

## Effect of probiotic (Poultrystar®) and heat stress on some blood parameters in common carp (*Cyprinus carpio L.*)

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### Abstract

This study was conducted to determine the effect of the heat stress on some blood parameters and role of probiotics (Poultrystar®) to reduce these effects, 120 common carp (*Cyprinus carpio*) with mean weight 150±10g were obtained from collage of Agriculture and forestry /University of Mosul. Fish were acclimated for at least 7 day in the laboratory and fed daily, the water temperature was maintained at 25 ±2°C. Three experiments have been done, each of 40 fish which have been divided randomly into four groups 10 for each. First experiment the four groups exposed to different water temperature (control 25, 30, 32, 34 °C), second experiment the four groups treated with (Poultrystar®) in a dose 1 g/ kg body weight by using stomach tube with exposed to heat stress (control 25, 30, 32 and 34 °C). In the third experiment the four groups have been treated with (Poultrystar®) in the same dose of the previous experiment for 7 days then exposed to heat stress. The results show there were significantly increase in hemoglobin concentration in three experiments, and also the Packed cell volume was significantly increase in three experiments, but the stress index was significantly decrease in three experiments, and the probiotics (Poultrystar®) improved the blood picture especially in the second and third experiment because no probiotic used in the first experiment.

**Keywords:** Probiotics; heat stress; blood picture; Common Carp fish.

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## تأثير المعزز الحيوي (Poultrystar®) والاجهاد الحراري على بعض معايير الدم في اسماك الكارب الشائع (*Cyprinus carpio L.*)

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### الخلاصة

أجريت هذه الدراسة لغرض معرفة تأثير الإجهاد الحراري على بعض مكونات الدم ودور المعزز الحيوي Poultrystar® للحد من هذه الآثار، استخدم في هذه الدراسة 120 سمكة كارب شائع بمتوسط وزن 150 ± 10 غم. تم اقلمة الأسماك في المختبر لمدة 7 أيام وتم تغذيتها يوميا وتم المحافظة على درجة حرارة الماء عند 25 ± 2 درجة مئوية. شملت الدراسة ثلاثة تجارب تضمنت كل منها 40 سمكة التي تم تقسيمها عشوائيا إلى أربع مجاميع شملت كل منها 10 اسماك. التجربة الأولى عرضت المجاميع الأربعة إلى درجات حرارة مختلفة (سيطرة 25، 30، 32، 34 درجة مئوية) بينما التجربة الثانية تم معاملة المجاميع الأربعة بالمعزز الحيوي Poultrystar® بجرعة 1 غم / كغم من وزن الجسم باستخدام أنبوب اللي المعدي وتم تعريضها للإجهاد الحراري (سيطرة 25، 30، 32، 34 درجة مئوية) في نفس الوقت، وفي التجربة الثالثة تم معاملة المجاميع الأربعة بالمعزز الحيوي Poultrystar® بنفس جرعة التجربة السابقة لمدة 7 أيام ثم تعريضها للإجهاد الحراري. وأظهرت نتائج هذه التجربة حدوث زيادة معنوية في تركيز الهيموغلوبين في التجارب الثلاث وأيضا حدوث زيادة معنوية في النسبة المئوية لحجم خلايا الدم المرصوفة في التجارب الثلاث بينما مؤشر الكرب Stress index اظهر انخفاض معنوي في التجارب الثلاث. وكان دور المعزز الحيوي Poultrystar® تحسين صور الدم في التجارب الثانية والثالثة.

## **Introduction**

Fish reaction to high sub-lethal temperatures has been increasingly important, and this is correlated with an average temperature increase tendency and other climate changes in many regions of the world (1), therefore, the interest in temperature influence research as one of the basic ecological factors is rising, especially for the effect of additional heating that surface layers and small freshwater bodies are exposed to, it is common for many water bodies (both natural and man-made) to experience daily fluctuations of  $>4$  °C during summer, and some may fluctuate as much as 10 °C and thus cause thermal stress (2).

Blood is an indicator of physiological condition of an animal, and hematological parameters are used as a diagnostic tool by fish biologists and researchers throughout of the world, this is because fish are closely associated with the aquatic environment and the blood will reveal conditions within the body of the fish long before there is any outward manifestation of diseases (3-5), changes of blood parameters depend on the fish species, age, the cycle of sexual maturity and health condition (6-8) showed that an important health indicator in fish is represented by hematologic profile, which could help the researcher in assessing their welfare.

A good definition of probiotic in aquaculture is “a live, or dead component of a microbial cell that when administered via the feed or to the rearing water benefits the host by improving either disease resistance, health status, growth performance, feed utilization, stress response or general vigor, which is achieved at least in part via improving the hosts microbial balance or the microbial balance of the ambient environment” (9). Another proposed definition of probiotics used in aquaculture is “live microbial cultures added to feed or environment (water) to increase viability (survival) of the host” (10), they may also provide energy for indigenous bacteria to proliferate and thereby to utilize ammonia and branch chain fatty acids for the synthesis of protein (11). The mucosal immune system has to retain the ability to respond actively to pathogens, while avoiding active potentially inflammatory responses to pathogens (12),

In view of insufficient knowledge of thermal stress in fish, numerous experiments were conducted with the aim to contribute to better understanding of physiological conditions of the fish after thermal stress (2), this research have been done to study the effect of heat stress on the blood parameters and the effect of probiotic (Poultrystar®) to reduce these effects.

## **Materials and methods**

### **Fish**

A total of 120 fish of common carp *Cyprinus carpio* with mean weight  $150 \pm 10$ g were obtained from collage of Agriculture and Forestry /University of Mosul, fish were acclimated in dechlorinated tap water for at least 7 day in the laboratory before experimental time and fed daily, and the water temperature was maintained at  $25 \pm 2$  °C.

### **Blood component analysis**

Blood samples were quickly collected from the caudal vein of fish and the samples saved in tubes contain anticoagulant Heparin for hematological parameters that includes:

### **Hemoglobin concentration**

Drabkin hemoglobin- kit (Atlas Medical Co.) were used, United Kingdom, According to the equation: Hemoglobin (g/dl) = sample / standard \* 15 (standard concentration).

### **Stress index according to the equation:**

$$\text{Stress index} = \text{Heterophil} / \text{Lymphocyte} * 100 \text{ (13).}$$

### **Packed Cells Volume (PCV) according to (14).**

### **Probiotic (Poultrystar®)**

Obtained from Biomin Austerin company. Consist of Lactobacilli, Bifidobacteria and Fructooligosaccharides.

## **Experimental Design**

### **First experiment**

Effect of heat stress on common carp *cyprinus carpio* for 7 days, we have 40 fish divided into 4 group and exposed to water temperature (control, 25, 30, 32 and 34°C) each group is consisting of 10 fish and after the end of this experiment blood have been collected to determine the hemoglobin concentration, PCV and, stress index.

### **Second experiment two**

Effect of probiotic treatment with heat stress at same time for 7 days. 40 fish have been divided into 4 group and exposed to water temperature (control 25°C, 30°C, 32°C and 34°C) each group is consist of 10 fish, at the same time they treated with the probiotic by stomach tube (15) in a dose of 1g/ kg of body weight (16) and after the end of this experiment the blood was collected to determined hemoglobin concentration, PCV and stress index.

### **Third experiment**

Effect of probiotic treatment for 7 days before heat stress exposure for 7 days (time of this experiment is 2 weeks). Each of 40 fish have been divided into 4 group each group is consist of 10 fish, the first week the fish

treated with the probiotic in a dose of 1g/ kg of body weight by stomach tube and then in second week the treatment was stopped then exposed to water temperature (control 25°C, 30°C, 32°C and 34°C) and after end of this experiment was collected to determined hemoglobin concentration, PCV and stress index.

### Statistical Analysis

The use of Analysis of variance and Duncan test multiple ranges were applied with SAS program (17).

## Results

### First experiment

Table (1) shows the effect of heat stress on some blood parameters of common carp exposed to different temperature ranged between (30, 32 and 34 °C) for 7 days Which leads to changes in hemoglobin which represented by significantly ( $P<0.05$ ) increase in hemoglobin concentration of groups vulnerable to heat stress from control group, which was 21.6 - 24.2 - 27.8 g/dl, respectively, while the concentration of hemoglobin in control group was 16.6 g/ dl, and the hemoglobin concentration of group exposed to (34°C) is significantly ( $P<0.05$ ) increase from group exposed to (30°C), but there was no significantly difference of group exposed to (32°C), the results of the PCV in table (1) shows significantly increase ( $P<0.05$ ) in group exposed to (34°C) compared with other three groups and there was no significantly different in group which exposed to (30°C) and the group exposed to (32°C) which were significantly difference compared with the control group, and stress index results in the table (1) shows significantly decrease ( $P<0.05$ ) in group exposed to (34°C) compared with other three groups, while there was no significant difference ( $P<0.05$ ) in stress index of groups exposed to a rise in water temperature.

### Second experiment

Table (2) shows the effect of probiotic during heat stress, the hemoglobin concentration of fish group vulnerable to heat stress (34°C) was significantly increased ( $P<0.05$ ) which was (25.4) g/ dl compared with the control group (18.6) g/ dl, while the hemoglobin concentration of two groups exposed to heat stress (30 and 32°C) there were no significantly difference from control group which was (21.6 - 21) g/ dl, respectively, but the results of the PCV and stress index in table (2) shows significantly increase ( $P<0.05$ ) in groups vulnerable to heat stress (30 - 32 and 34°C) but there was no significantly difference from the control group.

### Third experimental

Table (3) shows the effect of probiotics after heat stress exposure, the hemoglobin concentration of group exposed

to heat stress (34°C) was significantly increased ( $P<0.05$ ) which was (27) g/ dl compared with the control group (15.8) g/ dl, while the hemoglobin concentration of two groups exposed to heat stress (30 and 32°C) no significantly different from control group which was 20.2-22.6 g/ dl, respectively, but the results of the PCV and stress index in table (3) shows significantly increase ( $P<0.05$ ) in groups exposed to heat stress (30 - 32 and 34°C) but there was no significantly difference from the control group.

## Discussion

Hematology is a tool that makes it possible to study organisms' physiological responses to pathogens. It may assist in making diagnoses and prognoses on diseases in fish populations. Red blood cells can be used to identify conditions that cause stress to the fish, and consequently disease (4,5).

The results shows that there were variation in hemoglobin concentration with variation in water temperature, this could be due to the relationship between hemoglobin and environmental temperature (18,19) pointed that the hemoglobin levels increased with temperature increase which may be due to the increase in oxygen demand in common carp *Cyprinus carpio*, and (20) pointed that when the temperature increase the hemoglobin content was increased, and (21) pointed the temperature increase, the erythrocytes content increase due to increase in erythrocytes population, the hemoglobin content was also found to be increase. Those results an agreement with the results of (22) who had studied the influence of thermal stress on hematological profile of trout (*Oncorhynchus mykiss*) where hemoglobin concentration and PCV increase with increasing in water temperature, the increase of PCV is accompanied by the blood viscosity being considered the superior limit of quantitative adaptation strategy, due to the additional cardiac effort needed in order to pump more viscous blood (22).

As the stress index is obtain by the equation of the total number of lymphocyte and the heterophil number (23), and the reduction in total leukocyte production with increasing of total temperature lead to decrease in non-specific immunity of fish as indicated (24) this is an agreement with our results.

The improvement of hematological parameters (hemoglobin, PCV, and stress index) in the second and third experiment compared with those results in the first experiment could be due to the effect of probiotics it was used for contribute the health or the well-being by stimulating the immunity and improving disease resistance in fish, allowing the fish to utilize metabolic energy to fight the effect of environment stress (25), and as the Poultrystar® consist of harmless microorganism cells in this

content they regard direct benefits to the host as immunostimulants (26), and our results are agreement with (16) who pointed that probiotics improved the blood picture of

common carp when fed on a meal containing Poultrystar<sup>®</sup> of (1g/ kg body weight).

Table 1: Effect of heat stress on the blood parameters of common carp *Cyprinus carpio*

Treatment	PCV	Hb	Stress index
Control 25°C	27.800±1.39 c	16.600±1.93 c	0.110±0.02 a
30 °C	31.800±1.07 b	21.600±1.03 b	0.078±0.02 ab
32 °C	34.600±1.50 b	24.20±1.59 ab	0.052±0.01 b
34 °C	38.600±0.92 a	27.800±1.02 a	0.038±0.01 b

Different letters vertically refer to significant differences P<0.05 among groups.

Table 2: Effect of probiotic treatment with heat stress at same time for 7 days

Treatment	PCV	Hb	Stress index
Control 25°C	28.600±0.51 a	18.600±1.96 b	0.070±0.02 a
30 °C	32.200±1.68 a	21.600±2.24 ab	0.056±0.02 a
32 °C	32.000±2.21 a	21.000±1.87 ab	0.032±0.01 a
34 °C	33.200±1.28 a	25.400±0.98 a	0.024±0.01 a

Different letters vertically refer to significant differences P<0.05 between groups.

Table 3: Effect of probiotic treatment for 7 days before heat stress exposure for 7 days (Time of experiment 14 days)

Treatment	PCV	Hb	Stress index
Control 25°C	33.400±0.74 a	15.800±0.73 b	0.074±0.01 a
30 °C	36.400±3.04 a	20.200±1.59 ab	0.052±0.02 a
32 °C	35.600±1.36 a	22.600±3.45 ab	0.048±0.02 a
34 °C	35.600±1.28 a	27.000±2.46 a	0.034±0.01 a

Different letters vertically refer to significant differences P<0.05 between groups.

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