Effect of Different Stocking Densities on Hematological and Biochemical Parameters of Silver Carp, *Hypophthalmichthys molitrix* Fingerlings

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Abstract: The impact of rearing silver carp, *Hypophthalmichthys molitrix* to the fingerling stage under three different stocking densities was investigated depending on the hematological and biochemical parameters as indicators of general health state of fish. The present study was carried out for 12 weeks to determine the most optimum stocking density for rearing silver carp. Fish were cultured in duplicates of cement ponds under stocking densities of 3, 6 and 9 fish/m^3 as T_1, T_2 and T_3 respectively. The ponds were fertilized weekly with organic fertilizer at rate of 50 g/m^3. Results of hematological analyses showed significant increase in T_1 for values of RBCs count, hemoglobin, hematocrit and mean corpuscle volume (MCV) while the lowest values were recorded in T_3 for all these parameters. Changing the stocking density had non-significant effect on values of WBCs count, mean corpuscle hemoglobin (MCH) and mean corpuscle hemoglobin concentration (MCHC). Plasma biochemical analyses showed that increasing the stocking density caused significant increase in values of plasma glucose, total protein, albumin (A) and globulin (G) associated with significant decrease in values of cholesterol and triglycerides. The highest values of A/G ratio were recorded non-significantly in T_1 and T_3 while the lowest values were recorded significantly in T_2. Meanwhile T_1 showed a significant decrease in values of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and uric acid. Values of creatinine showed non-significant increase among treatments. The findings suggest that the most optimum condition was detected in T_1 (3 fish/m^3) where most of the studied hematological and biochemical parameters were essentially normal and within the range consistent with good fish health.

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Key words: Silver carp, *Hypophthalmichthys molitrix*, Stocking density, Hematology, Biochemical parameters.

1. Introduction:

Aquaculture is currently the largest single source of fish supply in Egypt accounting for almost 65% of the total fish production of the country. Most aquaculture production in Egypt depends on freshwater or brackish water species in the Nile delta region with tilapias, mullets and carps making up more than 97% of the total country production in 2007 (GFCM, 2010). Silver carp, *Hypophthalmichthys molitrix* is a native species in China and Eastern Siberia but has been intentionally distributed all over the world for aquaculture and to control algal blooms. It is not only utilized as human food but also appreciated by its ability to clean water reservoirs from clogging algae (FAO, 2005). Carps have advantageous biological characteristics such as wide tolerance to environmental conditions, high growth rate and efficient filter feeding (Shen, 2009).

Silver carp is a typical planktonivore that consumes diatoms, dinoflagellates, chrysophytes, xanthophytes, some green algae and cyanobacteria. In addition, detritus, conglomerations of bacteria, rotifers and small crustaceans constitute major components of their natural diet. Generally, there is no need to provide supplementary formulated diet in silver carp culture which makes its production costs lower than most other cultured species (FAO, 2005).

The researches devoted to the evaluation of some biochemical parameters in cultured fish are justified by their significance for estimating the general health condition of the animals, as well as the possible effects of food availability, stocking density and exposure to stressors (Kebus *et al*., 1992; Roche and Boge, 1996; Chen *et al*., 2004; Tintos *et al*., 2006). Some investigations have evidenced important modifications of such indices under over-density stress in cultured cyprinids (Misaila *et al*., 2005).

Many studies were concerned with evaluation of hematological and biochemical parameters of carps in normal conditions or when exposed to stressors (Koedprang *et al*., 2002; Kopp *et al*., 2009; Nicula *et al*., 2010). Most of these studies were conducted on more than 3 years old carps but relatively few information in the literature has reported these parameters in carp fry or fingerlings such as the work of Kopp *et al*. (2011) although it is

commonly known that modification of fish blood constituents during these early stages of development could affect the fish performance in the future.

The aim of the present work was to evaluate the effects of different stocking densities on hematological and biochemical parameters of silver carp, *H. molitrix* fingerlings reared in cement ponds under normal environmental conditions.

2. Materials and Methods:

The present study was conducted during summer season, 2009 for 12 weeks at the Research Unit of Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abo-Hammad, Sharkia Governorate, Egypt.

Experimental design:

A total number of 2160 silver carp with mean body weight of 1.2 ±0.2 g/fish were divided into three treatments with stocking densities of 3, 6 and 9 fish/m^3 as T_1, T_2 and T_3 respectively. Fish were randomly distributed at the desired stocking density and acclimated for two weeks in duplicates of cement ponds each measuring 20 x 2.5 x 1.2m (length x width x water depth). The total volume of each pond was 60 m^3. Before starting the experiment all fish ponds were drained completely and then were exposed to solar radiation for 2 weeks till complete dryness. Ponds were then refilled with freshwater from Ismailia Canal (branched from the River Nile).

Fish ponds were fertilized weekly with Rigirs at rate of 50 g/m^3. Rigirs is an organic fertilizer produced by Misr El-Salam International Company for producing organic fertilizers. Rigirs consist of compressed and pelleted chicken manure also heat treated in order to be free from parasites, Salmonella, Shigella and *E. coli*.

Sampling and laboratory methods:

At the end of experiment, blood was collected from the caudal vein of 8 fish per treatment using sodium citrate as anticoagulant and examined directly for total red blood cells (RBCs) and total white blood cells (WBCs) using improved Neubauer Hemocytometer and expressed as the number of cells/mm^3. Hematocrit (Ht) was determined through microhematocrit tubes using hematocrit centrifuge for 5 min. Hemoglobin concentration (Hb) was estimated using cyanomethemoglobin method (Blaxhall and Daisley, 1973). The red cell indices; mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH) and mean corpuscle hemoglobin concentration (MCHC) were calculated using standard formulae (Coles, 1986). Blood samples were centrifuged to get plasma for the determination of glucose (Trinder, 1969), cholesterol (Allain *et al*., 1974), triglycerides (McGowan *et al*., 1983), total protein (Bradford, 1976), albumin content (Dumas and Biggs, 1972), alkaline phosphatase activity (ALP, EC. 3.1.3.1) (Bergmeyer, 1974), aspartate aminotransferase activity (AST, EC. 2.6.1.1), alanine aminotransferase activity (ALT, EC. 2.6.1.2) (Reitman and Frankel, 1957), creatinine (Henry *et al*., 1974) and uric acid (Barham and Trinder, 1972) using enzymatic-colorimetric methods by means of commercial kits (Biodiagnostic Co, Egypt). The globulin content (G) was estimated by subtracting the albumin content (A) from total protein content then A/G ratio was calculated.

Statistical analysis:

The results were expressed as mean ±S.E. Data were statistically analyzed using one-way analyses of variance (ANOVA) and significance was expressed using F-value at $P<0.05$. Duncan's multiple range test was used to evaluate the comparison between means as indicated by different case letters in a descending order A, B and C using Statistical Analysis System, version 9.1 (SAS, 2006).

3. Results:

During the experimental period all treatments showed non-significant mortality with over than 83% survival in all groups also water quality parameters remained well within limits recommended for freshwater fish culture. Results of hematological analyses are presented in Table 1. Statistical analysis showed significant decrease ($P<0.05$) in values of RBCs with the highest values recorded in T_1 and the lowest values were recorded non-significantly (Duncan's test) in T_2 and T_3. Meanwhile, changing the stocking density had non-significant effect on values of WBCs, MCH and MCHC. Values of Hb and Ht showed highly significant decrease ($P<0.01$) among treatments with the highest values recorded in T_1 and the lowest ones recorded in T_3 for both parameters. Values of MCV showed significant decrease ($P<0.05$) among treatments with significantly lower values recorded in T_3.

As indicated in Table 2, the increase in stocking density was associated with highly significant increase ($P<0.01$) in plasma glucose and highly significant decrease ($P<0.01$) in plasma cholesterol and triglycerides. The highest values of cholesterol were recorded non-significantly in T_1 and T_2 while those of triglycerides were recorded significantly in T_1.

Results of the plasma protein content (Table 3) showed highly significant increase ($P<0.01$) in values of total protein, albumin and globulin associated with the increase in stocking density. Meanwhile, values of A/G ratio showed significant difference among

treatments (P<0.05) with the highest values recorded non-significantly in T_1 and T_3 and the lowest values recorded significantly in T_2.

In case of plasma indicative parameters of liver functions (ALP, AST and ALT) (Table 4), values of ALP, AST and ALT showed highly significant increase (P<0.01) with the lowest values recorded significantly in T_1 for ALP and AST. Meanwhile, the lowest values were recorded non-significantly in T_1 and T_2 for ALT. The measured plasma creatinine and uric acid (Table 4) were used as indicators of fish kidney functions. Values of creatinine showed non-significant increase among treatments. Meanwhile, values of uric acid showed highly significant increase (P<0.01) with the highest values recorded non-significantly in T_2 and T_3 while the lowest values were recorded significantly in T_1.

Table 1: Effect of different stocking densities on hematological indices of silver carp

	RBCs ($X10^6$cells/mm^3)	WBCs ($X10^3$cells/mm^3)	Hb (g/dl)	Ht (%)	MCV (μm^3)	MCH (pg)	MCHC (%)
T_1 (3 fish/m^3)	1.04 ±0.01 A	21.33 ±1.76 A	2.97 ±0.09 A	16.32 ±1.68 A	158.61 ±16.63 A	28.62 ±0.85 A	18.66 ±4.51 A
T_2 (6 fish/m^3)	0.68 ±0.11 B	23.33 ±4.67 A	1.87 ±0.07 B	8.35 ±1.17 B	149.73 ±25.42 A	29.94 ±2.78 A	23.0 ±5.09 A
T_3 (9 fish/m^3)	0.64 ±0.08 B	17.33 ±2.91 A	1.57 ±0.09 C	4.07 ±0.46 C	81.44 ±12.57 B	24.15 ±3.14 A	38.31±12.5 A
F-value	8.30*	0.84	81.50**	32.19**	5.96*	1.51	1.58

Data are represented as means of eight samples ±S.E.
Means with the same letter in the same column for each parameter are not significantly different.
* Significant difference (P<0.05) ** Highly significant difference (P<0.01)

Table 2: Effect of different stocking densities on plasma glucose, cholesterol and triglycerides of silver carp

	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
T_1 (3 fish/m^3)	72.90 ±7.43 C	174.09 ±3.06 A	181.43 ±6.99 A
T_2 (6 fish/m^3)	97.52 ±5.32 B	150.01 ±2.55 A	101.79 ±29.23 B
T_3 (9 fish/m^3)	102.33 ±10.21 A	57.72 ±5.63 B	64.17 ±3.50 B
F-value	13.94**	12.63**	11.75**

Data are represented as means of eight samples ±S.E.
Means with the same letter in the same column for each parameter are not significantly different.
* Significant difference (P<0.05) ** Highly significant difference (P<0.01)

Table 3: Effect of different stocking densities on plasma protein content of silver carp

	Total protein (g/dl)	Albumin (A) (g/dl)	Globulin (G) (g/dl)	A/G ratio
T_1 (3 fish/m^3)	3.08 ±0.27 B	1.75 ±0.18 B	1.33 ±0.13 C	1.32 ±0.13 A
T_2 (6 fish/m^3)	4.91 ±0.16 A	2.22 ±0.19 A	2.69 ±0.10 A	0.83 ±0.10 B
T_3 (9 fish/m^3)	5.54 ±0.15 A	3.39 ±0.28 A	2.15 ±0.13 B	1.58 ±0.23 A
F-value	36.23**	15.03**	33.88**	5.79*

Data are represented as means of eight samples ±S.E.
Means with the same letter in the same column for each parameter are not significantly different.
* Significant difference (P<0.05) ** Highly significant difference (P<0.01)

Table 4: Effect of different stocking densities on indicative parameters of liver and kidney functions in plasma of silver carp

	ALP (U/l)	AST (U/l)	ALT (U/l)	Creatinine (mg/dl)	Uric acid (mg/dl)
T_1 (3 fish/m^3)	53.32 ±1.92 B	16.63 ±4.28 C	14.88 ±3.17 B	0.28 ±0.12 A	2.21 ±0.19 B
T_2 (6 fish/m^3)	82.26 ±4.70 A	21.75 ±4.96 B	15.88 ±1.30 B	0.31 ±0.08 A	3.15 ±0.40 A
T_3 (9 fish/m^3)	83.76 ±5.70 A	25.13 ±6.89 A	23.25 ±2.98 A	0.35 ±0.10 A	2.82 ±0.43 A
F-value	12.72**	13.61**	10.61**	0.68	11.82**

Data are represented as means of eight samples ±S.E.
Means with the same letter in the same column for each parameter are not significantly different.
* Significant difference (P<0.05) ** Highly significant difference (P<0.01)

4. Discussion:

Fish production capacity can increase by identifying environmental factors and providing appropriate environmental conditions for the fish. During the 12 weeks experimental period of the present study, water quality parameters were within the acceptable range for freshwater fish culture as indicated by Boyd (1990) and Delince (1992) making the stocking density as the main variable affecting the hematological and biochemical parameters of the studied fish.

The physiological system of fish can be severely challenged by a variety of biological, chemical and physical factors. When the physiological tolerance limits of fish are surpassed, reproductive success, growth, activity, resistance to infectious diseases and survival can all be impaired. Stress elicits a generalized endocrine response in fish which in turn induces a suite of secondary effects including rapid mobilization of energy reserves. The secondary responses of fish to the stressors can be evaluated by measurement of secondary biochemical indicators, such as changes in hematology and plasma chemistry (Wedemeyer *et al*., 1990).

Fish hematology is gaining great attention in fish culture because of its importance in monitoring the health status of fish (Hrubec *et al*., 2000). In case of cultured species, occasionally health issues arise that necessitate clinical evaluation of the fish under such captive conditions. Lack of published species-specific normal reference ranges remains the primary reason that blood testing is not routinely performed in fish health evaluations (Mauel *et al*., 2007). The knowledge of the hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fish (Kori-Siakpere *et al*., 2005).

Alteration of fish blood biochemistry may be indicative of unsuitable environmental conditions or the presence of stressing factors (Barcellos *et al*., 2004). Our results showed significant higher values of RBCs, Hb and Ht in the statistical evaluation of T_1 compared to the other two treatments which indicates that increasing the stocking density over 3 fish/m^3 affected consequently these relevant physiological parameters of fish. This is confirmed by the significant decrease in values of RBCs, Hb, Ht and MCV at stocking density of 6 fish/m^3 (T_2) followed by 9 fish/m^3 (T_3). Similar results have been reported by Kopp *et al*. (2010) who studied the influence of cyanobacterial water bloom on hematological and biochemical indices of two years old silver carp, *H. molitrix* although they reported higher values of normal RBCs, Hb and Ht than the present study which may be attributed to the age difference of the studied fish and changes of the experimental conditions.

According to Coles (1986) the calculated blood indices have particular importance in describing anemia in most animals. The detected non-significant differences in values of MCH and MCHC support the assumption that increasing the stocking density did not pose much challenge to the erythrocytes and did not indicate any pathological condition in the studied fish, even if the decrease is significant in values of RBCs, Hb and Ht separately. This is also confirmed by the recorded non-significant difference among values of WBCs. According to Weber and Jensen (1988) erythropoiesis and hemoglobin synthesis require long time to complete and can only be involved in long-term adaptation.

Blood glucose level has been used as an indicator of environmental stress to reflect changes in carbohydrate metabolism under stress conditions (Kavitha *et al*., 2010). Tintos *et al*. (2006) reported significant increase in plasma glucose of immature gilthead sea bream, *Sparus auratus* after short-term and long-term stress conditions. This strongly supports the present findings with the detected highly significant increase in values of plasma glucose in T_2 and T_3 which were much higher than the normal reference interval of cyprinids (63-86 mg/dl)

indicated by Nicula *et al*. (2010) and T_1 showed the optimum condition regarding this parameter.

The measurement of total protein, albumin and globulin in serum or plasma is of considerable diagnostic value in fish as it relates to general nutritional status as well as the integrity of the vascular system and liver functions (Abdel-Tawwab *et al*., 2008). According to Firat and Kargin (2010) the stress response of Nile tilapia, *Oreochromis niloticus* exposed to individual and combined mixtures of heavy metals was expressed by significant increase in glucose, total protein, albumin, AST and ALT as well as a significant decrease in cholesterol levels. This strongly supports the present findings where the significant increase in values of total protein, albumin and globulin was associated with increasing the stocking density of silver carp. However, the detected values of total protein were within the normal physiological range of cyprinids (2.10-5.76 g/dl) while values of albumin in case of T_2 and T_3 were marginally higher than the normal range of cyprinids (0.53-2.20 g/dl) indicated by Nicula *et al*. (2010).

Cholesterol is one of the structural components of cell membranes as well as the outer layer of plasma lipoproteins and is the precursor of all steroid hormones (Yang and Chen, 2003). Triglycerides function primarily in providing cellular energy and can be used as an indicator of nutritional status. In fact the greatest change in body composition is usually produced in the lipid fraction (Wallaert and Babin, 1994). The measured values of cholesterol and triglycerides showed highly significant decrease among treatments. Similar trend in cholesterol values of stressed fish was described by Zhang *et al.* (2008) in crucian carp, *Carassius auratus* and by Qiu *et al*. (2009) in silver carp, *H. molitrix* and bighead carp, *Aristichthys nobilis*. During sexual maturation lipid stores are mobilized and directioned to the gonads to sustain their development (Guijarro *et al*., 2003). This can support the present findings in T_1 and T_2 where the silver carp in this premature developmental stage had higher levels of cholesterol and triglycerides in preparation to this shift of body stores of lipid. The negative effect of stressors on fish may be indicated by a decrease in body lipid content (Svobodova *et al*., 2006). The detected values of cholesterol in T_1 and T_2 were within the normal physiological range of cyprinids (65-264 mg/dl) while it was lower than this range in case of T_3 which may be attributed to the increased metabolic rate and decreased food availability. Meanwhile, all measured values of triglycerides were within the normal range of cyprinids (68-200 mg/dl) indicated by Nicula *et al*. (2010).

AST and ALT are frequently used in the diagnosis of damage caused by pollutants in various tissues such as liver, muscles and gills (De la Tore *et al.,* 2000). It is generally accepted that increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of even minor cellular damage in the liver of fish (Palanivelu *et al.,* 2005). AST and ALT are quantitatively important in transamination of amino acids thereby allowing interplay between carbohydrate and protein metabolism during the fluctuating energy demands of the organism in various adaptive situations (Verma *et al*., 1981). Moreover, Palikova *et al*. (2010) stated that an increased value of alkaline phosphatase indicates incorrect secretion of bile due to lower food intake which may be associated with higher stress. This coincides with the recorded increase in values of glucose, total protein, ALP, AST and ALT with increasing the stocking density of silver carp in the present study. Acclimation of fish to different environmental factors results in increased metabolic activity correlated with changes in the quality and quantity of certain enzymes involved in energy metabolism and with compensating modifications in the rate of protein synthesis (Wallaert and Babin, 1994).

AST and ALT are the most frequently tested enzymes in fish for indication of cyanobacterial toxicity. The acute toxicity of microcystins (known cyanobacterial toxin) is unlikely to occur in silver carp and chronic exposure to cyanobacteria will not be detected by changes in ALP, AST and ALT in the blood plasma (Kopp *et al*., 2011). This supports our assumption that stocking density was the main variable affecting these parameters in the present study. The detected steady increase in values of ALP, AST and ALT with increasing the stocking density of silver carp, even if this increase was non-significant in some cases, indicates the consistent stress effect of increasing the stocking density on liver functions of the fish.

Nicula *et al*. (2010) indicated acceptable reference intervals of cyprinids as 46-83 U/l, 26-54 U/l and 9-23 U/l for ALP, AST and ALT respectively. Generally, values of ALP in T_2 and T_3 only were marginally higher than normal values while values of AST in the three treatments were lower than normal. The values of ALT were normal in T_1 and T_2 but marginally higher in T_3. According to Kopp *et al*. (2011) small differences among biochemical indices in a fish population are considered normal.

Plasma creatinine and uric acid levels can be used as rough indicators of glomerular filtration rate and kidney functions (Maita *et al*., 1984). Low levels of creatinine and uric acid have no significance but increasing their values indicates several disturbances in renal system (Maxine and Benjamin, 1985).

Increased level of creatinine is an indicator of kidney damage, muscular dystrophia and physical exertion of organisms (Masopust, 1998). Our results of total protein, ALP, AST, ALT, creatinine and uric acid support the assumption that the liver and muscle tissues of silver carp were markedly affected with increasing the stocking density even with the detected non-significant increase in values of creatinine. According to Nicula *et al*. (2010) the normal physiological ranges of creatinine and uric acid in cyprinids are 0.03-0.29 mg/dl and 0.78-2.20 mg/dl respectively. The detected values of creatinine and uric acid in T_1 were marginally within the upper limits of these physiological ranges while T_2 and T_3 values were higher than the normal physiological ranges of both parameters.

In conclusion, the most optimum condition was detected in T_1 (3 fish/m^3) where most of the studied hematological and biochemical parameters were essentially normal and within the range consistent with good fish health. Moreover, measurement of RBCs count, Hb, Ht, plasma glucose, triglycerides, total protein, albumin, ALP, AST and uric acid proved to be more sensitive and reliable in the detection of even minor stress response of fish.

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