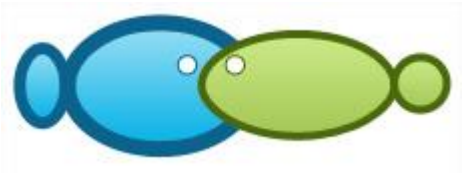

Growth performances, feed utilization and hematological parameters of the carp (*Cyprinus carpio*), according to the dietary glutamate

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Abstract. The purpose of this study was to determine the effects of dietary glutamate supplementation on the growth performances, feed utilization efficiency and hematological parameters of carp (*Cyprinus carpio* L.). Two hundred carps were allocated to four experimental groups, each consisting of five replicates. The four treatments were dietary glutamate supplementation of 0.0, 0.5, 1.0 and 1.5%, respectively, each with 10 fish in one experimental unit. The initial weight of the trial fish ranged between 2.54 to 2.61 g fish^{-1} ($P<0.05$). Each group of fish was cultured in a 10 L aquarium container of (60x45x45) cm^3 for 42 days. The fish were fed on the test diets three times a day by at satiation method. The experiment was carried out to study the effects of glutamate supplementation on the final weight gain, total feed consumption, feed utilization efficiency, relative growth rate, survival and hematological parameters (i.e. consisting of aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), bilirubin, blood glucose) of the carp. The data obtained on growth performances were analyzed by ANOVA and followed by Duncan's test using SPSS 22.0. Hematological and water quality data were analyzed descriptively. This study showed that dietary glutamate supplementation resulted in the increase of feed utilization efficiency and relative growth rate. Dietary glutamate affected the concentrations of AST, ALT, bilirubin and blood glucose, but had no impact on the fish survival. Based on the ECR and RGR regression, it was found that the optimal level of dietary monosodium glutamate (MSG) varied from 0.99 to 1.16%. The maximum value of feed utilization efficiency and relative growth rate were 59.79 and 3.48% day^{-1}, respectively.
Key Words: glutamate, relative growth, feed utilization efficiency, AST, ALT, bilirubin, blood glucose.

Introduction. The common carp (*Cyprinus carpio*) is a freshwater fish species that plays an economically important role as aquacultural fish commodity in Indonesia. The carp has been cultivated and consumed for a long time by Indonesian people. The prospect on the increasing of the fish production was quite large, as the demand for the carp was high, reaching 50 tons per day for Jakarta, Bogor, Depok, Tangerang and Bekasi areas (Jabodetabek), Indonesia (Ramadhan & Lutfiana 2018). Furthermore, the demand for the carp was estimated to increase in the range of 100 tons per day. Therefore, a high production of carp was needed to meet its demand. The large increase in the production of the fish can be gained by applying an intensive culture technology and by increasing the fish growth by using better quality diets (Nguyen et al 2021).

Technological advances and intensification of the production systems will increase the fish production. It was closely related to the development production of high-quality fish feed for aquaculture (Beklevik 1999; Citarasu et al 2003; El-Dakar et al 2007; Zhelyazkov et al 2015; Staykov et al 1998; Nguyen et al 2021). Several studies have been developed with various additive supplements for fish feed. Therefore, the feed utilization was much more efficient (Polat & Beklevik 1999; Mousavi et al 2016). These additives are expected to promote the growth of cultivated species and to reduce the feed conversion ratio, without negative effects on the survival rates of the fish species. Moreover, supplements in fish feed should ultimately increase the efficiency of its utilization (Zhelyazkov 2018). The use of supplements in the diet of fish is beneficial. Some amino acids can be used as beneficial supplements, including glutamate

(Zhelyazkov & Stratev 2019). Glutamate plays a key role in amino acid metabolism through its conversion to α-ketoglutarate or other non-essential amino acids (NEAAs) (Solares et al 2015).

Monosodium glutamate (MSG) is a source of glutamate. The MSG was found in all protein feeds, representing the sodium salt of glutamic acid (Henry-Unaeze 2017). Natural food contains free and bound MSG, and, in some foods, free MSG was found in large quantities (Shi et al 2014). The MSG was widely used as a feed additive to improve taste. Its presence in fish feed will increase its consumption (Masic & Yeomans 2013; Peng et al 2018). The use of MSG supplements in fish diets is beneficial. The amino acid glutamate from MSG can be used as a supplement. Glutamate plays an important role as a regulator of amino acid metabolism (De Silva & Anderson 1995; Oehme et al 2010) and is required for the maintenance, growth, reproduction and immune response of fish (Oehme et al 2010). It acts directly either after conversion to other amino acids or in intracellular metabolism, as an energy source for enterocytes and nucleic acid precursors (De Silva & Anderson 1995; Yan & Qiu-Zhou 2006; Yoshida et al 2016). The purpose of this experiment was to evaluate the effects of dietary glutamate supplementation on the growth performances, feed utilization efficiency and hematological parameters of *C. carpio*.

Material and Method

Experimental fish and rearing. *C. carpio* as much as 200 fish were used in this experiment. The initial body weight of the trial fish ranged from 2.54 to 2.61 g fish^{-1}. The fish was kept for 42 days in 20 aquarium containers, with dimensions (length x width x height) of 60x45x45 cm^3. Each aquarium was filled with 10 L of water and 10 individual test fish. Therefore, the fish density was 1 fish L^{-1}. During the experimental period, the fish were fed on the test diet containing 0.0, 0.5, 1.0 and 1.5% dietary glutamate. The feeding frequency was three times a day, by applying the at satiation method.

Experimental diet. The test diet used was a basic feed containing 30% protein, with added glutamate (from MSG), by repelling. The glutamate concentration in each test diets was A (0.0% kg^{-1}), B (0.5% kg^{-1}), C (1.0% kg^{-1}) and D (1.5% kg^{-1}). MSG contains 78% glutamate, 12% sodium and 10% water. Therefore, the MSG used in treatments A, B, C and D was 0.00, 0.64, 1.28 and 1.92%, respectively. The feed composition and proximate analysis of the trial diets were presented in Table 1.

Table 1

The feed composition and proximate analysis of the trial diets (% dry weight basis)

Feed composition	*Treatments of dietary glutamate*			
	A (0.00%)	*B (0.50%)*	*C (1.00%)*	*D (1.50%)*
Basic feed (commercial feed)	98.00	98.00	98.00	98.00
MSG	0.00	0.64	1.28	1.92
CMC	0.50	0.50	0.50	0.50
Albumin	1.50	0.86	0.22	0.00
Total	100	100	100	100
Glutamate	0.00	0.50	1.00	1.50
Proximate analysis				
BETN (%)	48.68	45.87	43.28	46.14
Protein (%)	31.96	33.87	34.4	33.82
Lipid (%)	6.97	6.10	5.45	6.22
Ash (%)	9.33	11.11	10.58	8.99
Fiber (%)	3.06	3.05	6.29	4.83
Energy (kcal)	290.02	282.63	272.75	284.1
Ratio E/P (kcal g^{-1}protein)	9.07	8.34	7.93	8.40

MSG-monosodium glutamate; CMC-carboxymethyl cellulose; NFE-nitrogen free extract; E/P-energy protein ratio.

This experiment used a completely randomized design (CRD) with four treatments and five replicates. This study was conducted at the Teaching Factory of Diponegoro University, Semarang, Central Java, Indonesia, from November to December 2020.

Experiment parameters. The experimental parameters measured to evaluate the treatment responses were the relative growth rate (Effendi 1997), feed utilization efficiency (NRC 2011), total feed consumed (Pereira et al 2007; Subandiyono & Hastuti 2020), protein efficiency ratio (Tacon 1987) and survival rate (Panigrahi et al 2017). Hematological responses of fish were observed through blood glucose parameters, amino transferase enzymes (Hastuti & Subandiyono 2020) and bilirubin (Ngashangva et al 2019).

Water quality. Observations of the water qualities included temperature (°C), pH, dissolved oxygen (DO, mg L^{-1}) and ammonia (mg L^{-1}). Temperature, pH and DO were measured daily, using a Water Quality Checker, while ammonia was measured every 14 days.

Statistical analysis. Results of the calculated variables were presented as mean±SD. Statistical analysis was performed using the one-way analysis of variance (ANOVA) followed by Duncan's test with the SPSS 22.0 software. The significance test was performed with a probability level of $p<0.05$. Furthermore, the relative growth rate (RGR) and feed utilization efficiency (FUE) values were calculated using a polynomial regression model (Robbins et al 1979) to estimate the optimal dietary glutamate requirement. Hematological variables consisting of ALT, AST, bilirubin, blood glucose and water qualities were analyzed descriptively.

Results

Growth parameters and feed utilization. After 42 days feeding period, the growth performances and feed utilization efficiency of *C. carpio* were affected by the level of glutamate in the diet ($p>0.05$). Final body weight, total feed consumption, protein efficiency ratio (PER) and survival rates (SR) were not affected by the level of glutamate in the diet ($p<0.05$). The growth parameters and feed utilization are displayed in Table 2.

Table 2

Growth performances and feed utilization of *Cyprinus carpio* after consuming trial feeds containing glutamate

Parameters	*Treatments of dietary glutamate*			
	A (0.00%)	*B (0.50%)*	*C (1.00%)*	*D (1.50%)*
Initial body weight (g $fish^{-1}$)	2.54±0.12^{a}	2.55±0.16^{a}	2.61±0.12^{a}	2.58±0.12^{a}
Final body weight (g $fish^{-1}$)	6.48±0.22^{a}	6.68±0.38^{a}	6.76±0.30^{a}	6.57±0.36^{a}
Total feed consumption (g $fish^{-1}$)	6.76±0.32^{a}	6.66±0.28^{a}	6.59±0.36^{a}	6.65±0.38^{a}
Feed utilization efficiency (FUE, %)	54.58±2.17^{a}	58.39±2.23^{ab}	59.91±1.65^{bc}	58.33±2.38^{abc}
Protein efficiency ratio (PER)	1.71±0.07^{a}	1.72±0.07^{a}	1.74±0.05^{a}	1.72±0.07^{a}
Relative growth rate (RGR, % day^{-1})	3.15±0.19^{a}	3.32±0.12^{ab}	3.51±0.07^{bc}	3.43±0.20^{abc}
Survival rate (SR, %)	92.00±4.47^{a}	90.00±7.07^{a}	92.00±4.477^{a}	94.00±5.48^{a}

The polynomial regression model of the RGR and FUE values were shown in Figure 1 and Figure 2. Based on the RGR regression equation in Figure 1, the optimum dietary glutamate concentration is 1.16%. The regression model of the FUE (Figure 2) shows that the optimum value is 0.99%. Therefore, the optimum value of dietary glutamate ranges from 0.99 to 1.16%.

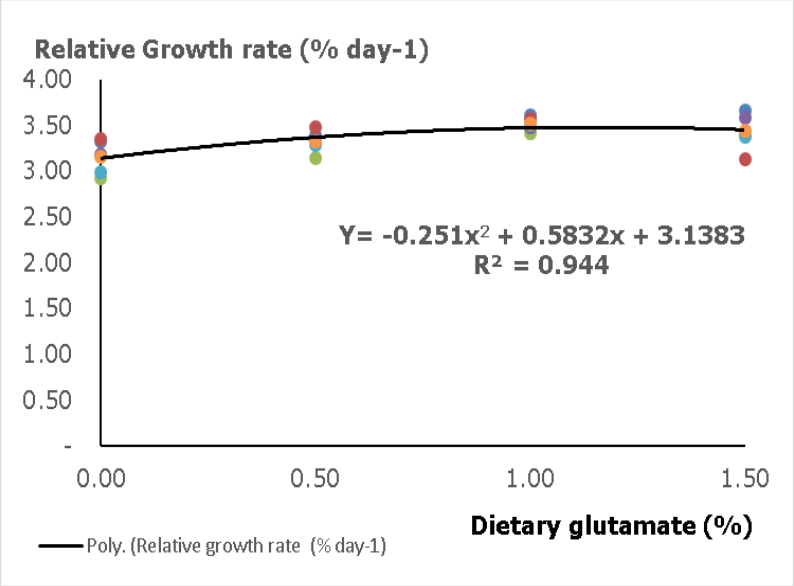

Figure 1. Regression model of the relative growth rate of *Cyprinus carpio* according to the dietary glutamate.

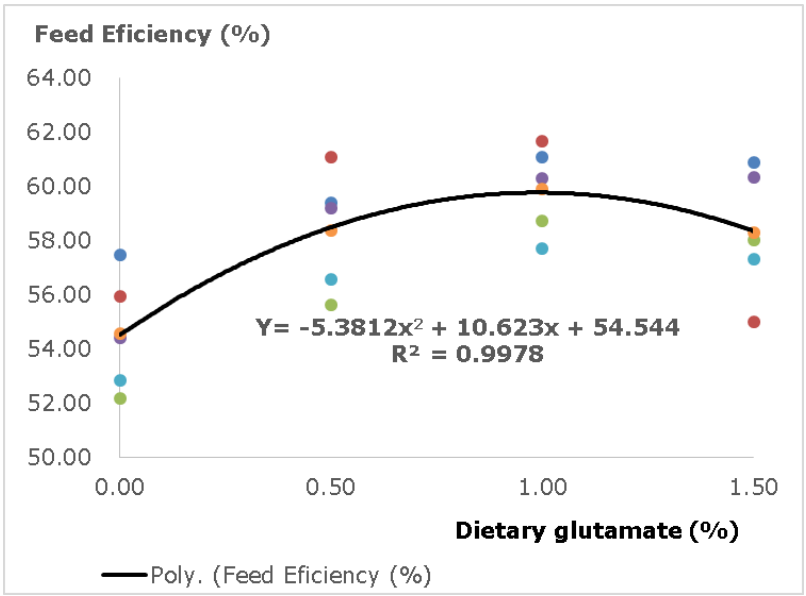

Figure 2. Regression model of feed efficiency in *Cyprinus carpio* according to the dietary glutamate.

Hematological parameters. The hematological variables of *C. carpio* consuming dietary glutamate, consisting of blood glucose, amino transferase and bilirubin are shown in Table 3. The activity of (ALT) and (AST) enzymes was lower in fish that consumed dietary glutamate, as well as direct bilirubin. In contrast, blood glucose concentrations were higher in fish that consumed dietary glutamate.

Table 3

Hematological variables of *Cyrprinua carpio* consuming dietary glutamate

Variables	*Dietary glutamate (%)*			
	0.0	*0.5*	*1.0*	*1.5*
ALT (U L^{-1})	94	12.8	13.1	12.2
AST (U L^{-1})	48.8	39.6	27.7	41.8
Bilirubin total (mg dL^{-1})	0.5	1	1	0.3
Bilirubin direct (mg dL^{-1})	0.4	0.03	0.4	0.1
Bilirubin indirect (mg dL^{-1})	0.1	0.67	0.6	0.2
Blood glucose (mg dL^{-1})	40	118	106	70
ALT (U L^{-1})	94	12.8	13.1	12.2

Water quality. Water quality in aquaculture is the main factor for fish life, which affects the survival, health and growth of fish. The results of measurements of various water quality parameters are presented in Table 4.

Table 4

Water quality values in *Cyprinus carpio* culture fed with various dietary glutamate contain

Variables	*Dietary glutamate (%)*				*References*
	0.0	*0.5*	*1.0*	*1.5*	
Temperature (°C)	28.0±1.5	28.5±1.4	28.0±1.0	28.4±1.5	26-32[(a)]
pH	7.1±0.1	7.1±0.1	7.1±0.1	7.1±0.1	7-9[(b)]
DO (mg L^{-1})	3.3±0.2	3.3±0.1	3.2±0.1	3.2±0.2	>3[(b)]
Ammonia (mg L^{-1})	0.0029±0.0005	0.0025±0.0003	0.0025±0.0002	0.0028±0.0003	<0.1[(c)]

[a)]SNI (1999); [b)]Boyd (1990); [c)]Naeem et al (2016).

Discussion. Feeding with diets containing glutamate of 0.0, 0.5, 1.0 and 1.5% in carp did not affect feed consumption, protein efficiency ratio and fish survival. However, this dietary glutamate supplementation affects feed efficiency (FE) and relative growth rate (RGR) (Table 2). The FE value was significantly improved by dietary glutamate supplementation. The feed efficiency values were 58.39±2.23, 59.91±1.65 and 58.33±2.38% in groups of *C. carpio* fed with glutamate (MSG) in concentrations of 0.5, 1.0 and 1.5%. This value was higher than in the group of fish fed with the control diet (0.0% glutamate), with 54.58±2.17% ($P>0.05$) (Table 2). Other studies have reported positive effects of dietary glutamine supplementation for feed utilization and growth performance in several fish species, for example, in the red drum (*Sciaenops ocellatus*) (Cheng et al 2011) and in gilthead sea bream (*Sparus aurata*) (Coutinho et al 2016). In these fish species, feed efficiency was improved in dietary glutamine supplementation, although growth rate did not increase (Cheng et al 2011; Coutinho et al 2016). Furthermore, final weight, growth rate and feed intake of *Sparus aurata* juveniles were not statistically different among the dietary glutamin treatments (Solares, et al 2015; Coutinho et al 2016). The FUE regression equation (Figure 2) was $Y= -5.3812x2 + 10.623x + 54.544$, with $R^2 = 0.9978$. The optimum dietary glutamate concentration based on FUE regression was estimated at 0.99% and the maximum FUE value was estimated at 59.79%.

Glutamate is an important neurotransmitter in the central nervous system 0f mammals and it also acts as a signaling molecule regulating appetite and digestion (Brosnan & Brosnan 2013; Torii et al 2013). However, a dietary glutamate supplementation up to 1.5%, in this study, did not significantly affect the total feed consumption of *C. carpio*. The use of dietary glutamate plays an important role in the intestinal and systemic metabolism (Zhelyazkov & Stratev 2019). The Relative Growth Rate (RGR) value was significantly improved by a dietary glutamate supplementation. Similar results were found in half-smooth tongue sole (*Cynoglossus semilaevis)* post

larvae: dietary glutamate supplementation can increase growth (Liu et al 2015). The RGR regression equation (1) Figure was $Y= -0.251X^2 + 0.5832X + 3.1383$, with $R^2 = 0.944$. The optimum dietary glutamate concentration was estimated at 1.16%. Therefore, the maximum RGR value was estimated at 3.48% day^{-1}. This study used juvenile *C. carpio* with an initial body weight of 2.55±0.12 g $fish^{-1}$. Whereas optimal dietary free glutamate level based on SGR for *C. semilaevis* (initial body weight 10.64±0.86 g) about 0.63% dry diet (Liu et al 2015). In other research on rainbow trout (*Oncorhynchus mykiss*), the optimum level of dietary glutamate was of 0.5% (Zhelyazkov & Stratev 2019). The mechanism of influence of dietary glutamate supplementation on growth is mediated through an increased activity of the digestive enzymes, an increasing antioxidant capacity and a hypoxic stress resistance (Liu et al 2015).

As a non-essential amino acid, the glutamate becomes an important nutrient during the periods of rapid growth, stress or critical illness. Thus, the consumption of a diet containing glutamate is essential for maintaining the survival of fish (Liu et al 2015; DeBerardinis & Cheng 2010; Lacey & Wilmore 1990). The culture of carp used water with the same quality in each group of dietary glutamate supplementations. Water quality parameters were suitable for life of *C. carpio* (Table 4). Survival values were not significantly different, ranging between 90.00 and 94.00%. Fish survival was not affected by dietary glutamate. Otherwise, dietary glutamin supplementation can increase survival of *C. semilaevis* post larvae (Liu et al 2015). During the experimental period, the fish were kept in an aquarium with continuous aeration and water change system to manage its quality. Water temperature ranged from 28 to 28.5°C, pH from 7.0 to 7.2, the dissolved oxygen content was about 3.3 mg L^{-1} and the ammonia ranged from 0.0025 to 0.0029 mg L^{-1} (Table 4). The water quality parameter values during the study, including temperature, pH, DO and ammonia, are considered suitable for carp rearing (Boyd 1990; SNI 1999; Naeem et al 2016).

Transaminase, also known as aminotransferase, is an enzyme that catalyzes transamination reactions. There are two types of serum transaminase enzymes, namely the serum glutamate oxaloacetate transaminase, which is known as alanine aminotransferase (AST), and alanine aminotransferase (ALT), both produced by the liver. Furthermore, if hematocyte damage occurs, it causes release of AST and ALT into the bloodstream, so that serum ALT and AST increases (Al-Mousawi 2017; Al-Mamary et al 2002; de la Torre et al 2000; Lemaire et al 1991). Additionally, the most important hepatic ALT and AST activities were as degrading enzymes in the amino acid catabolism (Metón et al 1999; Hastuti & Subandiyono 2020b). Results of the present study showed that the highest level of AST in *C. carpio* fed on diet control (0.0% MSG) was of 94 UL^{-1} (Table 3). AST and ALT in *C. carpio* that received dietary glutamate supplementation exhibited a lower activity than in the control (Table 3), which was an indicator of the diet quality. Dietary glutamate supplementation increased the diet quality. The high level of AST was as indicator of malnutrition (low dietary protein level) and responsible for the poor growth performance (Hastuti & Subandiyono 2020b). Additionally, AST was more active than ALT in *C. carpio* and was consistent with the values found in the *O. niloticus* fingerlings (Hastuti & Subandiyono 2020b), and in tiger puffer (*Takifugu rubripes*) juveniles (Kim & Lee 2009).

Dietary glutamate supplementation significantly decreased AST and ALT activity in carp (Table 3). Similarly, a decrease in ALT and AST activity with the dietary glutamate supplementation was found in the catfish (*Clarias gariepinus*) (Ngaddi et al 2019). Therefore, it affects the metabolism of amino acids in the body fish. Furthermore, it affects the growth of *C. carpio* (Table 2). A better growth rate was found in *C. carpio* who received a dietary glutamate supplementation than in the control group. This is due to a better conversion of the feed nutrients and increased the metabolism of experimental fish. Carbohydrate metabolism and digestibility were predicted to be better in *C. carpio* that received a dietary glutamate than in controls. It was seen that the blood glucose concentration increased in *C. carpio* that received dietary glutamate supplementation (Table 3), but it did not affect the value of protein efficiency ratio (PER) (Table 2). Therefore, dietary glutamate supplementation improves the diet quality indicated by the

value of feed utilization efficiency, relative growth rate, blood glucose, hepatic enzymes (ALT and AST) and billirubin.

Billirubin is secreted by the gallbladder and has a function in binding the cholesterol in the lipid digestion, together with the bile. The results of this study (Table 3) showed an increase in the value of the total and indirect bilirubin, according to the dietary glutamate. It shows increasing the digestibility of feed nutrients, especially carbohydrates and lipids. Direct bilirubin values decreased in fish that received dietary glutamate supplementation. That means dietary glutamate decreasing of bilirubin to be excreted together with fish faeces. It is normally released as bile in the feces (Hastuti & Subandiyono 2020a).

Glutamate from the dietary MSG plays a key role in the amino acid metabolism, through its conversion to α-ketoglutarate or other NEAA. These conversions are mediated by different transaminases (ALT and AST) and glutamate dehydrogenase (Brosnan 2000; Brosnan & Brosnan 2009). By the action of these enzymes, carbon skeletons from amino acids can be used to replenish the TCA cycle or can be derived to glucose production, through the gluco-neogenic pathway. Therefore, the blood glucose of *C. carpio* that received dietary glutamate increased significantly. The blood glucose value of *C. carpio* was 40 mg dL^{-1} in the control group (0.0% dietary glutamate) and the value increased to 118, 106 and 70 mg dL^{-1} in the dietary glutamate supplementation group at 0.5, 1.0 and 1.0%, respectively (Table 3).

Conclusions. The results of the study showed that the diet supplementation with glutamate for *C. carpio* increased the feed utilization efficiency and the relative growth rate. Dietary glutamate supplements affected the concentrations of AST, ALT, bilirubin and blood glucose and had no impact on the fish survival. The recommended range of optimal levels of dietary glutamate, based on the ECR and RGR regression, is 0.99 to 1.16%. The maximum value of the feed utilization efficiency and the relative growth rate were 59.79% and 3.48% day^{-1}, respectively.

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

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