infested with Ciliophora (*I. multifiliis* and *Trichodina* sp.) and double infestation with *Dermoargasilus* sp. and *A. coleostoma* metacercariae. This may be explained by increasing mucus production in the gills of infested fishes with ciliates which interferes with osmoregulatory functions in fish (Hughes, 1970). Ali (2001) recorded mortality in stinging catfish *Heteropneustes fossilis* when infested with heavy metacercariae of *A. coleostoma* (more than 1400 metacercaria/fish) due to destruction of gill lamellae respiratory dysfunction, while light (less than 800 metacercaria/fish) and mid (800-400 metacercaria/fish) infestations with the same parasite caused severe hyperplasia and infiltration with leucocytes in gill lamellae (Al-Salim and Ali, 2003b). High pressure existed on gill filaments caused by second antenna activity of *Dermoergasilus* sp. may cause inflammation which caused increasing percentage of monocytes. Khamees (1996) found no significant differences between Leucocyte counts in uninfested and infested fish with four species of Ergasilid copepods parasite freshwater mullet *Liza abu* in Basrah, Iraq. Same results were found by de Azevedo *et al.* (2006) in tilapid fish *Orechromus niloticus* infested with ciliates, Monogeneans and crustaceans in Brazil.

The data obtained in this study proved that common carp can stimulate the immune system during infestation with light intensity of ectoparasites (see Tables 1-3). The same result was recorded by Tavares-Dias *et al.* (2007) who found that tambaco fish parasitized by Branchiurid crustacean *Dolops carvalhoi* lead to an activation of cellular defense mechanism during mild infestation. However Almeida *et al.* (2011) indicate that the fish are responding to the parasite infestation by producing antibodies. On the other hand, many fish diseases were found to alter the concentration of total protein, albumin and globulin. It has been shown that total serum protein content in the fish infected with *Aeromonas hydrophila* decreased significantly (Maqsood *et al.*, 2009). Aydin *et al.* (2001) found a decrease total serum protein level and a significant increase in albumin level in rainbow trout infected with *Serratia liquefacieniens* in Turkey. The non-significant differences in the blood total protein, albumin and globulin that recorded in this study, revealed the good nutritional status of the cultured common carp (Osmani *et al.*, 2009). Similarly stress and infection can reduce appetite of fish and thus affect the serum protein level (Magnadottir *et al.*, 2010). The decrease in the concentration of total protein in many disease statuses is due to decrease capacity of synthesis and reduces absorption or protein loss through hemodilution (Patriche *et al.*, 2011; Stosik *et al.*, 2001). However, blood proteins act as buffer to maintain hydrogen ion concentration and osmotic pressure (De Lisle, 1971). The concentration of total protein decreased in many diseases status due to decreased capacity of synthesis, reduced absorption or protein loss (Yang and Chen, 2003). In this study the concentration of blood globulin in blood serum of infested fish have been close to those indicated as standard as good physiological status the index of A/G ratio is an index used to track relative change in the composition of serum or plasma. The increase in A/G ratio refers to the decrease in globulin and that infestation could inhabit immune response (Aydin *et al.*, 2001). However, Law A/G ratio may indicate inflammation and liver disease (Osmani *et al.*, 2009) or a shift from albumin production to globulin in response to infection (Foott *et al.*, 1996). White blood cell number and percentage affected by a variety of physiological and environmental factors (Rowan, 2007; Homatowska *et al.*, 2002). Lower temperatures also inhabit antibodies production (Cecchini and Saroglia, 2002). Crowding stress increases the susceptibility of fish to infestation with *Ichthiophthirius* (Witeska *et al.*, 2010). In the present study, the low density conditions and optimal water quality may result in a healthy situation that induced the innate immunity during parasite infection (Campbell, 2004). Witeska *et al.* (2010) noticed that fish with minor symptoms of disease had a significant increase in leucocytes without an alternation in the differential count. While those of severe symptoms had a significant decrease in leucocyte accompanied with lymphopenia and an increase in neutrophil and monocyte.

In this study Lymphocyte was the most abundant of the leucocytes in the peripheral blood of the healthy fish followed by granulocytes and then monocytes. Lymphocyte is responsible for a wide variety of immune system functions (Martins *et al.*, 2008; Savaei, 2011). Less percentage of lymphocyte and monocyte may result in a less efficient functioning of immune system (Homatowska *et al.*, 2002). The finding of high proportion of neutrophils in peripheral blood of cod, lead Ronneseth *et al.* (2007) to suggest that these cells might have a central role in the defense and protection against infection. However, the primary function of neutrophil is phagocytosis of small articulate matter (bacteria) (Savari *et al.*, 2011). Besides, increases of neutrophil mean environmental stress (Ponsen *et al.*, 2009). The non-significant differences in the lymphocyte and granulocyte percentage between non-infested and infested fish in the present study suggest that ectoparasites infestation in common carp lead to an activation of the cellular defense mechanism during light infection. The same result was recorded by Foott *et al.* (1996) who showed that juvenile Chinook salmon can withstand severe infection of *Nanophyetus salmincola* in freshwater. A significant increase in the percentage of monocytes was recorded (in the present study) in fish infested with Ciliophora (group 3) and *Dermoergasilus* sp. with *A. coleostoma* (group 6).

The same result was recorded by Silva-Souza *et al.* (2004) who found that increasing of monocyte percentage values occurred in the infested fish by a copepod *Lernaea*. Increasing monocyte was recorded in infected fish suffer from fin rot (Ranzani-Paiva *et al*., 2004). Also, Tambacu fish infested with a fish louse *Dolops carvalhoi* showed an increase in number of monocytes and granulocytes (Tavares-Dias *et al*., 2007). 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 (Zhou *et al*., 2010; Ellis, 2001). To sum up, this study actually has shown that common carp can withstand of light infestation of different group of ectoparasites. This study also upholds that ectoparasites infestation represents an important challenge parasite for stimulated the immunity in fish and we can consider common carp among a fish category has very good resistibility toward ectoparasites.

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بروتينات الدم والعد التفريقي لخلايا الدم البيضاء في اسماك صغار الكارب الشائع (*Cyprinus carpio* L.) المصابة بالطفيليات الخارجية

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المستخلص- جمع 48 نموذج من الكارب الشائع *Cyprinus carpio* بطول يتراوح بين (2.5-11.8) سم من مزرعة اسماك جامعة البصرة خلال ايار-حزيران 2011. فحصت الاسماك ولوحظ اصابتها بأربعة مجاميع رئيسية من الطفيليات شملت حاملات الاهداب (نوعين) والديدان المسطحة (ثلاثة انواع غير مشخصة من الجنس *Dactylogyrus* ونوع غير مشخص من الجنس *Gyrodactylus*) ونوع من يرقات المخرمات (*Ascocotyle coleostoma*) ونوعين غير مشخصة من القشريات (أحدهما من الجنس *Dermoergasilus* وآخر من الطور اليرقي الرابع للقشري *Lernaea* sp.). اظهرت النتائج عدم وجود فروقات معنوية في العدد التفريقي لخلايا الدم البيض والبروتين الكلي والبومين وكلوبيولين مصل الدم بين الاسماك السليمة والمصابة بست مستويات مختلفة من الاصابة باستثناء عدد خلايا الدم الوحيدة عند مستوى الاصابة الثالث (الاصابة بنوعين من الهدبيات) ومستوى الاصابة السادس (الاصابة المزدوجة بيرقة المثقوبة *A. coleostoma* والقشري *Dermoergasilus* sp.). وجد أن الكارب الشائع يقاوم مستويات الاصابة الخفيفة والمتوسطة للطفيليات الخارجية لذا يمكن أن يعد هذا النوع من الاسماك التي تمتلك مقاومة جيدة جداً تجاه الاصابة الخفيفة من الطفيليات الخارجية.