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Effects Of Stocking Density On The Growth Performance, Physiological Parameters And Antioxidant Status Of Juvenile Common Carp (Cyprinus Carpio L.) Reared In The Cage System

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Abstract

Stocking density is one of the most important factors influencing growth performance, health status and welfare of fish in aquaculture. This research was carried out to investigate the effect of different stocking densities on growth performance, feed utilization, survival rate, haematology, serum biochemistry and serum antioxidants enzyme responses in common carp juveniles (*Cyprinus carpio* L.) reared in outdoor cage culture. The fish were randomly stocked in four density groups; 7F fish/0.2m^3 (Control group), 14F fish/0.2m^3, 21F fish/0.2m^3 and 28F fish/0.2m^3 in 12 cylindrical cages (0.2m^3) in concrete pond, with three cages in each density for 84 days. The results of the 14f density group significantly improved the FW, WG, SGR, FCR, FCE, PER, FI, PI, WBC, RBC, Hct, GSH and SOD activities. The 21F and 28F had significant impacts on FW, WG, SGR, FCR, FCE, PER, FI, PI, glucose, cortisol, ALT, AST, ALP, TG, MDA and GSH increased significantly with increasing stocking density. In contrast, the survival rate, TP, TG, HDL, GPx and SOD activities were significantly decreased. In general, the results demonstrated that the best stocking density is 14F fish/0.2m^3 which provided a better growth performance and health status of *C. carpio* cultured in the cage in the concrete pond.

Keywords: Common carp, stocking density, growth performance, physiological parameters, antioxidant enzyme, outdoor cage

1. INTRODUCTION

Stocking density is a key factor in fish farming systems that can significantly impact fish growth, physiological responses, and production efficiency. The common carp (*Cyprinus carpio* L.) is a fish species that is widely cultivated and has a significant economic impact on aquaculture (FAO, 2019). Understanding the impact of stocking density on the growth performance, physiological parameters, and intestinal alterations of juvenile common carp is essential for maximizing fish welfare and production results. Stocking density is an essential

factor affecting growth performance, physiological function, behaviour, and welfare in aquatic animals. Aquafarms frequently elevate fish farming practices to increase yield and profit. However, extremely high stocking density can have negative impacts and a reduction in economic benefits from aquaculture (Dai et al., 2023).

Stocking density significantly impacts fish performance, physiological function, behaviour, and welfare (Liu et al., 2016a). High stocking level leads to stress which results in higher energy requirements and reduces growth and feed utilization (Maragoudaki et al., 1999: El-Saidy and Gaber 2002). On the other hand, increasing stocking density is a common practice in fish culture to boost productivity and profitability. Excessive stocking density can reduce the economic benefits of fish culture.

Stocking density refers to how many fish are placed in a given area or amount of water. High stocking densities can cause a variety of stressors, including a lack of space, competition for resources, and deterioration in water quality. These stressors have the potential to impede fish growth, modify physiological parameters, and even cause pathological changes in certain organs, such as the intestines (Li et al., 2019).

Recent studies have investigated the effects of stocking density growth, physiology and immunity parameters on some fish species; for instance, Wang et al., (2018) showed that high stocking density (90 and 120 fish/m^3) led to decrease specific growth rate, weight gain and increase stress response in blunt snout bream in cages system in a concrete pond. Also, Swaine et al., (2022) have reported lower growth performance and feed efficacy and increased glucose, stressors such as cortisol, superoxide dismutase and catalase at high stocking densities in Labeo rohita reared in inland open-water cages. Furthermore, the water quality, growth performance, feed utilization, body composition and blood indices of African catfish raised at low stocking density (1kg/m^3) were positively improved compared to fish raised at high stocking density (2kg/m^3) in concrete ponds under three feeding regimes (Abutalb et al., 2022).

In this study, the effects of different stocking densities on physiological changes, performance, feed efficacy, body composition and antioxidant capacity of juvenile common carp cultured in cages were examined.

2. MATERIALS AND METHODS

2.1 Fish and Experimental Design

Juvenile common carp (*Cyprinus carpio* L.) were obtained from Tarjan fish farm and hatchery in Erbil, Kurdistan Region Iraq, and transported using a plastic tank. The experiment was conducted in a cage system placed in the aquaculture unit, Grdarasha station, department of Fish Resources and Aquatic Animals, College of Agricultural Engineering Sciences, Salahaddin University-Erbil Kurdistan Region, Iraq. The fish were reared in cages for 21 days to acclimatize to experimental conditions before the feeding trial and fed a commercial diet for the entire study (Kosar Company limited, Erbil Kurdistan Region- Iraq) contained 32% protein and 8% lipid (Table 1). A total of 210 Fish with initial weights (22.87± 0.08) were randomly assigned into 12 cylindrical cages (0.2m^3) for 84 days. The fish were divided into four densities; 7F (7 fish/0.2m^3; control), 14F (14 fish/0.2m^3), 21F (21 fish/0.2m^3) and 28F (7 fish/0.2m^3), three replicates of each density. At the start of the feeding trail fish were starved for 24 hrs before weighting. The juveniles were hand fed twice daily at 9.00 at 16.00 of 3% live body weight, and the fish were weighed every week for 24 hrs starvation was applied before weighing and the feeding ratios were adjusted. During the feeding trial, water change was made twice a week, and water quality ranges as follows; water temperature was 20-26 ℃, PH 6.0 - 8.5, dissolved oxygen 10 - 20 mg\L, EC 360 -550 uS\cm, TDS 195 -295 and ammonia levels, 0.01 mg\L, nitrate 12.5 mg\L, and nitrite 0.1 mg\L.

Table1. Formulation of commercial diet and proximate analysis of diet used in the feeding trial.

Ingredients g/kg	Commercial diet
Soybean meal	570
Corn meal	122
Fishmeal	100
Soya oil	45
Wheat flour	100
Wheat bran	10
PREMIX	20
Methionine	5
Lysine	3
Threonine	2
Enzyme	1
Mono calcium	5
Salt	1

Limestone	16
Feed Ratio formulation (g)	1000
Proximate analysis	
Moisture %	7.42
Crude protein %	31.05
crude lipid %	8.73
Crude fiber %	4.42
Crude ash %	6.99
Metabolic Energy (kcal/kg)	3457

Vitamin Premix is sourced in Kosar Company and originally sourced in BAF in Turkey and consists of Vitamin D3 (300000 IU per kg), Vitamin A (2000000 IU per kg), Vitamin K3 (1600 MG per kg), Vitamin E (40000 MG per kg), Vitamin C (150000 MG per kg), Vitamin B6 (2000 MG per kg), Vitamin B2 (3000 MG per kg), Vitamin B1 (2000 MG per kg), Pantothenic acid B5 (20000 MG per kg), Niacin B3 (8000 MG per kg), Folic acid (800 MG per kg), Choline (45000 MG per kg), Biotin (2000 MG per kg).
Mineral premix consists of 1-trace minerals consisting of selenium (60 MG per kg), manganese (3000 MG per kg), Cobalt (20 MG per kg), Iodine (200 MG per kg), Zinc (6000 MG per kg), Copper (30000 MG per kg)2-calcium carbonate 41% 3- salt 1g per kg limestone 14g per kg.

2.2 Proximate Composition Analyses

The nutritional content of the diet and the whole-body composition of the fish samples (six fish per treatment) were analysed according to AOAC (2010) standard methods. All samples were in three replicates. Moisture content was measured by drying the material in a fan-assisted oven at 105°C to a constant weight. Crude protein (N×6.25) content was measured the Kjeldhal method (Kjeldahltherm microsystem 40, C. Gerhardt GmbH, KG, Germany). Crude fat content was determined by the Soxhlet gravimetric method (1356, Parr Instrument Company, IL, and the USA). Crude ash content was extracted by incineration in a muffle furnace at 550°C for 24 hours. Crude Fiber content was determined gravimetrically after chemical digestion and solubilization of other materials present. All analyses were conducted at the Barash Feed Company Laboratory Department in Erbil Kurdistan Region of Iraq.

2.3 Growth Performance

After a 12-week experiment, mean fish weight was calculated using the sum of a distinct fish weight divided by the fish number in each cage. Growth performance was examined in terms of initial weight, final weight, feed intake, protein efficiency ratio, protein intake, survival growth rate, survival rate, feed conversion efficiency, feed conversion ratio (FCR) and weight gain. The following formulate were used. WG (%) 100 [(FW IW)/IW], FCR =FI\ (FW – IW), and SGR (%d -1) =100× [(ln FW–ln IW)/d]. FI =Feed given – Remaining feed, PER =feed consumption \feed intake.

2.4 Hematology and Biochemical Analysis

Six fish were selected randomly from each treatment (two in a cage) selected and anaesthetized with clove powder (200 ppm). Blood was taken from the fish caudal vein taken using a sterile 3ml hypodermic syringe. A part of the blood was transferred to a heparinized vial for haematology analysis and another part was placed into un-heparinized vials and allowed to clot. Blood samples were carefully located on ice in a Vacutte k3EDTA container. The separation of serum samples was completed by centrifugation for five minutes at 3000 rpm. For the haematology test the following parameters were examined; leukocytes (WBC), erythrocytes (RBC), haematocrit (Hct), haemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cells distribution width (RDW) and platelet (PLT) using fully automatic haematology analyzer BC-2800 (Fish Laboratory-Department of Fish Resources and Aquatic Animals- College Agricultural Engineering Science Salahaddin University-Erbil Kurdistan region-Iraq). Serum samples were used to measure the Glucose, total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (ALT), alkaline phosphate (ALP), cholesterol (CHOL), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (HDL) using Cobas c111 Accent 200 (CORMAY) in Alpha Laboratory for Disease Diagnosis at 100m street in front of East Emergency Hospital, Erbil Kurdistan Region- Iraq.

2.5 Serum Antioxidants Enzyme Activities

The serum malondialdehyde (MDA) activities were measured using the thiobarbituric acid method (Ohkawa et al. 1979). The levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GSH-Px) were measured using a microplate reader (Rayto RT-6100 system, Shenzhen, China) at 550, 405, 420, 412, nm, respectively. MDA and antioxidant-related parameter detection kits (SOD, CAT, GSH and GSH-Px) were purchased from NanJing JianCheng Bioengineering Institute, Nanjing, China.

2.6 Statistical Analysis

The survival rate, growth performance, feed efficacy, haematology parameters, serum biochemical indices and serum antioxidant enzyme activities data were analyzed by one-way ANOVA test using SPSS program (Statistical package for social science, version 26, IBM Company, 2019) to find significant effect of stocking density. The means hypothesis for normality and homogeneity were validated. Significant differences (P< 0.05) among the means were determined by the Duncan test. The results were expressed as mean ± standard deviation.

3. RESULTS

3.1 Growth Performance

The growth performance, feed efficacy and survival of common carp juveniles stocked at different densities in cages are shown in Table 1. The final weight, weight gain and specific growth rate of the 14F group were significantly (P<0.05) higher than those of the 7F group, but they were significantly lower in the 21F and 28F groups than in the 7F group. The feed conversion ratio of 14F was significantly (P<0.05) higher than that of the control group, whereas the feed conversion ratio of 21F and 28F was significantly (P<0.05) lower than that of the control group. The feed intake and protein intake of the 14F group were significantly (P<0.05) higher than those of the 7F and 21F groups and the feed intake and protein intake were significantly (P<0.05) higher in the 28F group than that of the control group. The feed conversion efficiency of the 14F group was significantly (P<0.05) higher than those of the 7F group, but there was significantly (P<0.05) lower in the 21F and 28F groups than in the control group. The survival rate of 14F group was slightly lower but in the 21F and 28F groups was significantly (P<0.05) lower than in the control group. The PER of the 7F control group and the 14F group did not differ significantly(P<0.05), but the PER of the 21F and 28F groups were significantly lower than those of the 7F group (P<0.05).

Table (2): Growth performance, feed utilization and survival rate of common carp fingerlings for reared at different stocking densities for 12 weeks.

Parameters	7 F	14F	21F	28F
IW	22.86± 0.14	22.88 ± 0.11	22.89 ± 0.027	22.85 ± 0.021
FW	47.76 ± 0.58^{b}	51.20 ± 1.92^{a}	45.12 ± 0.49^{c}	44.85 ± 0.28^{c}
WG	24.90 ± 0.54^{b}	28.32 ± 1.81^{a}	22.24 ± 0.47^{c}	22.1 ± 0.26^{c}
SGR	1.02 ± 0.02^{b}	1.12 ± 0.05^{a}	0.94 ± 0.01^{c}	0.94 ± 0.01^{c}
FCR	2.88 ± 0.02^{b}	2.67 ± 0.08^{c}	3.18 ± 0.03^{a}	3.31 ± 0.12^{a}
FCE	34.74 ± 0.23^{b}	37.56 ± 1.11^{a}	31.54 ± 0.31^{c}	30.27 ± 1.09^{c}
PER	1.12 ± 0.01^{a}	1.19 ± 0.07^{a}	0.92 ± 0.02^{b}	0.82 ± 0.10^{c}
FI	71.67 ± 1.09^{b}	75.34 ± 2.60^{a}	70.51 ± 1.43^{b}	72.74 ± 1.79 ^{ab}
PI	22.25 ± 0.34^{b}	23.40 ± 0.81^{a}	21.89 ± 0.44^{b}	22.59 ± 0.56^{ab}
Survival rate	100.00 ± 0.00 ^{a}	97.62 ± 4.12^{ab}	92.06 ± 2.75^{bc}	86.90 ± 5.46^{c}

3.2 Proximate Composition

There were no significant differences in the moisture content, ether extract, crude ash, fibre and energy in the whole body composition of common carp after 84 days of rearing as the stocking density increased (Table 3). However, the ether extract content increased in the different stocking density groups compared to the control group. The crude protein content in fish reared at the 28F density group was significantly (P<0.05) higher than the fish in the 7F group. While the crude protein content in the 14F and 21F density groups was significantly (P<0.05) lower than in the control group.

Table (3): Proximate composition of common carp fingerlings for reared at different stocking densities for 12 weeks

Parameters	7F	14F	21F	28F
Protein	61.01 ± 0.39^{b}	59.43 ± 0.40^{d}	60.13 ± 0.27^{c}	62.49 ± 0.23^{a}
Fat	16.73 ± 3.54	20.17 ± 4.01	18.12 ± 4.36	17.97 ± 2.38
Ash	12.74 ± 1.41	12.62± 0.81	13.61 ± 0.17	12.80 ± 0.55
Fibre	0.56 ± 0.05	0.29 ± 0.22	0.50 ± 0.48	0.64 ± 0.32
Moisture	78.66 ± 0.67	78.33 ± 2.19	78.22 ± 2.85	77.80 ± 1.19
Energy	3583.33 ± 155.80	3614.67 ± 163.23	3507.33 ± 53.01	3536.33 ± 89.79

3.3 Hematology and Biochemistry Parameters

Fish reared at different stocking densities observed a significant increase in the WBC, RBC and HCT levels (Table 4). However, no significant differences in HGB, MCV, MCH, MCHC, RDW and PLT were observed at the different stocking densities (P<0.05).

Table (4): Hematology parameters of common carp fingerlings for reared at different stocking densities for 12 weeks

Parameters	7F	14F	21F	28F
WBC	63.73 ± 2.10^{b}	78.90 ± 3.03^{a}	76.23 ± 1.72^{a}	65.67 ± 3.58^{b}
RBC	1.22 ± 0.07^{c}	1.65 ± 0.10^{ab}	1.76 ± 0.05^{a}	1.57 ± 0.07^{b}
Hgb	11.87 ± 1.59	14.03 ± 1.45	13.87 ± 1.27	13.37 ± 0.93
Hct	22.100 ± 0.80^{b}	30.93 ± 1.63^{a}	31.80 ± 1.95^{a}	29.23 ± 0.85^{a}
MCV	181.07 ± 4.62^{ab}	186.83 ± 5.22^{a}	174.60 ± 5.07^{b}	184.43 ± 6.45^{ab}
MCH	86.83 ± 7.25	84.67 ± 3.72	78.20 ± 3.69	84.80 ± 1.35
MCHC	49.03 ± 4.22	45.60 ± 3.38	44.73 ± 1.82	46.80 ± 1.15
RDW	9.57 ± 1.48	9.70 ± 1.40	9.70 ± 1.61	9.00 ± 0.70
PLT	5.67 ± 3.06	5.33 ± 2.52	4.67 ± 2.08	6.00 ± 1.00

The serum biochemical indices showed significant differences between stocking density groups (Table 5) The different stoking density groups had a significant (P<0.05) increase in TG and cortisol when compared to the control group. TP, CHOL and HDL showed a significant (P<0.05) decrease trend as stocking density increased. LDL showed an increasing trend from the control to 21F groups, and it showed an increase in the 28F group. Only the 21F group achieved a significant (P<0.05) increase when compared to the control group. Glucose levels increased significantly in the 21F group compared to the control group, while they decreased significantly in the 14F group (P<0.05). The AST and ALT levels exhibited a significant (P<0.05) increase trend as the stocking density increased. ALP levels increased significantly in all stocking densities when compared to the control group, with the highest level achieved in the 21F group.

Table (5): biochemistry parameters of common carp fingerlings for reared at different stocking densities for 12 weeks

Parameters	7F	14F	21F	28F
TP	4.68 ±0.18 ^{a}	3 .95 ± 0.13 ^{b}	3.16 ± 0.86 ^{c}	3.00 ±0.17^{c}
Cortisol	23.58 ± 1.47^{d}	35.70 ± 1.53^{c}	45.50 ± 1.09^{b}	56.73 ± 1.90^{a}
Glucose	148.02 ± 0.70^{b}	87.59 ± 0.96^{c}	152.52 ± 0.52^{a}	148.29 ± 1.06^{b}
ALP	13.95 ± 0.99^{d}	19.37 ± 1.40 ^{c}	29.15 ±1.43^{a}	24.98 ± 0.75^{b}
AST	13.87 ± 1.55^{d}	18.85 ± 0.85^{c}	21.38 ± 0.83^{b}	23.70 ± 1.18^{a}
ALT	20.95 ± 1.47^{d}	26.00± 1.07^{c}	32.50 ± 1.24^{b}	35.95 ± 1.49^{a}
CHOL	345.17 ± 22.15^{a}	296.50 ± 11.01^{b}	239.00 ± 6.36^{c}	214.83 ± 8.01^{d}
TG	88.83 ± 6.18 ^{d}	118.83 ± 6.11 ^{c}	154.33 ± 5.28 ^{b}	184.50 ± 3.21^{a}
HDL	52.52 ± 1.87 ^{a}	46.93 ± 2.40 ^{b}	39.58 ± 1.39^{c}	39.23 ± 1.73 ^{c}
LDL	23.53 ± 2.37 ^{b}	26.93 ± 3.54^{ab}	28.93 ± 3.97 ^{a}	25.88 ± 2.13 ^{ab}

3.4 Serum Antioxidants Enzyme Activities

The immunological indices of the trial groups showed significant differences are presented in Table 6. All stocking density groups showed significantly higher MDA than the control group (P<0.05). The GSH-Px activity increased significantly (P<0.05) in all experimental groups compared with the control group. GPX activity was significantly (P<0.05) lower in the 14F and 28F groups compared to the control group. The 14F group showed a significant (P<0.05) increase of CAT activity than the control group, whereas the 21F and 28F groups had significantly (P<0.05) lower CAT activity. SOD activity was significantly (P<0.05) higher in the 14F and 21F groups compared to the control group, while it was significantly (P<0.05) lower in the 28F group (P>0.05).

Table (6): serum antioxidants enzyme activities of common carp fingerlings for reared at different stocking densities for 12 weeks

Parameters	7F	14F	21F	28F
MDA	57.12 ± 0.34^{d}	61.28 ± 0.18^{c}	118.13 ± 1.40^{a}	99.33 ± 1.46^{b}
GSH	0.48 ± 0.007^{c}	0.53 ± 0.006^{a}	0.50 ± 0.003^{b}	0.50 ± 0.002^{b}
GPX	9.71 ± 0.13^{a}	6.95 ± 0.04^{c}	9.68 ± 0.08^{a}	8.67 ± 0.048^{b}
CAT	51.05 ± 0.02^{c}	51.30 ± 0.03^{b}	52.13 ± 0.08^{a}	50.79 ± 0.07^{d}
SOD	5.65 ± 0.06^{b}	6.03 ± 0.08^{a}	5.24 ± 0.07^{c}	3.71 ± 0.04^{d}

DISCUSSION

Stocking density is one of the most important factors influencing growth indices and feed efficacy in rearing fish in cage culture (Oliveira et al., 2012; Oliveira et al., 2013). The final weight, specific growth rate, weight gain and feed conversion efficiency of the 14F group were significantly higher when compared with the 7F group. but they were significantly lower in the 21F and 28F groups than in the 7F control group. These results are in agreement with the findings of Wang et al., (2018) who observed that when blunt snout bream juveniles reared at different stocking densities for 6 weeks, the final weight, specific growth rate, weight gain, feed efficiency ratio, and food intake of the 90F and 120F groups were significantly decreased when compared control group. However, the 60F group was significantly increased than those of the (30F) control group. One of the reasons could be observed in the experiment schooling behaviours such as aggregating, scattering, resting, and group swimming. Fish schooling behaviours can improve feeding efficiency and food intake. Consequently, the growth performance enhanced the increase in group size and the 14F group exhibited significantly higher growth performance than the 7F group. Thus, in limited space, the competition for food and space resources intensifies as the group size increases (Grand and Dill, 1999). Then, in the big group size, fish consumption will be high, metabolic expenditure will change and food intake will be low (Li and Brocksen, 1977). Thus, in this study, the growth performance reduced significantly when the density was over 14F fish/0.2m^3.

In contrast, the findings of the current study disagree with the results of Oliveira et al. (2020) who observed a significant improvement with an increase in the stocking density (3.6, 6 and 10 fish/m^3) on final weight and weight gain for pirarucu juveniles cultured for 180 days in net pens. although these authors did not observe effects on production and feed conversion rate (FCR). Also, Paredes-López et al., (2021) showed that juvenile *Arapaima gigas* were not affected by increasing stocking density on WG, FCR, SGR, biomass gain, and relative growth rate. The 7F group indicated significantly higher FCR than the control group, while the 21F and 28F groups showed significantly lower FCR than the control (7F) group. This result is in line with a previous study in which a blunt snout bream juvenile was cultured in a cage (Wang et al. 2018). The survival rates decreased with increasing stocking rate, and significant differences appeared in the 21F and 28F groups compared to the control group. A good example to support our study is that high stocking density in blunt snout bream culture results in a decrease in survival rate (Wang et al. 2018).

The proximate composition of whole body common carp fingerlings in terms of crude protein has significantly fluctuated, the crude protein content in the group with 28F density was significantly higher than that of the control groups. On the contrary, Seo and Park (2022) indicated that the effect of stocking density on proximate composition in juvenile olive flounder *Paralichthys olivaceus* had a significant decrease in the crude protein content at the density of 8.56 kg/m^3. Also, Liu et al., (2016b) found that rainbow trout *O. mykiss* had significantly reduced the crude protein content at the high density. In addition, Ghozlan et al., (2018) showed that Meagre *Argyrosomus regius* fingerlings reared in cages with different stocking densities (50, 150, and 250 fish/m^3) had significantly lower protein contents in groups with higher stocking densities.

The crude fat content of the stocking densities (14F, 21F and 28F/0.2m^3) treatments slightly increased when compared with the control group, but no significant differences were found. Similar findings were observed by Eissa and Abdel-Rahman (2020) evaluated that Nile tilapia cultured at high stocking densities had higher fat content and lower protein content, which might be due to changes in feeding behaviour and nutrient absorption at these densities. Increased stocking density might require high energy expenditure to obtain food and coordinate interspecific relationships under stress stimulation resulting in increased metabolic levels (Jørgensen et al. 1993).

The haematological parameters are produced by very dynamic functional mechanisms, as a result, when the organism is exposed to external and internal factors, they are extremely vulnerable to variation, which makes them very important indicators for interpreting the fish response, as is the case here, under the effect of the rearing stocking density factor (Davis et al. 2008). In the current study, a significant elevation of Hct and RBCs was exhibited with increasing stocking densities. The results of our study agree with the findings of (Pinho et al. 2016) who indicated that RBCs of *Centropomus parallelus* in the 500 fish/m^3 group were higher than in the 200 and 350 fish/m^3 groups reared in a recirculation rearing system. Increased RBCs may improve blood oxygen capacity and oxygen delivery to the tissue (Ni et al., 2014). The haematocrit level is related to the number of RBCs because it is the ratio of blood plasma to red blood cells. An increase in the number of RBCs is followed by an increase in the haematocrit percentage (Fitria et al.,2019). An increased level in the fish blood is correlated to a higher number of red blood cells produced by the fish hematopoiesis tissue, as the number of red blood cells is proportional to the haematocrit value (Fadil et al., 2011). The Hgb level elevated with increasing stocking densities but no significant difference was found. Similarly, Wang et al., (2019) found

an increased trend in the Hb level with no significant differences in juvenile genetically improved farmed tilapia (GIFT) (*Oreochromis niloticus*) reared at different stocking densities in in-pond raceway recirculating systems. In general, blood haemoglobin is associated with stress responses, and the adrenaline release and the adrenalin released during stress cause in vivo swelling of erythrocytes (Nikinma and Huestis 1984), which then stimulates the erythrocytes to synthesize more Hb in order to maintain the optimal dissolved oxygen level in the blood (Ni et al. 2014; Nicula 2004). In our study, the differential white blood cells showed significantly increased trends in the density of 14F and 21F fish/0.2m^3 groups com compared to the control group. An increase in WBCs with increasing stocking density is favorable response to acute stress stimuli such as that with overcrowding (Tort 2011). However, prolonged overcrowding of the fish might conversely reduce the immune response (Caipang et al. 2009). Similar findings have been revealed in juvenile *A. gigas* (Paredes-López et al. 2021).

In the current study, the blood glucose levels fluctuated with different stocking densities, a significant elevation was found in the 21F fish density group while a significant retardation was found in the 14F fish density group. The level of glucose varies with the effects of different stocking densities which depends on the species, for example, the level of glucose in *Acipenser schrenckii* was similar at low, medium, and high densities (Ni et al. 2014), but in *Clarias gariepinus* glucose the level will increase with the increasing stocking densities (Abutalb et al. 2022) while the glucose trends decrease in *Megalobrama amblycephala* as the stocking density increased (Wang et al. 2018). The total protein trend decreased with increasing stocking density in this study. The reason could be explained by enhanced levels of glucose with reduced levels of total protein are a classic indicator of stress conditions, however, both are used as a source of energy to deal with stress (Barton 2002). The cortisol is considered an important indicator of stress response in organisms, it is typically increased under stress conditions (Zarkasi et al. 2016). In this study, serum cortisol levels were increased significantly with increasing stocking density in the 14F, 21F and 28F groups than those in the 7F (control) group. The high levels of cortisol in the high stocking cage (28F) suggest that fish suffered from crowding stress during the period in the cage culture. The same results were conducted by previous studies on Nile tilapia (Abu Zafar et al. 2022), Atlantic salmon (Liu et al. 2017) and blunt snout bream (Wang et al. 2018). On the contrary, no significant differences in serum cortisol were found in genetically farmed tilapia reared at all stocking densities (Wang et al., 2019). The LDL levels usually are linked with cortisol levels and therefore used to calculate the fish stress levels. Our findings revealed the same phenomenon; the 14F, 21F and 28F stocking density increased the level of LDL but significant elevation was reported at 21F density. This means that as an anti-stress response, serum LDL levels were enhanced for energy utilization and LDL was used for energy production to resist external stimuli (Sangiao Alvarellos et al. 2005). Meanwhile, The CHOL and HDL levels decreased significantly and TG increased significantly as the stocking density increased. Under stressful conditions, TG serves as a crucial energy source to deal with increased energy demands (Dehler et al. 2017).

All stocking density groups achieved significantly higher levels of ALT, AST and ALP than the control group. The results of this study are similar to the study done by Wang et al., (2018) investigated that the levels of ALT, AST and ALP were significantly elevated in *M. amblycephala* juveniles reared at high stocking densities. A good explanation for this phenomenon is that indicates the impairment of liver activity and myocardium functions or damage to the liver at high-density stocking conditions (Wang et al., 2018), or the reason could be that increased liver enzyme activity reflects the use of excess hydrocarbon from amino acids to meet energy requirements (Abdel-Tawwab et al., 2014). The serum ALP level is a vital metabolic enzyme that plays a key role in nutrient absorption and utilization ALP is an important metabolic. Elevation of ALP levels could be due to liver damage (Liu et al., 2016c) as well as activation of metabolic and immune functions (Xing et al. 2002). Differences in the results between these studies might be due to fish species, variations in physiological response, age of fish, as well as, the experimental conditions and the period of experiment.

Serum antioxidant enzyme activities are directly linked to the health status of aquatic animals including fish (Henrique et al.1998). Results from this study showed that the MDA and GSH activities improved with the increasing stocking density. Metabolic activities of the fish body will continue to generate oxygen free radicals, under normal physiological functions. Concurrently, the oxygen free radicals are cleared by using the cellular antioxidant system, ensuring that the oxygen free radical level in the fish body is the oxygen free radical content in the body is remains in a state of active balance, protecting the fish body from damage (Olsen et al. 2005). The MDA level in fish is an important indicator of the free radical level of oxygen (Cemek et al., 2011). Higher levels of oxygen free radicals elucidate why the high density group have lower levels of the higher level of oxygen free radicals explains the lower levels of SOD and GPx in the high-density groups. Liu et al., (2016d) found that SOD, CAT and GPx activities markedly reduced in the fish cultured at high stocking density on day 120, this supports the results of our study. The reduction of antioxidant enzyme levels could be a response to

the continuous stocking stress and imitates the low antioxidant capacity of aquatic animals for these harmful superoxide radicals scavenging which eventually leads to oxidative damage (Andrade et al., 2015; Costas et al. 2013).

CONCLUSION

In conclusion, the findings of this study exhibited that the 14F fish/0.2m^3 density group significantly promoted growth performance and feed utilization and health status in *C. carpio* juveniles which was more suitable for growing fish in cages. Higher stocking density (21F and 28F fish/0.2m^3) showed negative effect on fish and the stress levels increased significantly, and subsequent changes appeared in terms of haematology, serum biochemical, serum antioxidant activities and growth performance. According to the results of the current study, to obtain the possible growth and good health status, the best stocking density for rearing common carp juveniles in the cages is 14F fish/0.2m^3. Likewise, further study requires to focus on comparing physiological responses to stocking density in other outdoor culture systems, different sizes, social interaction, stress mechanism, and improving common carp stocking management strategies.

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