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Effects of yeast nucleotide on growth performance, serum immune index and muscle composition of *Ancherythroculter nigrocauda* Yih & Wu

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Abstract

A 50-day feeding trial was conducted to evaluate the effects of yeast nucleotide in diets on growth performance, serum immune indices and muscle composition of *Ancherythroculter nigrocauda* (mean initial body weight, 23.30±0.59 g). Seven isonitrogenous (approximately 42.76% crude protein) and isoenergetic (17.43 KJ g^{-1} gross energy) experimental diets with varying levels of yeast nucleotide (0[control], 150, 300, 450, 600, 750 and 900 mg kg^{-1}) were fed near to satiation to triplicate groups of fish. The results showed that the highest weight gain ratio (WGR), specific growth rate (SGR), protein efficiency ratio (PER), and best feed conversion ratio (FCR) were evident in fish fed 450-600 mg kg^{-1} yeast nucleotide diet. The intramuscular protein and fat contents of data also supported the above level. The serum enzymes showed that activities of lysozyme (LZS) and superoxide dismutase (SOD) in fish increased first and decreased afterwards with the dietary nucleotide supplemental level increasing. The LZS activity in serum was found to be significantly ($p<0.05$) greater in fish fed yeast nucleotides at 450-750 mg kg^{-1}. The fish fed the diet with 600 mg kg^{-1} yeast nucleotide had higher SOD level ($p<0.05$). Yeast nucleotides supplementation did not significantly influence acid phosphatase (ACP) activity of fish ($p>0.05$). Alkaline phosphatase (ALP) activity showed significantly ($p<0.05$) greater but continuous decrease with the increase in the levels of dietary yeast nucleotide. We therefore recommend dietary yeast nucleotide administration at (450 – 600 mg kg^{-1}) to promote growth, enhance immunity and intramuscular protein and fat content.

Keywords: *Ancherythroculter nigrocauda*, Yeast nucleotide, Growth performance, Serum immune index, Muscle composition

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Introduction

Nucleotides are an important component of genetic material DNA and RNA. It is a necessary intermediate in the protein synthesis, and it also plays an important role in the cellular structure, metabolism, energy, and regulation of physiological function. There are three ways that an animal can obtain the required nucleotides. These include recycling from dead cells known as the salvage pathway, direct *de novo* synthesis from amino acids, or through the diet (Quan *et al*., 1990). Although initial efforts in evaluation of dietary supplementation of nucleotides for fishes could be traced to the early 1970s, research at that time mainly focused on the possible chemo-attractive effects of these compounds (Mackie, 1973; Kiyohara *et al*., 1975; Mackie and Adron, 1978). However, research into potential growth and health benefits of dietary nucleotides in aquaculture species did not begin until the early 2000s.

In aquaculture, nucleotides were found to improve growth and immunity, particularly under stress conditions, in Atlantic salmon (*Salmo salar*) (Burrells *et al*., 2001a,b), red drum (*Sciaenops ocellatus*) (Li *et al*., 2007a) and grouper (*Epinephilus malabaricus*) (Lin *et al*., 2009) To date, numerous studies with several different aquatic species have reported that dietary nucleotides can enhance growth performance (Adamek *et al*., 1996; Cosgrove, 1998; Carver, 1999), immune responses (Ramadan *et al*., 1994; Sakai *et al*., 2001; Leonardi *et al*., 2003; Li *et al*., 2007b), disease resistance (Irianto and Austin, 2002) and even gastrointestinal physiology and morphology (Borda *et al*., 2003).

A. nigrocauda as an important aquaculture species, is a cyprinid fish endemic to the upper reaches of the Yangtze River (Chen, 1998). Unfortunately, due to long term overfishing, water pollution and habitat degradation, its habitat was reduced by approximately 25 % after the completion of the Three Gorges Reservoir (Eiadcas and Ripywr., 1995). The natural population of the species has decreased noticeably since the early 1990s. Fortunately, cultivation thereof has only recently been developed because the local populace prefer wild-caught products. With the success of captive breeding in *A. nigrocaudas*, large-scale, intensive breeding farms have developed rapidly. Most current researches on *A. nigrocauda* have focused on culture in Hubei Province of China (Zeng *et al*., 2010), carcass composition and embryonic development of *A. nigrocauda* (Tan *et al*., 2004a; Yin and Lu, 2010), and some studies focused on artificial propagation, and digestive enzyme of this species (Tan *et al*., 2004b; Bai *et al*., 2007), but little information is available on its growth performance and the increased immunity which might be stimulated by immunostimulants. Therefore, this research investigated the effects of yeast nucleotide on *A. nigrocauda* in terms of: growth performance, muscle composition, and the effects of yeast nucleotide on some serum immune parameters.

Materials and methods

Fish and experimental conditions

Six hundred and thirty healthy *A. nigrocauda* were obtained from Yangqiao Fingerling Station (in Luzhou's research workstations of The Institute of Hydrobiology, Chinese Academy of Sciences). The fish were transported to the laboratory in Henan University of Technology, acclimated to laboratory conditions and fed the basal experimental diet without supplement nucleotides for two weeks. At the end of the acclimation period, fish with an average weight of approximately 23.30 g were randomly selected and stocked in 21 1000-L tanks (triplicate groups per dietary treatment) at a density of 30 fish $tank^{-1}$. The aerated tap water was used in this study and its quality parameters were maintained as follows: pH between 7.2 and 7.4, dissolved oxygen 6.5-7.0 mg l^{-1}, temperature 22.5 - 23.5 °C and a 12L:12D photoperiod. Micro water flow was maintained during the trial and 30 % of the water in the tank was exchanged every day.

Feed and feeding

Using fish meal as protein sources, fish oil and corn oil as lipid sources, and dextrin as the main carbo fuel, the basal diet was formulated to contain approximately 42.76% crude protein and 17.43 KJ g^{-1} gross energy (Table 1). The basal diet was used as the control diet. Out of the control diet, six diets containing different gradient levels of yeast nucleotide (150, 300, 450, 600, 750 and 900 mg kg^{-1}) were considered. The yeast nucleotide at a purity of 99%, derived from the cell of yeast, *Saccharomyces cerevisiae*, was provided by Nanjing Master Biotechnology Co. Ltd (Nanjing, China). This product contained adenosine monophosphate, inosine monophosphate, guanosine monophosphate, cytidine monophosphate, uridine monophosphate and ribonucleic acid (RNA). All the ingredients were ground into a fine powder through a 120 mm mesh and were thoroughly mixed with fish oil, corn oil, and water was added to produce a stiff dough. The dough was then pelleted with an experimental feed mill (SLX–80, SLX-80, Luoyang Foodstuff Machinery Factory, Luoyang City, Henan Province, China). After air-drying at 60°C for 12 h, the diets were broken up and sieved into the appropriate pellet size and were stored at –20 °C until used. Fish were fed by hand to apparent satiation two times per day (at hours: 08:30 and 17:30) with one of the seven experimental diets over 50 days (Ahmad *et al*., 2011). Troughs were siphoned off to remove fecal matter before daily feeding. Any uneaten feed was siphoned off immediately 1.5 h of feeding time, dried in a hot air oven and reweighed to measure the amount of feed consumed.

Table 1: Ingredients composition of tested diets fed by *Ancherythroculter nigrocauda*.

	Yeast nucleotide level (mg kg^{-1})						
	0	**150**	**300**	**450**	**600**	**750**	**900**
Ingredients (% dry weight)							
Fish meal[1]	66.29	66.29	66.29	66.29	66.29	66.29	66.29
Fish oil	2.16	2.16	2.16	2.16	2.16	2.16	2.16
Corn oil	2.16	2.16	2.16	2.16	2.16	2.16	2.16
Choline chloride (50%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Dextrin	14.46	14.46	14.46	14.46	14.46	14.46	14.46
Vitamin premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[3]	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Amino acid premix[5]	1.50	1.50	1.50	1.50	1.50	1.50	1.50
α-cellulose	8.43	8.415	8.400	8.385	8.370	8.355	8.340
Adhesives	1.00	1.00	1.00	1.00	1.00	1.00	1.00
yeast nucleotide[4]	0	0.015	0.030	0.045	0.060	0.075	0.090
Analyzed proximate composition (% dry weight, N=3)							
Dry matter (%)	89.39	89.39	89.39	89.39	89.39	89.39	89.39
Crude protein (%)	42.76	42.76	42.76	42.76	42.76	42.76	42.76
Crude lipid (%)	9.61	9.61	9.61	9.61	9.61	9.61	9.61
Ash (%)	12.86	12.86	12.86	12.86	12.86	12.86	12.86
Gross energy (KJ·g^{-1} diet)	17.43	17.43	17.43	17.43	17.43	17.43	17.43

[1] Fish meal (dry weight, %): protein 73.78, crude lipid 9.20

[2] Vitamin provides for per kg diet: VA 5 000 IU, VD1 000 IU, VE 30 IU, VK 2.5 mg, VB_1 5 mg, VB_2 8 mg, VB_6 7 mg, VB_{12} 0.01 mg; niacin 30 mg; pantothenic acid 25 mg; folic acid 0.5 mg; biotin 0.2 mg; VC 35 mg; inositol 50 mg; choline chloride 700 mg.

[3] Mineral provides for per kg diet: Mn 10 mg, Zn 30 mg, Fe 60 mg, Cu 3 mg, I 1 mg, Se 0.2 mg.

[4] Nanjing Master Biotechnology Co. Ltd (Nanjing, China).

[5] Amino acid premix (g 100g^{-1} diet): Lysine, 0.25; Isoleucine, 0.25; Leucine, 0.30; Methionine, 0.20; Threonine, 0.20; Valine, 0.25; Phenylalanine, 0.1; Arginine, 0.40.

Growth and feed utilization

All fish in the different experimental groups were weighed at the end of the 50 day feeding trial for estimation of growth. Based on the recorded weight and feed intake of each fish, WGR, SGR, PER and FCR were calculated for each group according to Cho (1992) as follows:

Weight gain ratio (WGR, %)=$(W_t - W_0)\times 100/ W_0$;

Specific growth ratio (SGR, %/d)=$(\mathrm{Ln}W_t - \mathrm{Ln}W_0)\times 100/t$;

Protein efficiency ratio (PER, %)=$(W_t - W_0) \times 100/F\times P$;

Feed conversion ratio (FCR)=$F/(W_t - W_0)$.

W_0 is the initial body mass (g), W_t the final body mass (g), F the feed intake (g), and t the time in days (d).

Functional immune assay

Following the feeding trial, after being fasted for 24 h, blood samples were collected from the caudal vein of six fish per tank with a 27-gauge needle and 1 ml syringe, and centrifuged at 3500×g 4 °C for 15 min after being maintained at 4 °C for 12 h. The serum was frozen at −80°C until used. The LZS activity, SOD activity, ACP activity and ALP activity were determined using a LZS, SOD, ACP, ALP detection kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), respectively. Results were expressed in units of LZS ml^{-1}. The reaction was carried out and absorbance was measured at 530 nm after 0.5 and 4.5 min in a spectrophotometer at room temperature. One unit is defined as the amount of sample causing a decrease in absorbance of 0.001 min^{-1} at 530 nm compared to the control.

SOD activity unit was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome c reduction rate measured at 550 nm. Enzyme activity was expressed as units per ml serum (U ml^{-1}). ACP activity unit is defined as per 100 ml of serum at 37°C with the substrate for 60 min, resulting in l mg phenol as a unit of enzyme activity; the ALP activity unit is defined as 15 min per 100 ml of serum at 37°C and substrate effects, produce l mg of phenol by a unit of enzyme activity.

Muscle composition

At the end of the experiment, three subsamples of each replicate (n=3×3) were pooled separately and analyzed for final muscle composition. Muscle tissue samples were taken from behind the head and above the lateral line, within 5 min of slaughter, snap frozen in liquid nitrogen and stored at −80°C until further analysis. The analysis methods of crude protein, moisture, and ash contents were as described by the AOAC (1995). Moisture contents were determined by oven-drying at 105°C for 24 h. Crude protein (N×6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid contents were determined by ether-extraction using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). The ash contents were determined by gravimetric analysis after 24 h in a muffle furnace at 550°C. Gross energy contents were analyzed using an adiabatic bomb calorimeter (Parr; Moline, IL, USA).

Statistical analysis

All data were analyzed by SPSS 17.0 for windows. One-way analysis of variance was used to determine whether significant differences existed between the treatments. When overall differences were found, differences between means were tested by Duncan multiple range test. All differences were considered significant at $p<0.05$ and the results are presented as means with pooled standard error of the mean.

Results

Growth performances

After 50 days of trial, final weight, SGR, PER and FCR of *A. nigrocauda* were significantly different when fed with 300, 450, 600, 750 and 900 mg kg^{-1} diets ($p<0.05$) and fish fed the diet with yeast nucleotide had significantly higher WGR compared with the control group ($p<0.05$) (Table 2). The maximum final weight (41.10 g), WGR (74.31%), SGR (1.11%), PER (1.75%), and best FCR (1.33), were obtained for the fish fed diet with 450 mg kg^{-1} yeast nucleotide. Fish fed lower levels of yeast nucleotide in the diet exhibited significantly lower ($p<0.05$) growth and reduced feed utilization efficiency. However, the poorest final weight (37.52 g), SGR (0.94%), PER (1.49%) and FCR (1.60) in fish fed the diet with 150 mg kg^{-1} yeast nucleotide were not significantly different from the control group ($p>0.05$).

Table 2: Effect of different yeast nucleotide levels on growth performance of *Ancherythroculter nigrocauda.*

Dietary yeast nucleotide (mg kg^{-1})	Initial weight (g)	Final weight (g)	WGR (%)	SGR (%)	PER (%)	FCR
0	23.64±0.29	36.89±0.53^{d}	56.05±1.67^{d}	0.89±0.02^{b}	1.45±0.07^{c}	1.67±0.04^{a}
150	23.42±0.38	37.52±0.43^{d}	60.12±1.40^{c}	0.94±0.05^{b}	1.49±0.07^{bc}	1.60±0.08^{a}
300	23.49±0.33	39.68±0.80^{c}	67.91±1.85^{b}	1.04±0.07^{a}	1.61±0.09^{ab}	1.36±0.09^{c}
450	23.58±0.23	41.10±0.47^{a}	74.31±1.37^{a}	1.11±0.04^{a}	1.75±0.08^{a}	1.33±0.03^{c}
600	23.57±0.23	40.79±0.48^{ab}	73.07±1.17^{a}	1.10±0.03^{a}	1.72±0.09^{a}	1.40±0.07^{bc}
750	23.63±0.29	39.96±0.67^{bc}	69.34±0.81^{b}	1.07±0.01^{a}	1.65±0.07^{a}	1.46±0.02^{b}
900	23.57±0.26	39.91±0.42^{bc}	69.24±1.79^{b}	1.05±0.02^{a}	1.64±0.11^{ab}	1.47±0.02^{b}

Note: Values are mean±S.E. of three replicate groups. Mean values with different superscripts are significantly different from each other (Significance level is defined as $p<0.05$).
WGR: weight gain ratio, SGR: specific growth rate, PER: protein efficiency ratio, FCR: feed conversion ratio.

Serum enzyme activity

LZS activity

The serum LZS activity of fish fed diets with 450， 600 and 750 mg kg^{-1} were significantly higher than those of fish fed 150, 300 and 900 mg kg^{-1} diets and control diet after 50 d (Table 3).

SOD activity

The SOD activity in the serum was found to be significantly ($p<0.05$) greater in fish fed diets with 600 mg kg^{-1} compared with those of other experimental groups (Table 3).

ACP activity

At the end of the trial there were no significant differences in ACP activity among groups ($p>0.05$) (Table 3).

ALP activity

The ALP activity showed a continuous decrease with the increase in the levels of dietary yeast nucleotide (Table 3). Fish fed diets containing 150, 300, 450, 600 and 750 mg kg^{-1} yeast nucleotide diet did not show any significant change ($p>0.05$) in ALP activity. However, significant falls in ALP activity was recorded in fish fed diet containing 900 mg kg^{-1} yeast nucleotide and in the control group ($p<0.05$).

Table 3: Effect of different yeast nucleotide level on Immunological index of *Ancherythroculter nigrocauda*.

Dietary yeast nucleotide (mg kg^{-1})	LZS(U/ml)	SOD (U/ml)	ACP (U/mg)	ALP (U/mg)
0	0.56±0.10^{d}	56.57±2.22^{e}	3.50±0.14	2.32±0.07^{bc}
150	0.59±0.08^{d}	59.55±0.41^{d}	3.56±0.09	2.45±0.08^{a}
300	0.79±0.08^{c}	68.83±1.51^{c}	3.68±0.12	2.44±0.06^{ab}
450	0.99±0.10^{ab}	76.23±0.24^{b}	3.66±0.12	2.41±0.08^{abc}
600	1.12±0.06^{a}	79.02±0.57^{a}	3.62±0.11	2.39±0.04^{abc}
750	1.03±0.14^{ab}	75.72±0.31^{b}	3.63±0.11	2.38±0.06^{abc}
900	0.89±0.07^{bc}	74.35±0.38^{b}	3.62±0.11	2.3±0.06^{c}

Note: Values are mean ± S.E. of three replicate groups. Mean values with different superscripts are significantly different from each other (Significance level is defined as $p<0.05$).
LZS: Lysozyme, SOD: superoxide dismutase, ACP: acidic phosphatase activity, ALP: alkaline phosphatase.

Nutritional composition in muscle

The muscle composition of *A. nigrocauda* fed diets containing different levels of yeast nucleotide are presented in Table 4. The muscle composition showed that activities of crude protein and crude lipid in fish increased in 150, 300 and 450 mg kg^{-1} and then decreased afterwards with the increasing dietary yeast nucleotide supplemental level. The maximum muscle protein content was recorded at the 450 mg kg^{-1} yeast nucleotide level. The protein content of fish fed 450 mg kg^{-1} dietary yeast nucleotide level was not found to be significantly ($p>0.05$) higher compared to those fed other dietary levels except for the control. A similar trend was evident for crude lipid. No significant differences were observed in moisture and ash between the different dietary treatments ($p>0.05$).

Table 4: Effects of yeast nucleotide on muscle composition of *Ancherythroculter nigrocauda*.

Dietary yeast nucleotide (mg kg^{-1})	Crude protein	Crude lipid	Crude Ash	Moisture
0	15.57±0.39^{b}	2.46±0.32^{b}	1.39±0.10	78.26±0.42
150	16.32±0.41^{ab}	2.65±0.42^{ab}	1.43±0.17	78.17±0.32
300	16.63±0.77^{ab}	2.84±0.37^{ab}	1.54±0.24	78.03±0.39
450	16.96±0.52^{a}	3.12±0.09^{a}	1.76±0.28	77.94±0.55
600	16.74±0.63^{a}	3.08±0.19^{a}	1.69±0.31	78.16±0.30
750	16.53±0.68^{ab}	2.99±0.31^{ab}	1.53±0.48	78.30±0.50
900	16.53±0.55^{ab}	2.86±0.24^{ab}	1.42±0.12	78.33±0.17

Note: Values are mean±S.E. of three replicate groups. Mean values with different superscripts are significantly different from each other (Significance level is defined as $p<0.05$.)

Discussion

Results of the present study showed that the *A. nigrocauda* fed diets supplemented with yeast nucleotides exhibited improvement in growth and food utilization compared to those fed the basic diet. Feeding of 450- 600 mg yeast nucleotide kg^{-1} resulted in better growth performance and PER, and best FCR were also obtained for the fish fed diet with 300-600 mg yeast nucleotide kg^{-1} compared to other experimental groups, suggesting that supplementation of yeast nucleotide at 450-600 mg kg^{-1} was optimal for the growth of *A. nigrocauda*. Similarly, a growth-promoting effect was noted in grouper (*Epinephelus malabaricus*) fed nucleotides at level of 1.5 g mixed-nucleotides kg^{-1} (Lin *et al*., 2009). Burrells *et al*. (2001b) also reported that dietary supplementation of 0.25% exerted a positive effect on weight gain of Atlantic salmon (*Salmo salar*) after 8 weeks of feeding. Although the administration of dietary nucleotides has been reported to enhance the growth performance of cultured species such as tilapia (Ramadan *et al.*, 1991), red drum red drum (*Sciaenops ocellatus*) (Li *et al*., 2007a) and white shrimp *Litopenaeus vannamei* (Boone) (Li *et al*., 2007b), the growth promoting effect of nucleotides has not been observed in hybrid striped bass *Morone chrysops*× *M. saxatilis* (Li and Gatlin, 2004) and juvenile red drum (*Sciaenops ocellatus*) (Li *et al*., 2005). It seems that growth enhancing effect of nucleotides may vary in different species and depends on feeding period, life stages and the type of nucleotide

supplement (Li and Gatlin, 2006). Borda *et al*. (2003) presumed that an exogenous supply of nucleotides may promote growth of fish and crustaceans in early stages to meet their high rate of cell replication. Based on current knowledge, the mechanism of growth-promotion by dietary nucleotides remains to be identified in fish. Anyhow, there is no exact explanation on how nucleotides work to enhance growth rate.

LZS is one of the earliest known antibacterial proteins, and being an enzyme with antimicrobial activity, it can split peptidoglycan in bacterial cell walls, especially in gram positive species, and can cause lysis of the cells (Chipman and Sharon, 1969). Sakai *et al*. (2001) showed that yeast nucleotides could increase serum LZS activity of common carp (*Cyprinus carpio* L.). Similarly, rainbow trout (*O. mykiss*) fed diets containing nucleotides at doses of >0.5 g kg^{-1} for 8-week showed increased LZS activity (Ahmad *et al*., 2011). In the present study, *A. nigrocauda* fed diets containing yeast nucleotides at doses of 150-900 mg kg^{-1} for 50 days showed increased LZS activity. The facts suggest that increased LZS activity may be attributed to the supplemented nucleotides, confirming the benefit for the non-specific innate immunity of this fish.

SOD is one of the critical antioxidant enzymes in the body, which can catalyze the reaction of super anion transforming to H_2O_2 and O_2, so it can scavenge the super anion in the tissue. Therefore, it plays an important role in the self defense system of living bodies and it also possesses a vital function in the immune system (Lin *et al*., 2011). Common carp fed diets containing nucleotides showed increased superoxide dismutase activity (Sakai *et al*., 2001). Mo *et al*. (2013) also reported that dietary supplementation of 0.3g kg^{-1} exerted a positive effect on SOD activity of juvenile turbot (*Scophthalmus maximus* L.) after 60 - day of feeding. Similarly, Xu *et al*. (2011) reported serum and *hepatopancreas* showed similar increased levels of SOD activity after dietary nucleotides for 5-weeks. It also demonstrated the immunomodulatory action of SOD and its possible use as an indicator of immune responses in juvenile *L.vannamei*. In the present study, SOD activity in serum of *A. nigrocauda* was tended to be positively influenced by dietary yeast nucleotides, therefore, nucleotides might have a significant effect on the superoxide dismutase of serum.

ACP and ALP, two typical hydrolases, are capable of assisting, modulating and accelerating immunity, besides, they are also involved in nutrient transport and digestion (Chen *et al*., 2007; Xing *et al*., 2008). ALP activity was reported to be an indicator of the intensity of nutrient absorption in enterocytes of fish (Harpaz and Uni, 1999; Gawlicka *et al*., 2000). In our present study ALP activity did not change significantly among the treatments except that 150 mg kg^{-1} compared to the control group content in *A. nigrocauda* by dietary yeast nucleotides. Blier *et al*. (2002) also did not find any significant difference in ALP activity in growth hormone transgenic coho salmon (*O. kisutch*) that was supposed to have higher growth rate compared to non-transgenic coho salmon. But, the growth rate of Atlantic cod was found to be positively correlated with ALP

activity (Lemieux *et al*., 1999). ACP act as marker enzymes is known to be localized in the lysosomes and surrounded by a lipoprotein membrane (Blasco *et al.,* 1993), and is involved in killing and digesting microbial pathogens during immune responses (Cheng and Dougherty, 1989). In the present study, it was shown that dietary levels of yeast nucleotides from 150 to 900 mg kg^{-1} could not significantly influence the immunity of *A.nigrocauda* through affecting ACP activity. This result was consistent with that of Wei *et al*. (2007) who demonstrated that the dietary exogenous nucleotides at 86-430 mg kg^{-1} had not significantly increased the ACP activity in *Carassius auratus gibelio*. As the precise mechanism of how the exogenous nucleotides affect the ACP activity of fish were not clear yet, it was hard to explain accurately.

A number of studies have investigated the effect of nucleotides on fish body composition (Tacon and Cooke, 1980; Peres and Oliva-Teles, 2003). Li *et al*. (2007a) found that the crude fat and ash contents of muscles in juvenile red drum increased by feeding on yeast nucleotides. Similarly, Cao *et al*. (2011) showed that exogenous nucleotides could increase crude fat and ash contents in *Litopenaeus vannamei*. The lack of any significant difference in whole body composition is in agreement with observations from other similar studies on the use of nucleic acid in fish (Wei *et al*., 2007) except for the ash content of fish fed nucleic acid supplemented diets (Rumsey et al., 1992). Similarly, in the present study, it appears likely that dietary nucleotides can influence muscle lipids and/or protein as in whole body composition. These results suggest that nucleotide supplementation plays a role in enhancing growth performance of fish, which accordingly enhanced the lipids and/or protein of fish body/muscle composition.

The better muscle lipid and/or protein contents of the fish in nucleotide supplemented diets may have been due to increased fish nutrient utilization, and the high nutrient digestibility in a higher nucleotide supplement and therefore the increased deposited nutrients (Abtahi *et al*., 2013). In addition, biochemical analyses often provide vital information for muscle lipid and/or protein content of cultured fish. Previous studies demonstrated that nucleotides benefited the muscle proteome differential expression, such as glyceraldehyde -3- phosphate dehydrogenase, creatine kinase, nucleoside diphosphate kinase, and triosephosphate isomerase, etc. (Saeed and Ahmad, 2012). The altered expression of both metabolic and structural proteins in fish fed nucleotides may be related to higher protein deposited in fish. These findings may provide basic information to understand possible mechanisms of dietary nucleotides. However, information on exogenous nucleotides on lipid and/or protein metabolism of fishes is very limited, physiological consequences are not clear. Therefore, further investigation is warranted. Results of the present study showed moisture and ash contents in the muscle tissue of *A.nigrocauda* were not changed by the dietary supplementation of yeast nucleotides. Although fish size and age (Rønsholdt, 1995), diet (Rasmussen *et al*., 2000), and some experimental factors

can affect body composition, the main cause for the different results obtained by different authors may more likely be the different responses to yeast nucleotides from different fish species and nucleotides doses used in different experiments.

In conclusion, the results of this study show that the addition of yeast nucleotides to the diet of *A.nigrocauda* increased growth performance, feed utilization and intramuscular protein and fat content. Serum LZS and SOD also could be improved in fish fed yeast nucleotide level. The optimum supplementation level into a fish meal based diet appears to be between 450 and 600 mg yeast nucleotides kg^{-1} in practical fish feed formulation for *A. nigrocauda*.

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References

Adamek, Z., Hamackova, J., Kouril, J., Vachta, R, and Stibranyiova, I., 1996. Effect of Ascogen probiotics supplementation on farming success in rainbow trout (*Oncorhynchus mykiss*) and wels (*Silurus glanis*) under conditions of intensive culture. *Krmiva* (Zagreb), 38, 11-20.

Ahmad, T.K., Saeed, K., Amin, N., Nemat, M, and Hossein, P.Z., 2011. Dietary administration of nucleotides to enhance growth, humoral immune responses, and disease resistance of the rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Fish and Shellfish Immunology*, 30, 189-193.

AOAC, 1995., In: Cunniff PA (ed) Official methods of analysis of the association of official analytical chemists, vol 1,16th ed. AOAC International, Arlington, USA.

Bai, X.H., Wang, G.Y, and Xiong, C.X., 2007. Comparison of digestive enzyme activity in different age of *anchery*throculter *nigrocauda*. *Freshwater Fisheries*, 37, 30-33.

Blasco, J., Puppo, J, and Sarasquete, C., 1993. Acid and alkaline phosphatase activities in the clam *Ruditapes philippinarum*. *Marine Biology*, 115, 113-118.

Blier, P.U., Lemieux, H, and Devlin, R. H., 2002. Is the growth rate of fish set by digestive enzymes or metabolic capacity of the tissues? Insight from transgenic coho salmon. *Aquaculture*, 209, 379-384.

Borda, E., Martinez-Puig, D, and Cordoba, X., 2003. A balanced nucleotide supply makes sense. *Feed Mix*, 11, 24-26.

Burrells, C., Williams, P.D, and Forno, P.F., 2001a. Dietary nucleotides: a novel supplement in fish feeds 1. Effects on resistance to disease in salmonids. *Aquaculture*, 199, 159-169.

Burrells, C., Williams, P.D., Southgate, P.J, and Wadsworth, S.L., 2001b. Dietary nucleotides: a novel supplement in fish feeds 2. Effects on vaccination, salt water transfer, growth rates and physiology of Atlantic salmon (*Salmo salar L*.). *Aquaculture*, 199, 171-184.

Cao, J.M., Xu, D.D., Huang, Y.H., Lan, H.B., Chen, B., Zhao, H.X., Jiang, W.L. and Chen, X.Y., 2011. Effects of dietary nucleotides on growth performance, tissue biochemical composition and non-specific immunity of juvenile *Litopenaeus vannamei*. *Journal of Fisheries of China*, 35, 594-603.

Carver, J.D., 1999. Dietary nucleotides: effects on the immune and gastrointestinal systems. *Acta Paediatr*, 88, 83-88.

Chen, M.Y., Yang, H.S., Delaporte, M., Zhao, S.J, and Xing, K., 2007. Immune responses of the scallop *Chlamys farreri* after air exposure to different temperatures. *Journal of Experimental Marine Biology and Ecology*, 345, 52-60.

Chen, Y.Y., 1998. Fauna sinica, osteichthyes, cypriniforms Ⅱ. Beijing: Science Press, pp.112-207.

Cheng, T.C, and Dougherty, W.J., 1989. Ultrastructural evidence for the destruction of *Schistosoma mansoni* sporocysts associated with elevated lysosomal enzyme levels in *Biomphalaria glabrata*. *The Journal of Parasitology*, 75, 928-941.

Chipman, D.M, and Sharon, N., 1969. Mechanism of lysozyme action. *Science*, 165, 454–65.

Cho, C.Y., 1992. Feeding systems: energy and protein requirements. *Aquaculture*, 100, 107-23.

Cosgrove, M., 1998. Nucleotides. *Nutrition*, 14, 748-751.

Delliou, H., Huelvan, C., Desbruyeres, E, and Kaushik, S.J., 2002. Nitrogen utilisation and ureogenesis as affected by dietary nucleic acid in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). *Fish Physiology and Biochemistry*, 26, 177-88.

EIADCAS and RIPYWR (The environmental impact assessment department, Chinese Academy of Sciences, and the Research Institute for Protection for Yangtze Water Resources), 1995. Environmental impact statement for the Yangtze Three Gorges Project. Science Press, Beijing. 72P.

Gawlicka, A., Parent, B., Horn, M.H., Ross, N., Opstad, I, and Torrissen, O.J., 2000. Activity of digestive enzymes in yolk – sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): indication of readiness for first feeding. *Aquaculture*, 184, 303-314.

Harpaz, S, and Uni, Z., 1999. Activity of intestinal mucosal brush border membrane enzymes in relation to the feeding habits of three aquaculture fish species. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology*, 124(**2**), 155-160.

Irianto, A, and Austin, B., 2002. Probiotics in aquaculture. *Journal of Fish Diseases*, 25, 633.

Kiyohara, S., Hidaka, I. and Tamura, T., 1975. Gustatory response in the puffer-II. Single fiber analysis. *Nippon Suisan Gakkaishi*, 41, 383-391.

Lemieux, H., Blier, P. and Dutil, J.D., 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the

Atlantic cod (*Gadus morhua*)? *Fish Physiology and Biochemistry*, 20, 293-303.

Leonardi, M., Sandino, A, and Klempau, A., 2003. Effect of a nucleotide-enriched diet on the immune system plasma cortisol levels and resistance to infectious pancreatic necrosis (IPN) in juvenile rainbow trout (*Oncorhynchus mykiss*). *Bulletin- European Association of Fish Pathologists*, 23, 52-59.

Li, P., Burr, G.S., Goff, J., Whiteman, K. W., Davis, K.B., Vega, R.R., Neill, W.H. and Gatlin, D.M., 2005. A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). *Fisheries Research*, 36, 1120-1127.

Li, P. and Gatlin, D.M., 2004. Dietary brewers yeast and the prebiotic Grobiotic™ AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) to *Streptococcus iniae* infection. *Aquaculture*, 231, 445-456.

Li, P, and Gatlin, D.M., 2006. Nucleotide nutrition in fish: current knowledge and future applications. *Aquaculture*, 251, 141-152.

Li, P., Gatlin, D.M, and Neill, W.H., 2007a. Dietary supplementation of a purified nucleotide mixture transiently enhanced growth and feed utilization of juvenile red drum, *Sciaenops ocellatus. Journal of the World Aquaculture Society*, 38, 281-286.

Li, P., Lawrence, A.L., Castille, F.L, and Gatlin, D.M., 2007b. Preliminary evaluation of a purified nucleotide mixture as a dietary supplement for Pacific white shrimp *Litopenaeus vannamei* (Boone). *Fisheries Research*, 38, 887-890.

Lin, S.M., Pan, Y., Luo, L, and Luo, L., 2011. Effects of dietary β-1, 3-glucan, chitosan or raffinose on the growth, innate immunity and resistance of koi (*Cyprinus carpio* koi). *Fish and Shellfish Immunology*, 31, 788-794.

Lin, Y.H., Wang, H, and Shiau, S.Y., 2009. Dietary nucleotide supplementation enhances growth and immune responses of grouper, *Epinephelus malabaricus*. *Aquaculture Nutrition*, 15, 117-122.

Mackie, A.M., 1973. The chemical basis of food detection in the lobster *Homarus gammarus*. *Marine Biology*, 21, 103-108.

Mackie, A.M. and Adron, J.W., 1978. Identification of inosine and inosine 5'-monophosphate as the gustatory feeding stimulants for the turbot, *Scophthalmus maximus*. *Comparative Biochemistry and Physiology*, 60A, 79-83.

Mo, P., Wei, X., Ai, Q.H., Mai, K.S., Liufu, Z.G. and Zhang, K.K., 2013. Effects of nucleotides supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.). *Aquaculture*, 2, 51-68.

Peres, H, and Oliva-Teles, A., 2003. The effect of dietary ribonucleic acid incorporation in performance of

European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, 15, 245-253.

Quan, R., Barness, L.A. and Uauy, R., 1990. Do infants need nucleotide supplemented formula for optimal nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, 11, 429-434.

Ramadan, A., Atef, M. and Afifi, N.A., 1991. Effect of the biogenic performance enhancer (*Ascogen* "S") on growth rate of tilapia fish. *Acta Veterinaria Scandinavica*. 87, S304-S06.

Ramadan, A., Afifi, N.A., Moustafa, M, and Samy, A.M., 1994. The effect of ascogen on the immune response of tilapia fish to *Aeromonas hydrophila* vaccine. *Fish and Shellfish Immunology*, 5, 159-165.

Rasmussen, R.S., Ostenfeld, T.H., Rønsholdt, B, and McLean, E., 2000. Manipulation of end-product quality of rainbow trout with finishing diets. *Aquaculture Nutrition*, 6, 17-23.

Rønsholdt, B., 1995. Effect of size/age and feed composition on body composition and phosphorus content of rainbow trout, *Oncorhynchus mykiss*. *Water Science and Technology*, 31, 175-83.

Rumsey, G.L., Winfree, R.A. and Hughes, S.G., 1992. Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 108, 97-110.

Saeed Keyvanshokooh, and Ahmad Tahmasebi-Kohyani., 2012. Proteome modifications of fingerling rainbow trout (*Oncorhynchus mykiss*) muscle as an effect of dietary nucleotides. *Aquaculture*, pp. 324–325, pp. 79–84.

Sakai, M., Taniguchi, K., Mamoto, K., Ogawa, H, and Tabata, M., 2001. Immunostimulant effects of nucleotide isolated from yeast RNA on carp, *Cyprinus carpio* L. *Journal of Fish Diseases*, 24, 433-38.

Tacon, A.G.J. and Cooke, D.J., 1980. Nutritional value of dietary nucleic acids to trout. *Nutrition Republic Institute*, 22, 631-40.

Tan, D.Q., Wang, J.W. and Dan, S.G., 2004a. The ratio of flesh to body and analysis of the nutritive composition of muscle in *Ancherythroculter nigrocauda* Yih et Wu. *Acta Hydrobiologica Sinica*, 28, 240-246.

Tan, D.Q., Wang, J.W., Yan, T.M. and Dan, S.G., 2004b. Studies on the artificial propogation of *Ancherythroculter nigrocauda*; *Resources and Environment in the Yangtze Basin*, 2, 94-97.

Wei, W.Z., Luo, F.N., Yang, C, and Chen, B.G., 2007. Effects of dietary yeast nucleotides on growth and immune enzyme activities of *Carassiuo auratus gibelio*. *Freshwater Fisheries*, 37, 57-60.

Xing, J., Lin, T.T, and Zhan, W.B., 2008. Variations of enzyme activities in the haemocytes of scallop *Chlamys farreri* after infection with the acute virus necrobiotic virus (AVNV). *Fish and Shellfish Immunology*, 25, 847-852.

Xu, D.D., Huang, Y.H., Cao, J.M., Lan, H.B., Wang, G.X., Zhang, R.B., Chen, X.Y. and Yan, J., 2011. Nucleotide mixture supplementation affects non-specific immune and

antioxidant indices of juvenile *Litopenaeus vannamei*. *Chinese Journal of Animal Nutrition*, 23, 828-835.

Yin, H.C. and Lu, H.Y., 2010. Artificial propagation and embryonic development in *Ancherythroculter nigrocauda*. *Journal of Ocean University of China*, 25, 265-269.

Zeng, Q.X., Zhu, N.H., Zhsng, J.H, and Zhong, J.S., 2010. Culturing Experiment of *Ancherythroculter nigrocauda* in ponds. *China Fisheries*, 3: 37-38.