

Effect of Dietary Supplement of Probiotic (*Lactobacillus plantarum*) on Growth Performance, Feed Utilization and Survival Rate in Bocourti Catfish (*Pangasius bocourti* Sauvage, 1880)

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This study was investigated the effect of Probiotic (*L. plantarum*) supplementation at 0, 1×10^7 , 1×10^8 , 1×10^9 cfug⁻¹ diet on growth performance, feed utilization, survival rate and carcass quality in Bocourti's catfish (*Pangasius bocourti*). An average initial weight of fish 132.59 ± 1.20 gfish⁻¹ were divided into four treatments, three replication and fed for 8 weeks. The results showed that body weight, weight gain, daily growth rate, specific growth rate, survival rate, feed conversion ratio and feed conversion efficiency were not significant difference in all groups ($P > 0.05$). However, Fish fed dietary supplementation of probiotic at 1×10^7 cfug⁻¹ diet had trendy high in body weight, weight gain, daily growth rate and specific growth rate with 207.16 ± 16.31 g., 74.30 ± 15.13 gfish⁻¹, 1.33 ± 0.27 gday⁻¹ and 0.79 ± 0.13 %day⁻¹ respectively. The biochemical parameters in serum and carcass quality were did not significantly different for any of the diet treatment. Therefore, this study indicated that the optimum level of *L. plantarum* in diet for bocourti catfish culturing could be used for contains 1×10^7 cfug⁻¹ diet.

Keywords: Probiotic, Bocourti catfish (*Pangasius bocourti* Sauvage, 1880) growth performance.

Bocourti catfish or Basa or Pla-mong (Thai name) is common name of *Pangasius bocourti* Sauvage 1880 that number is Pangasiidae. This fishes are native species in Mekong and Chao Phraya basins in Indochina. In Thailand found in Mae Kong river, Chao Phraya river and some other smaller river such as Mun river and Chi river and other river in Northeastern in Thailand^{23,25}. This fish has a good taste and has been accepted of consumers and the demanded of globe has been increased in America and Europe¹⁵. In both foreign markets are mostly demanded in filet forms and has high price. In Thailand markets had price depended

on size of fish, e.g. 0.7-1.0 kg had about up to 50 baht and 1.5 to 2.0 kg had about 150 baht²¹.

Recently, Aquaculture is important sector and spreading area to providing human consumption and fisheries. The growing of aquaculture practices that from many factor :1) the demand of market because protein form the aquatic animals is good quality, 2) the security and safety food of consumer and 3) make income from aquaculture⁵. However, aquaculture practices in intensive system requires cultivation at high densities, which has caused destroyed the environment because the effluent water from the ponds containing organic wastes in high volume, effect on water quality such as reducing dissolved oxygen, giving the ammonia, nitrites and hydrogen sulfide from the metabolites of organics degradation that often

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are responsible for mortality²⁷. And, under these conditions of intensive system, aquatic animals has to high-stress, that easily infection by pathogens and high mortality rate and causing a decreasing productivity³. So, the owner of farm using antibiotic drug for preventing and treating which has seen the previous research that can be used to prevent infection. But, the using antibiotics will kill the bacteria in the digestive tract of aquatic animals and the accumulation of antibiotic residues in fish products to be harmful for human consumption. Form the this problem, the farmer has turned to use probiotics in aquaculture that it is non-pathogenic microorganisms in aquatic animals that are many proportion , such as improved growth of fish , preventing the pathogen , increasing utilization of nutrient , treat the water quality in pond, reduce stress of fish and improved reproductive system of aquatic animals⁵.

Probiotic is a relatively new term which is used to name microorganisms that are associated with the beneficial effects for the host. Probiotics have been introduced as a growth stimulant. Such as the study of Lara *et al.*¹⁷ that use of *Streptococcus* as growth promoters on Nile tilapia (*Oreochromis niloticus*) the result showed probiotic strain ,increasing significantly the content of crude protein and crude lipid in the fish, also weight has increased from 0.154 g to 6.164 g. in 9 weeks of culture. And ornamental fishes include swordtail (*Xiphophorus helleri*, *X. maculatus*) and guppy, (*Poecilia reticulata*, *P. sphenops*), their feed was supplemented with *Bacillus subtilis* and *Streptomyces*, can be increases in growth and survival of *Xiphophorus* and *Poecilia* after 90 and 50 days of experimental period, respectively^{7,12}.

Lactic acid bacteria (LAB) are most applied probiotics in for use enhanced growth or immune system for aquatic species^{8,9,24}. And they are producers of bacteriocins and organic acids (lactic and acetic acids) which have inhibitory effects *in vitro* on the growth of some pathogens in fish²⁰. And they have the previous study for use enhanced growth or immune system in fish . e.g Nile tilapia, *Oreochromis niloticus*¹, Grouper, *Epinephalus coioides*²⁶, Cobia, *Rachycentron canadum*¹¹ and European sea bass, *Dientrachus labrax*¹⁹. Therefore, The aim of this study was to determine the effect of *Lactobacillus plantarum* supplementation on growth performance, survival

rate, serum biochemical and carcass quality in Bocourti catfish (*Pangasius bocourti* Sauvage, 1880)

MATERIALS AND METHODS

Fish, diets and feeding protocol

This study was carried out with the used four different levels of *Lactobacillus plantarum* (0, 1×10^7 , 1×10^8 and 1×10^9 cfug⁻¹ diet). A Completely Randomized Design (CRD) with three replication. The basal diet was developed from formula diet of Boonarsa and Doolgidachbaporn⁴ and the nutritional value of basal diet had a crude protein and gross energy with 29.89% dry matter and 424.65 kcal per 100 g diet (Table 1). The experimental diets were prepared by mixing the dry ingredients with soybean oil and water by hands and then the wet dough was placed in a grinder and pelleted by mincer to pass through a 2-mm and finally the moist pellets were dried in hot air oven at 60 °C for 12 hr and then stored in the fridge at -20 °C for further uses.

Lactobacillus plantarum CR1T5 were provided from Department of Microbiology, Faculty of science, Khon Kaen University, Thailand. The bacterial suspension content was about 3.4×10^9 cfuml⁻¹ and the bacterial were prepared stocking every twice a weeks. After that diluted the bacteria in concentration of experimental by used sterilized MRS broth is dilutant. The dilution of bacteria sprayed on basal diet with 0, 1×10^7 , 1×10^8 and 1×10^9 cfug⁻¹ diet that test diets were daily prepared.

Bocourti catfish (*P. bocourti*) juvenile obtained from Phayao Inland Fisheries Research and Development Center, Phayao Province, Thailand . Fish were kept in 1000L fiber tank for acclimatization with commercial diet with 40% protein for four weeks. Then protein content in diet was decreased to 30 and fed for 8 weeks prior to feeding the experimental diets. After the acclimatization fish were randomly distributed into four groups with three replications that each replicate contained 10 fish (mean wet weight of 132.59 ± 1.20 g fish⁻¹) in an aquarium (60 x 150 x 60 cm³) filled the water 450 L. which 12 aquaria tanks. Water quality parameters such as dissolved oxygen (6.05 ± 0.28 mg L⁻¹), temperature (26.98 ± 0.19 °C) and pH value (7.37 ± 0.27) were measured every day during the experimental period.

During the trial, fish were fed *ad-libitum* by hand fed method twice a day (08:00 and 15:00 h) for 8 week. Feed consumption was recorded weekly and fish from each tank were weighed to measure growth every two weeks until the end of the experiment; growth performance and feed utilization were calculated.

Evaluation of growth performance and feed utilization parameters

The collected data on weight gained (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE) and survival rate (SR) were calculated.

WG (g) = Final Weight - initial weight

ADG (g/day) = (Final Weight - initial weight) / period of experiment

SGR (% /day) = (Ln final weight - Ln initial weight) / days x 100

FCR = total feed intake (g) / total wet weight gained (g)

FCE (%) = total wet weight gained (g) / total feed intake (g) x 100

Survival rate (%) = (final fish number) / (initial fish number) x 100

Serum biochemistry and carcass quality

At the end of the experiment, after fish were starved for 12 hours, randomized two fish per tank that were anesthetized with tricaine methanesulfonate (*MS-222*). And blood samples were collected from caudal vein that kept in non-heparinized tubes. Hematocrit was determined by using microhematocrit centrifuge. The serums were separated into aliquots and analyses of serum biochemistry. Serum was analyzed for total protein (TP) and alkaline phosphatase (ALP) activity. The serum biochemical indices were determined with an automatic biochemical analyser (BS-200; Mindray, Shenzhen, China). Then the fishes were killed and liver and visceral were kepted and test investigate weighed carcass quality by Hepatic somatic index (HSI), Visceral Somatic index (VSI), Visceral Fat index (VFI) and Muscle ratio (MR) by equation following:

HSI (%) = (Liver mass(g) / body mass (g)) × 100

VSI (%) = (total inner organ mass (g) / body mass (g)) × 100

VFI (%) = (Visceral Fat mass (g) / body mass (g)) × 100

MR (%) = muscle mass (g) / body mass (g) × 100

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and if significant differences ($p < 0.05$) were found, Duncan's multiple range test was used to rank the groups.

RESULTS

Growth performance, feed utilization and survival rate

The growth performance, feed utilization and survival rate of bocourti catfish (*P. bocourti*) with an initial average weight of 132.59 ± 1.20 g fish⁻¹ fed with diet containing *Lactobacillus plantarum* at 0, 1×10^7 , 1×10^8 and 1×10^9 cfug⁻¹ diet for 8 weeks is shown in Table 2. At the end of experiment found that growth performance base on the weight gain (WG), average daily gain (ADG) and specific growth rate (SGR) and feed utilization base on feed conversion ratio (FCR) and feed efficiency ratio (FCE) were not significant different ($p > 0.05$). But fish fed with *Lactobacillus plantarum* at 1×10^7 cfug⁻¹ diet had maximum growth performance and best feed utilization compared with fish in other group. The weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency ratio were ranged from 64.30 ± 9.66 to 74.30 ± 15.13 g fish⁻¹, 1.15 ± 0.17 to 1.33 ± 0.27 d day⁻¹, 0.70 ± 0.09 to 0.79 ± 0.13 % day⁻¹, $1.91 \pm .41$ to 2.17 ± 0.31 and 46.76 ± 7.04 to 54.06 ± 11.01 %, respectively. And survival rate was 100 % in all groups.

Carcass quality

The termination of experiment, the carcass quality was determine in Bocourti catfish (*P. bocourti*) fed with test diets containing *Lactobacillus plantarum* at 0, 1×10^7 , 1×10^8 and 1×10^9 cfug⁻¹ (Table 3). The Hepatosomatic index (HSI), vesicle somatic index (VSI), Vesicle fat index (VFI) and muscle ratio (MR) were not significant different ($p > 0.05$). Hepatosomatic index (HSI), Visceral somatic index (VSI), Visceral fat index (VFI) and Muscle ratio (MR) were ranged from 1.63 ± 0.35 to 2.08 ± 0.34 %, 9.17 ± 1.08 to 10.25 ± 0.68 %, 2.96 ± 1.41 to 3.67 ± 1.63 % and 32.68 ± 0.82 to 34.97 ± 1.70 %, respectively

Serum biochemistry

The hematocrite (Hct) of fish fed with

diet containing at *L. plantarum* at 0, 1×10^7 , 1×10^8 and 1×10^9 cfug⁻¹ were determined at the end of the experiment that was highly significant different ($p < 0.01$). and shown in Table 4. The hematocrit of fish fed with diet supplementary *L. plantarum* at 1×10^8 and 1×10^9 cfug⁻¹ diet differ control group (0 cfug⁻¹ diet) but did not differ with 1×10^7 cfug⁻¹ diet. And there were 32.25 ± 0.50 , 34.25 ± 1.26 , 36.75 ± 1.85 and 35.13 ± 0.25 % of fish fed with diet containing at *L. plantarum* at 0, 1×10^7 , 1×10^8 and 1×10^9 cfug⁻¹. respectively. The fish serum from all groups were analysed total protein (TP) and alkaline phosphatase (ALP) on the end of experiment period that were not significant different ($p > 0.05$). The total protein was ranging from 3.390 ± 0.577 to 4.107 ± 0.931 gd⁻¹ and alkaline phosphatase (ALP) was ranging from 51.000 ± 29.597 to 91.000 ± 38.158 UI⁻¹. (Table 4)

DISCUSSION

This study was evaluated the effect of using *Lactobacillus plantarum* levels on Growth performance, Survival rate, Serum biochemical and Carcass quality of bocourti catfish (*Pangasius bocourti* Sauvage, 1880). From the present study, the growth performance and feed utilizations of fish were not significantly different between groups. But, the growth and feed utilization of fish fed with *L. plantarum* at 1×10^7 cfug⁻¹ diet was better than other groups. Therefore, it can be concluded that giving optimal level of probiotics improved growth of fish which was consistent with study of Piccolo *et al.*¹⁹ that use *L. plantarum* in diet of European sea bass (*Dientrachus labrax*) and no did not significantly different between groups. However the additions *L. plantarum* to

Table 1. Ingredient and nutritional value of basal diet

Ingredients (%crude protein)	Content (gKg ⁻¹)
Fish meal (57.48)	300.00
Soybean meal (44.00)	180.20
Broken rice (6.62)	255.00
Rice bran (11.73)	11.00
Corn meal(7.22)	93.00
Full Fat Soybean(35.55)	68.16
Soybean oil	35.00
Alpha starch	50.00
Vitamin and mineral ¹	7.64
Proximate composition based on dry matter (%)	
Crude Protein	29.89
Moisture	6.79
Fat	7.26
Fiber	2.52
Ash	7.91
Nitrogen free extract (NFE) ²	45.63
Gross Energy (GE ;Kcal/100 g) ³	424.65

¹ Vitamin and mineral mixture (g) :retinyl acetate (100 IU.g-1) 0.03 g., Cholecalciferol (20IU.g-1) 0.03 g. D,L- α -tocopherol acetate 0.009 g., menadione 0.00525 g., thiamine nitrate 0.00375 g., riboflavin 0.006 g. Pydroxinehydrochlorite, 0.006 g., D-calciumpantothenate 0.015 g., Niacin 0.015 g., Folic acid 0.0015 g. Cyanocobalamin 0.00045 g. ascorbyl acetate 0.03 g. CaCO₃ 6.00 g. MgSO₄·7H₂O 1.00 g. ZnSO₄·7H₂O 0.60 g. FeSO₄·7H₂O 0.03 g. CuSO₄·5H₂O 0.06 g. and KI 0.001 g.

² NFE = 100-(% Moisture + % Crude Protein + % Fat + % Fiber + % Ash) 18, NRC (1993)

³ Gross Energy = (%crude protein x 5.64) + (% NFE x 4.11) + (%fat x 9.44) 18; NRC (1993)

diet increased growth in term of weight gain, specific growth rate and protein efficiency ratio were 3.6, 5.2 and 6.9 % respectively. Lara et al.¹⁷ reported that use of *Streptococcus* sp. as growth promoters on Nile tilapia (*Oreochromis niloticus*) increasing significantly the content of crude protein and crude lipid in the fish, also weight has increased from 0.154 g to 6.164 g. in 9 weeks of culture. Similar resulted were conducted in swordtail (*Xiphophorus helleri*, *X. maculatus*) and guppy, (*Poecilia reticulata*, *P. sphenops*), their feed was supplemented with *Bacillus subtilis* and

Streptomyces sp. can be improved in growth and survival of swordtail and guppy after 90 and 50 days of experimental period, respectively^{7,12}. These effects have been reported a similar response and positive effect on productive performance in other species. e.g. Alatic cod, *Gadus morhua*¹³, Juvenile Dentex, *Dentex dentex* L.¹⁴.

The carcass quality of fish in these study found that hepatosomatic index (HSI), vesicle somatic index (VSI) vesicle fat index (VFI) and muscle ratio (MR) were not significantly different between groups ($p > 0.05$). Likely, the

Table 2. Growth performance, feed utilization and survival rate of bocourti catfish (*P. bocourti*) fed with experimental diets for 8 weeks

Parameter	Levels of Probiotic(<i>L. plantarum</i>) supplement in diet (cfug ⁻¹)				p-value
	0	1x10 ⁷	1x10 ⁸	1x10 ⁹	
initial weight (g)	132.51±1.38	132.84±1.44	132.46±1.35	132.69±0.90	0.982
Final Weight (g)	197.86±9.56	207.16±16.31	198.25±8.64	196.99±10.35	0.690
WG (g)	65.37±8.23	74.30±15.13	65.80±8.50	64.30±9.66	0.660
ADG (gday ⁻¹)	1.17±0.15	1.33±0.27	1.17±0.15	1.15±0.17	0.657
SGR (%day ⁻¹)	0.72±0.07	0.79±0.13	0.72±0.08	0.70±0.09	0.661
FCR	2.13±0.26	1.91±0.41	2.11±0.28	2.17±0.31	0.747
FCE (%)	47.53±5.95	54.06±11.01	47.85±6.15	46.76±7.04	0.657
SR (%)	100	100	100	100	

Means within row followed by a common letter are not significantly different according to DMRT ($P < 0.05$)

Table 3. Carcass quality of bocourti catfish (*P. bocourti*) fed with experimental diets for 8 weeks

Parameter	Levels of Probiotic(<i>L. plantarum</i>) supplement in diet (cfug ⁻¹)				p-value
	0	1x10 ⁷	1x10 ⁸	1x10 ⁹	
HSI(%)	1.81±0.20	1.63±0.35	1.78±0.30	2.08±0.34	0.393
VSI(%)	9.92±3.33	9.17±1.08	9.51±1.47	10.25±0.68	0.908
VFI(%)	3.50±1.24	2.96±1.41	3.67±1.63	3.02±0.64	0.879
MR(%)	34.97±1.70	33.81±3.07	34.69±1.66	32.68±0.82	0.522

Means within row followed by a common letter are not significantly different according to DMRT ($P < 0.05$)

Table 4. Haematocrit and serum biochemistry of bocourti catfish (*P. bocourti*) fed with experimental diets for 8 weeks

Parameter	Levels of Probiotic(<i>L. plantarum</i>) supplement in diet (cfug-1)				p-value
	0	1x10 ⁷	1x10 ⁸	1x10 ⁹	
Hct(%)	32.25±0.50 ^a	34.25±1.26 ^{ab}	36.75±1.85 ^b	35.13±0.25 ^b	0.001
TP (gd ⁻¹)l	4.107±0.931	3.390±0.577	3.527±0.532	3.673±0.278	0.556
ALP (U ⁻¹)	51.000±29.597	61.333±50.402	82.667±31.943	91.000±38.158	0.579

Means within row followed by a common letter are not significantly different according to DMRT ($P < 0.05$)

study of Dawood *et al.*⁶ that used *L. rhamnosus* and *Lactococcus lactis* supplement in diet of red sea bream (*Pagrus major*) at 10^6 cell g^{-1} was found the hepatosomatic index (HSI) and somatic index (VSI) were not significantly different ($p > 0.05$) which was consistent with study of Piccolo *et al.*¹⁹ that use *Lactobacillus plantarum* in diet of European sea bass (*Dientrachus labrax*).

Hematocrit has been recognized as valuable tools for monitoring of fish health^{2,18}. From the present study the hematocrit value of fish from all groups and has significantly different ($p < 0.01$). And the trend of hematocrit increased on probiotics levels in diet. The results indicated that supplement of probiotic improved healthy of fish. The blood protein, albumin and globulin are the major proteins, which play a significant role in the immune response²². From this study showed not significantly different of total protein in fish serum. Because when liver is damaged, it will total protein, albumin and globulin in serum decreased¹⁶. The alkaline phosphatase (ALP) in these study was not significantly different. which was consistent with study of Piccolo *et al.*¹⁹ that use *Lactobacillus plantarum* in diet of European sea bass (*Dientrachus labrax*). However, the trend of ALP increased on supplementary *Lactobacillus* level in diet of bocourti catfish. From the reported of Piccolo *et al.*¹⁹ that highest values of ALP in fish resulted of increased growth rate and feeding level.

CONCLUSION

In conclusion, this present study indicated that supplementation of different concentrations *Lactobacillus plantarum* in diet of Bocourti catfish (*P. bocourti*) were not significantly different in growth performances, feed utilizations, carcass quality and serum biochemistry. The haematocrit values was increased with increased concentration of probiotics. Base on these results, use of *Lactobacillus plantarum* at 1×10^7 cfug⁻¹ supplementation in diet had maximum growth performance and best feed utilization.

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