



# Hematological and physiological characteristics of the common carp (*Cyprinus carpio*) reared in saline water and fed with fermented herbs

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**Abstract.** The quality of life of fish is strongly influenced by aquatic environmental conditions, such as salinity. This study aimed to analyze the changes in hematological, biochemical (serum), and physiological of common carp (*Cyprinus carpio*) feed with fermented herbs in saline media. The method used was a Completely Randomized Design (CRD) with five treatments, corresponding to salinity levels of 0, 3, 6, 9, and 12 ppt. The fish was kept in aquariums with the dimensions of 60x40x40 cm<sup>3</sup> and a stocking density of 1 fish 2 L<sup>-1</sup> for 60 days and fed with commercial fish feed that had been added with fermented herbs at a dose of 200 mL kg<sup>-1</sup> of feed. Parameters observed were hematological levels (total erythrocytes, leukocytes, hemoglobin, hematocrit), serum biochemistry (calcium, magnesium, phosphorus, and total protein) and physiological conditions (blood glucose, osmolarity, growth, and survival rate). The results showed that common carp could live in media with a salinity of up to 12 ppt, reaching a survival rate of 75% and hematological and physiological values in the normal ranges.

**Key Words:** brackish water, fish, immunitas, osmolaritas, serum biochemistry.

**Introduction.** The potential area for brackish water cultivation is currently recorded at 2,964,331 ha, but only 650,509 ha (21.9%) were utilized (KKP 2018). Considering the high demand for fish, these unutilized brackish water areas have a great value for fish farming. In 2020, the average fish consumption in Indonesia was 56.39 kg capita<sup>-1</sup> year<sup>-1</sup>. To ensure optimal production, the Ministry of Marine Affairs and Fisheries set the aquaculture production target at 10.32 million tons by 2024 (KKP 2020). Therefore, it is necessary to cultivate fish that not only live in fresh water, but can also live in brackish water. The fish whose adaptation to saline (brackish) media was demonstrated is tilapia (*Oreochromis niloticus*), while catfish (*Pangasianodon hypophthalmus*) (Riswan et al 2022) and common carp (*Cyprinus carpio*) (Alam et al 2020) acclimation is still in the experimental stage.

*C. carpio* is a freshwater fish that has a high demand because it has a savory meat taste and is easy to cultivate. *C. carpio* production in Indonesia in 2020 was around 127,772.13 tons, especially in Riau, where it reached 4,181.18 tons (KKP 2020). So far, *C. carpio* cultivation is carried out in fresh waters, such as ponds, cages, and floating net cages. The cultivation can be expanded if the fish can survive in saline water. Climate change and anthropogenic activities have recently affected the land use in the agricultural sector and aquaculture, through salinization of the water zones (Murmu et al 2019). In addition, around 6,500 ha of empty ponds (idle) are left by tiger shrimp farmers (Arafani et al 2016). This provides opportunities for the community or cultivators to carry out fish farming in the abandoned ponds.

Alam et al (2020) stated that *C. carpio* can adapt to 12 ppt salinity with a 60% survival rate. The low survival rate is influenced by the increase in salinity. High salinity increases osmotic pressure of water in the fish body and affects the osmolarity value as well as the gill histopathology (Handayani et al 2020). All of these pressures cause stress to fish, that leads to death (Alam et al 2020).

Based on the description above, the aim of the research was to assess the effect of salinity on the hematological, biochemical (serum), and physiological characteristics of *C. carpio* reared on saline media and fed with commercial pellets plus fermented herbs.

## Material and Method

**Place and time.** This study was conducted at the Laboratory of Aquaculture Technology, Faculty of Fisheries and Maritime, Universitas Riau in June-September 2022.

**Research design.** The method used was a Completely Randomized Design (CRD) with five treatments at the salinity levels of 0, 3, 6, 9, and 12 ppt, with three replications, referring to (Salati et al 2011). The common carp specimens were 6-8 cm long and weighed 8 g. They were kept in aquariums with a dimension of 60x40x40 cm<sup>3</sup> for 60 days. During the adaptation and rearing, the fish were fed 3 times a day with commercial pelleted feed added with fermented herbs at a dose of 200 mL kg<sup>-1</sup> (Syawal et al 2020).

**Procedure.** Firstly, fermented herbs were prepared following (Syawal et al 2020). The ingredients were 100 g *Curcuma zanthorrhiza*, 100 g *Kaempferia galanga*, 100 g turmeric, 175 mL molasses, 65 mL probiotic drink, 50 mg *Saccharomyces cerevisiae*, and 3 L water. Washed *C. zanthorrhiza*, *K. galanga*, and turmeric were milled, boiled with water, and filtered to obtain herbs extract. After the extract reached the room temperature, it was added with molasses, probiotic drink, and *S. cerevisiae*. The homogeneous mixture was then incubated at room temperature for 7 days.

After fermented herbs were produced, the fish specimens were adapted 3 days to the conditions of each treatment, before blood samples were collected. Blood was drawn twice for each sampling: with anticoagulant (EDTA) and without anticoagulant. The blood with anticoagulant was used for routine hematological analysis, such as total erythrocytes, total leukocytes, hemoglobin levels, and hematocrit values (Blaxhall & Daisleey 1973) and the plasma were used to measure the osmolarity value using an Automatic Osmometer and the glucose levels using the Glucodoc kit. Serum was obtained from the blood without anticoagulation. It was used for blood biochemical analysis, such as calcium, magnesium, phosphorus, and total protein. Serum and plasma that were not used immediately were stored at a temperature -20°C. Levels of magnesium, calcium, phosphorus, and total protein were analyzed using a photometer microlab 300 with kits (XL FS REF 1 4610 99 83 021 KIT LOT 60 148320 for magnesium, AS FS REF 1 1130 99 83 021KIT LOT 60 148385 for calcium, FS REF 1 5211 99 83 021KIT LOT 60147518 for phosphorus, and FS REF 1 231199 83026 KIT LOT 60143445 for total protein).

**Statistical analysis.** Data obtained from the hematological, physiological, serum biochemistry, growth and survival measurements were tabulated and then analyzed using SPSS and discussed descriptively.

**Results and Discussion.** The hematological parameters of *C. carpio* in this study are presented in Table 1. Based on the data, the hematological values, such as total erythrocytes, hematocrit and hemoglobin decreased until the 30<sup>th</sup> day, and increased again on the 60<sup>th</sup> day. The value fluctuations were due to the influence of media salinity, which caused stress to the fish. When fish experience stress due to changes in salinity, the osmoregulation process is disturbed, especially in the gill organs, so that the oxygen uptake process is disrupted. If the fish is deprived of oxygen, its response to eating is reduced and the metabolic process cannot run properly. This affects the growth and the hematological system (Kavya et al 2016).

The increase in the hematological values at the end of the study (day 60) was suspected to be related to the addition of fermented herbs to the feed. Fermented herbs could help to increase feed digestibility, increase fish growth and health, and reduce fish stress levels due to environmental changes (Kavya et al 2016; Syawal et al 2022). These herbs contain flavonoids that can increase the absorption of vitamin C, hence reducing stress levels in fish (Kavya et al 2016). Curcumin in fermented herbs is also able to

increase fish appetite. During the first month of fish rearing, although the hematological values decreased, the fish in saline water still ate the feed in the same manner as the fish in fresh water. The feed was suspected to provide energy for the fish during the adaptation period to maintain osmotic pressure and homeostatic conditions (Hasan et al 2016).

Table 1

Average hematological values of *Cyprinus carpio* reared in saline water and fed with fermented herbs

Treatments	Total erythrocyte ( $10^6$ cells $mm^{-3}$ )		
	0 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
P0 (0ppt)	2.57±0.02 <sup>d</sup>	2.63±0.32 <sup>c</sup>	2.65±0.44 <sup>b</sup>
P1 (3ppt)	2.37±0.02 <sup>c</sup>	1.51±0.29 <sup>ab</sup>	2.25±0.21 <sup>ab</sup>
P2 (6ppt)	2.41±0.03 <sup>c</sup>	1.66±0.21 <sup>b</sup>	2.34±0.21 <sup>ab</sup>
P3 (9ppt)	2.02±0.06 <sup>b</sup>	1.17±0.11 <sup>a</sup>	1.94±0.58 <sup>ab</sup>
P4 (12ppt)	1.61±0.08 <sup>a</sup>	1.22±0.08 <sup>a</sup>	1.70±0.44 <sup>a</sup>
Treatments	Hematocrit (%)		
	0 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
P0 (0ppt)	32.00±2.00 <sup>c</sup>	33.67±4.16 <sup>b</sup>	35.00±3.61 <sup>b</sup>
P1 (3ppt)	28.00±2.00 <sup>b</sup>	24.67±3.51 <sup>b</sup>	32.67±1.53 <sup>ab</sup>
P2 (6ppt)	27.00±1.73 <sup>ab</sup>	23.00±1.73 <sup>b</sup>	30.33±1.53 <sup>ab</sup>
P3 (9ppt)	26.00±2.00 <sup>ab</sup>	21.67±2.89 <sup>b</sup>	29.33±3.06 <sup>ab</sup>
P4 (12ppt)	23.00±1.00 <sup>a</sup>	16.00±2.00 <sup>a</sup>	26.33±1.53 <sup>a</sup>
Treatments	Hemoglobin (mg L <sup>-1</sup> )		
	0 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
P0 (0ppt)	7.80±0.20 <sup>c</sup>	7.87±0.12 <sup>c</sup>	10.27±0.42
P1 (3ppt)	7.53±0.11 <sup>bc</sup>	7.23±0.31 <sup>bc</sup>	10.07±0.50
P2 (6ppt)	7.42±0.34 <sup>bc</sup>	6.80±0.31 <sup>bc</sup>	9.73±0.42
P3 (9ppt)	7.11±0.23 <sup>ab</sup>	6.83±0.42 <sup>a</sup>	9.13±1.85
P4 (12ppt)	6.80±2.00 <sup>a</sup>	6.40±0.20 <sup>a</sup>	9.00±1.00
Treatments	Total leukocyte ( $10^4$ cells $mm^{-3}$ )		
	0 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
P0 (0ppt)	4.88±0.04 <sup>a</sup>	8.83±1.85 <sup>ab</sup>	5.55±0.61 <sup>ab</sup>
P1 (3ppt)	8.71±0.04 <sup>d</sup>	8.97±0.56 <sup>ab</sup>	6.47±0.91 <sup>b</sup>
P2 (6ppt)	7.85±0.04 <sup>c</sup>	6.13±1.72 <sup>a</sup>	3.67±0.47 <sup>a</sup>
P3 (9ppt)	7.51±0.04 <sup>b</sup>	9.20±0.60 <sup>ab</sup>	6.45±1.70 <sup>b</sup>
P4 (12ppt)	11.69±0.04 <sup>e</sup>	11.06±2.71 <sup>b</sup>	4.21±2.71 <sup>ab</sup>

Means with the same superscripts in the same column are not statistically different at p=0.05.

Total leukocytes fluctuated during the study. On the 30<sup>th</sup> day, the values were higher than the initial values, although they were still in the normal range. The total leukocytes in normal fish ranges from 20,000-150,000 cells  $mm^{-3}$  (Royan et al 2014). The increase in total leukocytes indicates that the fish' immune system undergoes a process of fighting against foreign objects that enter the body. The number of leukocytes in fish is a good indicator of physiological stress. The high leukocyte value at 12 ppt salinity was caused by the non-specific interaction between prolactin and cortisol (Soltanian et al 2016). Furthermore, physiological values of fish were also examined, such as glucose levels, overculum openings and osmolarity due to rearing in saline media. Data on fish physiology are shown in Table 2.

The average value of glucose levels increased until the end of the study in all treatments, especially in the 12 ppt salinity treatment. This indicated that the fish were experiencing secondary stress conditions, resulting in an increase in glucose levels. Glucose is needed as an energy source to maintain homeostasis. Thus, fish require high energy to carry out homeostasis by mobilizing glucose into the blood, both through the process of glycogenesis and gluconeogenesis (Esmaili 2021). This condition lasted until the end of the study, which agrees with a previous study (Alam et al 2020).

Table 2

Average physiological values of *Cyprinus carpio* reared in saline water and fed with fermented herbs

Treatments	Glucose level $g L^{-1}$		
	0 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
P0 (0ppt)	37.00±2.00 <sup>a</sup>	38.00±2.00 <sup>a</sup>	39.67±1.53 <sup>a</sup>
P1 (3ppt)	37.00±1.00 <sup>a</sup>	41.67±1.53 <sup>a</sup>	49.33±2.08 <sup>c</sup>
P2 (6ppt)	39.00±5.29 <sup>a</sup>	40.33±4.93 <sup>a</sup>	44.00±3.00 <sup>b</sup>
P3 (9ppt)	40.00±1.00 <sup>a</sup>	43.33±6.03 <sup>a</sup>	51.67±1.53 <sup>c</sup>
P4 (12ppt)	42.00±2.00 <sup>a</sup>	53.67±4.16 <sup>b</sup>	54.00±2.65 <sup>c</sup>
Treatments	Overculum openings		
	0 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
P0 (0ppt)	121.33±3.51 <sup>a</sup>	123.67±2.3 <sup>a</sup>	120.67±2.08 <sup>a</sup>
P1 (3ppt)	124.67±3.21 <sup>a</sup>	127.67±3.06 <sup>a</sup>	121.67±3.06 <sup>a</sup>
P2 (6ppt)	129.67±7.64 <sup>ab</sup>	126.67±2.31 <sup>a</sup>	122.33±2.08 <sup>a</sup>
P3 (9ppt)	136.33±4.73 <sup>b</sup>	127.33±5.03 <sup>a</sup>	120.67±3.79 <sup>a</sup>
P4 (12ppt)	145.67±4.04 <sup>c</sup>	128.00±3.61 <sup>a</sup>	124.00±1.73 <sup>a</sup>
Treatments	Osmolarity ( $mOsmol kg^{-1} H_2O$ )		
	0 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
P0 (0ppt)	135	135	137
P1 (3ppt)	243	237	236
P2 (6ppt)	299	294	269
P3 (9ppt)	301	292	285
P4 (12ppt)	338	334	331

Means with the same superscripts in the same column are not statistically different at  $p=0.05$ .

As the size of the fish increases, the rate of respiration/mouth opening will decrease. In addition, the fish's overculum opening is also influenced by the environment. The higher the salinity, the higher the fish's respiration rate (Seibel et al 2021). In this study, in the treatment with 12 ppt salinity, from the beginning of the adaptation period until the 30<sup>th</sup> day, the overculum openings were still high when compared to other treatments. This could be due to the increased need for oxygen to achieve physiological balance at the beginning of the adaptation phase. After the fish have adapted, the oxygen demand is relatively the same at various levels of salinity. The oxygen demand can also be influenced by the activity of the fish (Kültz 2015).

The stress due to change in fish habitat from fresh water to saline water also generate a compensatory osmoregulation response in fish. The osmolarity value of fish reared in saline media of 3, 6, 9, and 12 ppt ranges from 236-338  $mOsmol kg^{-1}$  (Table 2). Most fish (>25,000 extant species) are teleost and osmoregulatory, meaning that they can maintain their extracellular body fluids at a relatively constant osmolality of 300  $mOsmol kg^{-1}$  (isosmotic to 9  $g kg^{-1}$  salinity) (Evans 2008). Pamungkas (2012) states that the mechanism of osmoregulation by teleost fish in freshwater and marine water is different. If fish live in a freshwater environment, it will prevent salt loss by excreting a large amount of dilute urine and increasing ion absorption. In contrast, in a saltwater environment, fish body fluids are hypoosmotic and should reduce water loss and NaCl gain by ingestion of seawater and excretion of small volumes of ion-concentrated urine, as well as by an active secretion of NaCl through the gill epithelium. The high osmolarity value in the 12 ppt treatment, which ranged from 331-338  $mOsmol kg^{-1}$  had caused the fish to experience severe stress, which had an impact on their growth and survival. The growth data of fish reared in saline media is presented in Table 3.

Fish that were reared in saline media up to 12 ppt for two months in the aquarium could grow with an average absolute weight of 9.26 g and an absolute length of 3.8 cm. These results are better than in the case of tilapia (*Oreochromis niloticus*) that were reared at salinities of 0, 10, 20, and 30 ppt, in the study of Elarabany et al (2017). They found that the highest absolute weight growth was in the control treatment, with 3.39 g,

followed by the 10‰ treatment, with 3.22 g, 20‰ treatment, with 3.12 g, and the lowest was in the 30‰ treatment, with 2.65 g.

Table 3

Average growth in weight and length of *Cyprinus carpio* reared in saline water and fed with fermented herbs

Salinity treatments	Growth in weight ( $g\ ind^{-1}$ )		Absolute weight ( $g\ ind^{-1}$ ) $\pm$ SD
	Initial	End	
P0 (0ppt)	5.67	15.46	9.79 $\pm$ 1.27
P1 (3ppt)	5.90	15.85	9.95 $\pm$ 0.52
P2 (6ppt)	5.63	15.72	10.08 $\pm$ 0.75
P3 (9ppt)	6.28	15.54	9.47 $\pm$ 0.31
P4 (12ppt)	5.68	15.16	9.26 $\pm$ 0.64
Salinity treatments	Growth in length (cm)		Absolute length (cm) $\pm$ SD
	Initial	End	
P0 (0ppt)	7.70	11.40	3.87 $\pm$ 0.31
P1 (3ppt)	7.67	11.83	4.17 $\pm$ 0.12
P2 (6ppt)	7.60	11.67	4.07 $\pm$ 0.21
P3 (9ppt)	7.50	11.33	3.83 $\pm$ 0.67
P4 (12ppt)	7.53	11.33	3.80 $\pm$ 0.75

It is suspected that there is an effect of adding fermented herbs to feed. These herbs acted as a supplement, so that fish could adapt and grow in media with salinity up to 12 ppt. The ingredients such as curcuma, aromatic ginger, and turmeric could reduce adaption stress and increase appetite and absorption of nutrients in the intestine, thus increasing growth (Blaxhall & Daisleey 1973). Moreover, the resistance of these fish to environmental changes can also be seen from the biochemical content in the fish serum, such as levels of calcium, magnesium, phosphorus, and total proteins. For more details, biochemical data are shown in Table 4.

Table 4

Average biochemical values of *Cyprinus carpio* reared in saline water and fed with fermented herbs

Treatments	Calcium ( $mg\ dL^{-1}$ )	Magnesium ( $mg\ dL^{-1}$ )	Phosphorus ( $mg\ dL^{-1}$ )	Total protein ( $g\ dL^{-1}$ )
P0	7.1-8.1	2.8-3.2	2.5-2.4	2.7-2.9
P1	8.4-10.0	2.9-3.8	2.3-2.4	3.2-3.4
P2	9.5-10.8	2.4-2.7	2.2-2.5	3.2-3.6
P3	10.5-10.6	2.8-3.0	4.5-5.8	3.3-3.4
P5	13.0-13.5	3.0-3.5	4.0-6.8	3.3-3.5

Based on the data from Table 4, the concentration of calcium, magnesium, and phosphorus increased with the salinity levels of the rearing media. This indicated that the fish were in a state of physiological stress, which can affect homeostasis conditions and hydromineral imbalance. Seibel et al (2021) stated that the concentration of sodium, potassium, and calcium in fish *Nototerus notepterus* may increase with the salinity, resulting in loss or disruption of homeostasis. The concentration of calcium in the blood can be classified as low if it is  $<8.6\ mg\ dL^{-1}$ , normal if it is  $8.6-10.30\ mg\ dL^{-1}$ , and high if it is  $>10.30\ mg\ dL^{-1}$ . The concentration of calcium in this study increased in relation to the increase in salinity levels of the rearing media.

The concentration of magnesium in fish serum also increased during the observation, correlated with an increase in the salinity level of the rearing media. The highest magnesium concentration, which was  $3.5\ mg\ dL^{-1}$ , was obtained in the 12 ppt treatment. High concentrations of magnesium can cause lethargy (fish become weak)

and inactive (Immanuel & Iriani 2006). This could be seen from the movement of fish that are less active and stay still near the aeration source.

Phosphorus levels in this study were still in the normal range, except for the 12 ppt treatment. There was an increase in phosphorus levels at the end of the study due to fish being unable to tolerate high salinity levels. Serum phosphate levels exceeding 5.5 mg dL<sup>-1</sup> can increase mortality. Normal homeostatic conditions in fish require serum phosphate concentrations between 2.5 and 4.5 mg dL<sup>-1</sup>. High levels of phosphate in the blood are called hyperphosphatemia; this condition occurs due to a failure of excretion of phosphate in the organism due to a decreased kidney function.

The total protein in fish serum during the study (Table 4) was relatively the same between treatments (3.2-3.6 mg dL<sup>-1</sup>) except in the control group, which had 2.7-2.9 mg dL<sup>-1</sup>. The highest total protein value was found in the 6 ppt treatment. Total serum protein in normal fish fed with commercial feeds ranged from 2.4-4.5 mg dL<sup>-1</sup> (Dawood et al 2021). Total protein in serum can be influenced by the stress conditions, season, time of gonadal maturation, type of food given and eating habits (Pastorino et al 2022).

Salinity is the main abiotic factor in aquaculture. Its optimum degree is specific and can also affect the growth and the survival rate of a fish. The ability of fish to survive can be seen from the survival rate of fish at the end of the study, as shown in Table 5.

Table 5

Average survival rate of *Cyprinus carpio* reared in saline water and fed with fermented herbs

Treatments	Survival rate (%)		
	0 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
P0 (0ppt)	100	100±0.00 <sup>b</sup>	100±0.00 <sup>b</sup>
P1 (3ppt)	100	100±0.00 <sup>b</sup>	100±0.00 <sup>b</sup>
P2 (6ppt)	100	100±0.00 <sup>b</sup>	100±0.00 <sup>b</sup>
P3 (9ppt)	100	100±0.00 <sup>b</sup>	95±8.66 <sup>b</sup>
P4 (12ppt)	100	86.67±7.64 <sup>a</sup>	75±10.00 <sup>a</sup>

Means with the same superscripts in the same column are not statistically different at p=0.05.

The low survival rate of fish in 12 ppt salinity media, which was 75% at the end of the study, could be due to the fish failure to maintain homeostatic conditions when there is a high difference in the osmotic pressure between the environment and body fluids. Fish require high energy for regulation and adaptation in hypoosmotic or hyperosmotic conditions, so that it can affect metabolism and decrease growth performance (Dawood et al 2021; Pastorino et al 2022). This results in lower growth and higher mortality (Fazio 2019). Although the survival rate in the 12 ppt treatment was significantly lower than in control, this result was better than the in Alam et al (2020), where the survival rate of the fish drops to 60% in 35 days, when salinity is 12 ppt. The main difference between this study and Alam et al (2020) is the type of feed given to the fish. Alam et al (2020) only gave commercial pellets, while in our study the commercial pellets were added with fermented herbs. This strengthens our presumption that the fermented herbs could reduce the stress experienced by the fish due to the environmental changes.

**Conclusions.** Based on the results of the study, it can be concluded that *C. carpio* could live in media with a salinity of up to 12 ppt by feeding them with commercial feed fortified with fermented herbs at a dose of 200 mL kg<sup>-1</sup> feed to produce a survival rate of 75%, with normal hematological, physiological, and biochemical values of serum biomarkers.

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**Conflict of interest.** The authors declare no conflict of interest.

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