# The Efficacy of Organic Zinc Amino Acid Complex (AvailaZn<sup>®</sup>) on Growth Performance and Immunity of Pangasius Catfish (*Pangasianodon hypophthalmus*)

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#### Abstract

Orapint Jintasataporn, Terry Ward, and Supalug Kattakdad. 2014. The Efficacy of Organic Zinc Amino Acid Complex (AvailaZn®) on Growth Performance and Immunity of Pangasius Catfish (Pangasianodon hypophthalmus). Aquacultura Indonesiana, 15 (2): 94-97. The efficacy of zinc amino acid complex (ZnAA; AvailaZn<sup>®</sup>) on growth performance and immunity of Pangasius catfish (Pangasianodon hypophthalmus) was assigned in CRD with 4 treatments and 5 replications per treatment. The treatments were control with supplemental inorganic zinc sulfate (ZnSO4;100 mg/L Zn; T1), two treatments of 100 mg/L zinc at different proportions of ZnSO<sub>4</sub>:ZnAA 70:30 (T2) and 40:60 (T3) and a treatment of only ZnAA at 50 mg/L Zn (T4; 50% of control). Pangasius catfish with average weight of 209 g were stocked in 1,000 L fiber tanks at the density of 15 fish per tank and fed treatment diets 3% of body weight twice daily for three months. The results indicated that there were improved (P < 0.05) growth performance in terms of body weight gain, average daily gain and specific growth rate of Pangasius catfish fed supplemental ZnAA at different levels compared to fish fed supplemental ZnSO<sub>4</sub> (T1). The specific growth rates were 0.89, 1.20, 1.16 and 1.27 % in T1, T2, T3, and T4 respectively. Feed conversion ratio were not significantly different (P=0.08). Protein efficiency ratio in group of fish fed ZnAA(T2,T3,T4) were significantly better (P < 0.05) than inorganicZnSO<sub>4</sub>(T1). White blood cell count, serum protein, immunoglobulin (IgM), hemoglobin, and super oxide anion in fish fed supplemental ZnAA were better (P < 0.05) than fish fed inorganic ZnSO<sub>4</sub> (T1). Therefore, using zinc amino acid complex (AvailaZn<sup>®</sup>) in Pangasius catfish diets can enhance catfish growth performance (P<0.05) and supplemental zinc amino acid complex (Availa $Zn^{\text{(B)}}$ ) level in the catfish diet may be decreased by up to 50% of supplemental inorganic zinc levels. In conclusively, supplementation zinc amino acid complex (AvailaZn®) in Pangasius catfish diet is recommended at the level of 50 mg/L for ZnAA as the sole supplemental Zn source or 100 mg/L Zn in 70:30 proportion of ZnSO<sub>4</sub>:ZnAA.

*Keywords* : Growth performance; Immunity; Organic zinc; Pangasius catfish; Striped catfish; Zinc amino acid complex

#### Introduction

Zinc is a mineral that is required by animals for a number of biochemical processes. The function of zinc is related to growth performance: structure, nuclear transcription factors via different DNA binding proteins. Vitamin A, Vitamin D, steroid hormones, insulinlike growth factor-1, growth hormone, and immunity via all functions within monocytes, cytotoxicity in natural killer cells, phagocytosis in neutrophils, normal function of T-cells, B cells undergoing apoptosis, etc. Aqua species fed zinc deficient diets show decreased growth rate, anorexia, lower serum zinc, and decreased bone zinc and calcium deposition. In aquafeed, inorganic forms of zinc, such as zinc carbonate, zinc sulfate, and zinc oxide are routinely supplemented but the absorption of zinc is around 15% (National Research Council, 2011). The organic mineral, zinc amino acid complex, has been shown to have a higher absorption rate in animal intestine than inorganic forms of zinc (Ashmead, 1992). Hardy and Shearer (1985) found that feeding a zinc-amino acid chelate resulted in greater zinc deposition in body tissues than zinc sulfate or zinc sulfate EDTA in low calcium-phosphorus diets but not in high calcium-phosphorus diets. Gomes and Kaushik (1993) replaced inorganic zinc with ZnMet in rainbow trout diets containing vegetable or animal proteins and found no difference in zinc concentrations in plasma, whole body or viscera between fish fed the two sources of zinc. However, Paripatananont and Lovell (1995) reported increased relative bioavailability of ZnMet to ZnSO4 of over 500% in channel catfish based on bone zinc concentrations. There is no information on the effects of organic and inorganic sources of dietary zinc on growth and immunity in Pangasius catfish (Pangasianodon hypophthalmus). The objectives of the study are to evaluate the effect of zinc amino acid complex

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on growth performance, and immunity in Pangasius catfish.

### **Materials and Methods**

## Trial design

The experiment was conducted in a completely randomized design (CRD) by varying the level of zinc amino acid complex. The organic mineral, zinc amino acid complex (AvailaZn<sup>®</sup>), 12% Zn from Zinpro Corporation, USA was used in this experiment. The study used 4 treatments with 5 replications per treatment as follows.

Trt	Mineral type	Remark
1	Control = inorganic (ZnSO <sub>4</sub> ; 100 mg/L Zn)	1X
2	Availa- $Zn^{\otimes}30 = ZnSO_4$ , 70 mg/L Zn +Availa Zn <sup>®</sup> , 30 mg/L Zn	1X
3	Availa- $Zn^{\otimes}60 = ZnSO_4$ , 40 mg/L Zn + Availa Zn <sup><math>\otimes</math></sup> , 60 mg/L Zn	1X
4	Availa-Zn <sup>®</sup> 50 = Availa-Zn <sup>®</sup> , 50 mg/L	0.5X

# **Diet Preparation**

The test diet was formulated based on the catfish nutrient requirements (Paripatananont, 2002; NRC, 2011). Four isonitrogenous diets of 31.5% CP, 7.5% lipid and 4.5% fiber were pelleted for this study. Feed was composed of 5% fishmeal, 32% soybean meal, 5% rapeseed, 25% wheat flour. 10% ricebran, 10% soyprotein concentrate, 2% fish oil, 5% soya oil, 1% vitamin premix, dicalcium phosphate and 5% mineral premix without zinc. Each treatment was supplemented inorganic ZnSO<sub>4</sub> and zinc amino acid complex at different proportions as designated above. The ingredients were collected from commercial sources. All materials were grounded and passed through a 500 µm screen.

All dry feed ingredients were well mixed for 15 min, whereafter oil was gradually added while mixing constantly. Water was slowly added and then entire diet was extruded. Floating pellets of 5 mm were dried at 65°C and stored in plastic bags at room temperature until fed. The diets were determined feed compositions by proximat analysis follow methods of AOAC (2000)

# Experimental conditions

The experiment was carried out in 20 tanks with 1,000 L capacity. Tanks were filled with 700 L of freshwater then stocked with juvenile pangasius catfish 15 ind./tank, approx size of 209 g. Fish were fed the treatment diets 3 times per day at 3-4% of body weight for 12 weeks. Aeration was applied to all experimental units to maintain DO >5 mg/L in a closed system.

## Data collection

At the end of 12 weeks, growth performance was recorded on live weight gain, specific growth rate, survival rate, feed conversion ratio (feed consumption/fish production) and protein efficiciency ratio. Glycogen was determined following Dubois *et al.* (1956)

Fish immunity in terms of red blood cell count, white blood cell count, hemoglobin, total protein (Lowry *et al.*, 1951), immunoglobulin (IgM), superoxide anion and glutathione activity were determined follow method of Braxhall and Daisley (1973).

# Statistical Analysis

This study was conducted in completely randomized design (CRD). All data was analyzed by one-way ANOVA (analysis of variance). The Duncan's Multiple Range Test was used to determine the differences between the treatment means. The alphabetical notation was used to mark the differences at significant level of an alpha 0.05. All research was conducted at the Nutrition and Aquafeed Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

### **Results and Discussion**

The efficacy of zinc amino acid complex AvailaZn<sup>®</sup>) (ZnAA: on performance of marketable Pangasius Catfish size (Pangasianodon hypophthalmus) was conducted to evaluate the growth performance, feed utilization and immune status. The different proportion of inorganic ZnSO<sub>4</sub> and organic ZnAA was assigned to 100:0, 70:30, 40:60 and 0:50. The results as following:

### Growth performance

The effect of zinc amino acid complex (ZnAA; AvailaZn<sup>®</sup>) on growth performance and feed utilization is presented in Table 1. The results show that after 12 weeks of feeding trial, the weight gain and average daily gain were significantly difference ( $P \le 0.05$ ). Fish in group of inorganic Zn :organic ZnAA, 70:30, 40:60 and

0:50 showed the improved growth performance compared with fish fed inorganic ZnSO<sub>4</sub>. These related to the amount of feed consumption which was decreased in fish fed the inorganic ZnSO<sub>4</sub>. The reduction of feed consumption caused the low growth performance in the inorganic ZnSO<sub>4</sub> group ( $P \le 0.05$ ) whereas fish fed organic ZnAA at different levels had high growth performance (P < 0.05). The feed conversion ratio and survival rate were not significantly different (P>0.05). After 12 weeks of feeding trial, feed conversion ratio tended to decrease in group of fish fed organic ZnAA (P=0.08) and the protein efficiency ratio in fish fed organic ZnAA at different levels had higher  $(P \le 0.05)$  than inorganic ZnSO<sub>4</sub> group. These means the higher level of zinc amino acid complex (AvailaZn<sup>®</sup>) can promote growth performance and feed utilization.

#### Immune status

The immune status in term of red blood cell count, white blood cell count, hemoglobin, total protein, immunoglobulin (IgM), superoxide anion and glutathione in Table 2 showed significantly difference ( $P \le 0.05$ ). After 12 weeks of feeding trial, fish fed diets containing zinc amino acid complex (ZnAA; AvailaZn<sup>®</sup>) showed better immune status than fish fed diets

containing the inorganic ZnSO<sub>4</sub> in control group. For the superoxide anion which is the residule after oxidation, catfish fed organic ZAA had lower concentrations (P < 0.05) than fish fed the inorganic ZnSO<sub>4</sub>. Due to during the metabolism, animal tissues produce a lot of free radicals. The fish that has more free radical scavenging enzymes like super oxide dismutase, peroxidase, catalase and glutathione activity in animal tissues, can exhibit the more efficacy to reduce free radicals hence the decrease in the amount of super oxide anion. The high level of superoxide anion may be caused by pathogen infection or stress. This may imply that the free radical scavenging activity to reduce superoxide anion in catfish fed organic ZAA is more effective than inorganic ZnSO<sub>4</sub>. Glycogen level in fish fed  $ZnSO_4$  was higher level (P<0.05) than in fish fed ZnAA. The function of zinc in the body is accelerated during protein synthesis which thus reducing requires energy glycogen accumulation in the liver (O'Dell et al., 1987; Scarpa and Gatlin, 1992; Hidalgo et al., 2002; Marcelo et al., 2004). Therefore, supplemental zinc amino acid complex (AvailaZn<sup>®</sup>) can enhance fish immunity especially hematology parameter, immunoglobulin and free radical scavenging enzyme activity.

Table 1. Growth performance and feed utilization of Pangasius catfish (Pangasianodon hypophthalmus)fed different levels of zinc amino acid complex: AvailaZn<sup>®</sup> (mean<u>+</u>SD)

		Inorg 100	Inorg 70 mg/L+ AvailaZn <sup>®</sup> 30	Inorg 40 mg/L+ AvailaZn® 60	AvailaZn®	
Growth performance	Unit	mg/L	mg/L	mg/L	50 mg/L	P-value
Initial weight	g	$209.50^{a}$	209.59 <sup>a</sup>	209.36 <sup>a</sup>	209.61 <sup>a</sup>	1.000
Final weight	g	331.69 <sup>b</sup>	396.66 <sup>a</sup>	388.27 <sup>a</sup>	411.21 <sup>a</sup>	0.0337
Weight gain	g,	122.20 <sup>b</sup>	187.07 <sup>a</sup>	178.92 <sup>a</sup>	201.60 <sup>a</sup>	0.0337
Average daily weight gain	g/f/d	2.18 <sup>b</sup>	3.34 <sup>a</sup>	3.19 <sup>a</sup>	3.60 <sup>a</sup>	0.0038
Specific growth rate	%/d	$0.89^{b}$	$1.20^{a}$	$1.16^{a}$	$1.27^{a}$	0.0290
Feed consumed	(g/ind)	222.2 <sup>b</sup>	311.8 <sup>a</sup>	254.0 <sup>b</sup>	320.0 <sup>a</sup>	0.0001
Daily feed consumed	(g/ind/d)	2.65 <sup>b</sup>	3.71 <sup>a</sup>	3.02 <sup>b</sup>	3.81 <sup>a</sup>	0.0001
Feed conversion ratio		1.83 <sup>a</sup>	$1.68^{a}$	1.49 <sup>a</sup>	1.59 <sup>a</sup>	0.0897
Protein efficiency ratio		1.77 <sup>b</sup>	$1.98^{ab}$	$2.30^{a}$	2.06 <sup>ab</sup>	0.0354
Survival rate	(%)	100.0 <sup>a</sup>	$98.7^{\mathrm{a}}$	$98.7^{\mathrm{a}}$	98.7 <sup>a</sup>	0.8014

Means within the same row with different superscripts differ ( $P \le 0.05$ )

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Immune parameter	Unit	Inorg 100	Inorg 70	Inorg 40		P-value
L.		mg/L	mg/L+	mg/L+	AvailaZn <sup>®</sup>	
		-	AvailaZn®	AvailaZn <sup>®</sup> 60	50 mg/L	
			30 mg/L	mg/L		
Red blood cell count	X10 <sup>6</sup> cells/mL	2.24 <sup>d</sup>	3.17 <sup>b</sup>	3.00 <sup>c</sup>	3.39 <sup>a</sup>	0.0001
White blood cell count	X10 <sup>4</sup> cells/mL	3.14 <sup>c</sup>	$3.72^{a}$	3.31 <sup>b</sup>	3.71 <sup>a</sup>	0.0001
Hemoglobin	g/dL	3.53 <sup>c</sup>	$4.80^{a}$	4.51 <sup>b</sup>	4.46 <sup>b</sup>	0.0001
Total protein	g/dL	8.15 <sup>c</sup>	9.82 <sup>a</sup>	8.36 <sup>c</sup>	9.37 <sup>b</sup>	0.0001
Immunoglobulin M	g/L	0.37 <sup>c</sup>	$0.56^{a}$	0.46 <sup>b</sup>	$0.56^{a}$	0.0001
Super oxide anion	OD	$0.08^{\mathrm{a}}$	0.03 <sup>b</sup>	0.03 <sup>b</sup>	$0.02^{b}$	0.0001
	nmol/mg					
Glutathione activity	protein	$4.58^{\mathrm{a}}$	$4.79^{a}$	5.39 <sup>a</sup>	3.64 <sup>b</sup>	0.0038
	mg glucose/g					
Glycogen	tissue	118.32 <sup>a</sup>	50.25 <sup>b</sup>	54.99 <sup>b</sup>	45.74 <sup>b</sup>	0.0004

 Table 2. Immune status of Pangasius catfish (*Pangasianodon hypophthalmus*) fed different levels of zinc amino acid complex: AvailaZn® (mean+SD)

Mean values within the same row with different superscripts were significantly different ( $P \le 0.05$ )

In conclusion, using zinc amino acid complex (AvailaZn<sup>®</sup>) in Pangasius catfish diet can enhance growth performance (P < 0.05) immunity and decrease zinc level in the catfish diet by 50% of using inorganic zinc. Supplementation of zinc amino acid complex (AvailaZn<sup>®</sup>) in Pangasius catfish diet is recommended at the level of 50 mg/L when using zinc amino acid complex (AvailaZn<sup>®</sup>) as the sole supplemental zinc source. When inorganic zinc sulfate and zinc amino acid complex (AvailaZn<sup>®</sup>) are used as the supplemental zinc sources 100 mg/L Zn is recommended in the proportion of ZnSO<sub>4</sub>:ZnAA of 70:30.

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