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# Effect of dietary *Bacillus pumilus* A1_YM_1 on growth, intestinal morphology and some hematological parameters of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*)

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**Abstract**

A 60 day feeding trial was performed to examine the dietary supplementation of *Bacillus pumilus* A1_YM_1 (BP) on growth, intestinal morphology and some hematological parameters of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*). Fish with an average weight of 8.55±0.18 g were divided into 4 treatments with 3 replications. The control treatment was fed with the basal diets, whereas the experimental treatments were fed the basal diet incorporated with $1\times10^6$, $1\times10^7$ and $1\times10^8$ CFU/g. The results indicated that fish fed the diets supplemented with BP significantly enhanced final weight, weight gain, specific growth rate, average daily gain and feed conversion efficiency when compared to the control diet ($P<0.05$). There were no significant differences in survival rate and condition factor between the experimental and control groups ($P>0.05$). Hematological evaluation showed supplementation of BP in the fish diets significantly increased red blood cell, white blood cell, lymphocyte and neutrophil when compared to the control diet ($P<0.05$). Histological study of different parts (anterior, middle and posterior) of intestine proper revealed that dietary supplementation of BP significantly enhanced intestinal villi width, intestinal villi height, thicknesses of muscular layers, goblet cell number and microvillus height when compared to the control ($P<0.05$). General feed intake behavior and health as well as feed acceptability of the treatments were the same as the control. It was suggested that the optimum dietary level of BP for catfish cultivation observed was $1\times10^7$ CFU/g. Thus, the results of this study support the use of BP in diets as a probiotic in order to enhance growth, intestinal morphology and hematology of catfish.

**Keywords:** *Bacillus pumilus*, hybrid catfish, growth, intestinal morphology, hematology

## 1. Introduction

Hybrid catfish is widely cultivated by fish farmers in many regions of Thailand due to its high growth rate, low cost production and high economic value [1]. To support an increasing demand for domestic consumption of catfish, intensive culture system has continuously performed and developed [2]. However, this practice may exert profound effects on growth, productivity and health status of cultured fish [2]. Interestingly, in order to promote fish growth and health, a variety of chemical and natural feed additives can be applied to fish feeds and novel dietary supplementation compounds have long been investigating [2].

Probiotics can be defined as live microorganisms which when ingested in appropriate levels would produce a beneficial effect on health of hosts by improving microbial balance in the intestinal tract [2-5]. In aquaculture practices, probiotics have long been used as feed additives in order to improve growth performance, health status and disease resistance in many kinds of fish [2-5].

The common probiotics used in fish rearing are the members of the heterogeneous group of Lactobacillus or Bifidobacterium [3-5]. *Bacillus* sp. have been tested for their probiotic properties in several aquatic species [3-5]. Truong Thy et al. [6] reported that striped catfish (*Pangasianodon hypophthalmus*) were fed the dietary

supplementation of mixed probiotic spores of *Bacillus amyloliquefaciens* 54A and *B. pumilus* 47B for 90 days produced a significant increase in several immune parameters such as respiratory bursts, phagocytic activity and lysozyme activity when fish were challenged with *Edwardsiella ictaluri* compared to the control. In diet of catfish (*Clarias* sp.), the use of *B. megaterium* PTB 1.4 as a probiotic supplementation in the diet for 30 days significantly increased growth performance and digestive enzyme activity when compared to the control [7].

*B. pumilus*, a Gram-positive bacteria, has been isolated and evaluated as a fish probiotic [8-10]. Ghosh et al. [8] stated that *B. pumilus* isolated from the intestinal tracts of rohu (*Labeo rohita*) fingerlings produced some important digestive enzymes including protease, amylase and cellulase, which might be modulated nutrient digestion in fish. Aly et al. [9] indicated that dietary *B. pumilus* proved immune responses, disease resistance and general health status of Nile tilapia (*Oreochromis niloticus*). Additionally, Nile tilapia fed the diets supplemented with *B. pumilus* showed a disease resistance to *Aeromonas hydrophila* infection [9 & 10].

Various data indicated that *B. pumilus* has a potential use as a fish probiotic [8-10]. However, the use of *B. pumilus* in fish feed to improve growth and intestinal morphology of catfish is still very limited [8-10]. Therefore, the aims of this study were to evaluate the effects of one strain of *Bacillus* (*B. pumilus* A1_YM_1) (BP) on growth, intestinal morphology and some hematological parameters of hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*).

## 2. Materials and Methods

### *2.1 Diet preparation*

BP isolated from biofertilizer was used in this present research [11]. A commercial diet (containing 25% protein and 3% lipid) was purchased and mixed with the bacterial suspensions to produce the final concentrations of $1\times10^6$, $1\times10^7$ and $1\times10^8$ CFU/g by using a meat mincer. The diet samples were dried in hot air oven at 45°C for 24 h [12], broken down to small pellets (1-2 mm long) and then kept in the plastic bag at 4°C until used.

### *2.2 Fish preparation*

Hybrid catfish (8.55±0.18 g) were purchased from Ubon Ratchathani Fishery Cooperatives, Ubon Ratchathani, Thailand. They were acclimatized to the experimental conditions in the circular concrete tanks (255 L) for 2 weeks and then divided into 4 treatments with 3 replications (30 fish/tank). Treatment 1, the fish were fed the basal diet without BP. Treatments 2-4, the fish were fed the diets containing BP at the concentrations of $1\times10^6$, $1\times10^7$ and $1\times10^8$ CFU/g, respectively. Fish were fed the experimental and control diets at a rate of 5% of body weight for 8 weeks. Water qualities were kept in the standard criteria for catfish. Experimental procedures and animal manipulations used in this present research were performed as described by National Research Council of Thailand.

### *2.3 Growth performance analysis*

At the end of experiment, growth parameters were evaluated using the following equations [12].

$$\text{Weight gain (WG, g)} = W_f - W_i \quad (1)$$
$$\text{Specific growth rate (SGR, \%/day)} = 100 \times (\mathit{In}\ W_f - \mathit{In}\ W_i)/T \quad (2)$$
$$\text{Average daily growth (ADG, g/day)} = (W_f - W_i)/T \quad (3)$$
$$\text{Feed conversion ratio (FCR)} = FI/(W_f - W_i) \quad (4)$$
$$\text{Condition factor (K-factor)} = 100 \times (W_f/FL^3) \quad (5)$$
$$\text{Survival rate (SR, \%)} = 100 \times (N_f/N_i) \quad (6)$$

Where $W_i$ is the initial wet weight (g), $W_f$ is the final wet weight (g), T is the experimental days (day), FI is the feed intake (g), FL is the length (cm), $N_i$ is the initial number of fish and $N_f$ is the final number of fish.

### *2.4 Intestinal morphological analysis*

To investigate the effects of dietary supplementation with BP on intestinal morphology, the samples of fish intestines were divided into proximal, middle and distal parts. They were cut transversely into small sections and kept in 10% neutral buffered formalin. Preparations of histological slides were performed [13-15]. Morphological studies of the intestines were evaluated by using DinoCapture 2.0 software and the experimental procedures were followed as reported by Fang et al. [16] and Munglue [17].

*2.5 Hematological analysis*

To investigate the effects of dietary supplementation with BP on some hematological parameters, blood samples were collected from the caudal vein using a 1 mL heparinized needle. Red blood cell and white blood cell were counted using a hemocytometer [18]. Different white blood cell count was performed as previously described by Zhu et al. [19].

*2.6 Statistical analysis*

Data are expressed as mean±standard error of the mean (SEM) and analyzed by using one-way analysis of variance (ANOVA). Duncan's multiple-range test was used to determine differences among the treatments. If $P<0.05$ was indicated statistically significant.

**3. Results**

*3.1 Growth performance*

The effect of dietary BP on growth of catfish is displayed in Table 1. The results indicated that fish fed the diets supplemented with BP significantly increased final weight and WG and significantly decreased FCR compared to the control ($P<0.05$). SGR and ADG of fish fed $1\times10^7$ and $1\times10^8$ CFU/g were significantly higher than that of the control and $1\times10^6$ CFU/g BP ($P<0.05$). There were no significant differences in SR and K-factor between the experimental and control groups ($P>0.05$). It was observed that general feed intake behavior and health as well as feed acceptability of the treatments were the same as the control.

**Table 1** Growth of catfish fed the diets supplemented with BP for 8 weeks.

| Parameters | BP levels in the experimental diets (CFU/g) | | | |
|---|---|---|---|---|
| | 0 | $1\times10^6$ | $1\times10^7$ | $1\times10^8$ |
| IL (cm) | 8.44±0.24 | 8.44±0.29 | 8.44±0.24 | 8.66±0.23 |
| FL (cm) | $12.66\pm0.23^{ab}$ | $13.44\pm0.37^{bc}$ | $14.11\pm0.26^{c}$ | $13.44\pm0.24^{bc}$ |
| IW (g) | 6.55±0.17 | 6.55±0.17 | 6.55±0.17 | 6.33±0.66 |
| FW (g) | $12.77\pm0.61^{a}$ | $14.77\pm0.79^{bc}$ | $16.11\pm0.67^{c}$ | $15.66\pm0.55^{c}$ |
| WG (g) | $6.22\pm0.52^{a}$ | $8.22\pm0.68^{bc}$ | $9.55\pm0.58^{c}$ | $9.33\pm0.40^{c}$ |
| SGR (%/day) | $1.17\pm0.69^{a}$ | $1.43\pm0.66^{a}$ | $1.59\pm0.53^{b}$ | $1.61\pm0.02^{b}$ |
| ADG (g/day) | $0.11\pm0.01^{a}$ | $0.14\pm0.01^{b}$ | $0.17\pm0.01^{c}$ | $0.16\pm0.01^{c}$ |
| FCR | $1.77\pm0.17^{a}$ | $1.32\pm0.09^{c}$ | $1.02\pm0.05^{b}$ | $1.03\pm0.04^{b}$ |
| K-factor | 0.62±0.02 | 0.67±0.02 | 0.57±0.01 | 0.64±0.01 |
| SR (%) | 95.55±0.02 | 93.33±0.03 | 93.33±0.05 | 95.55±0.04 |

**Remarks:** Values are mean±SEM ($n$=9). Different superscripts ($^{a\text{-}c}$) are significant differences between the control and experimental groups ($P<0.05$).
IL, initial length (cm); FL, final length (cm); IW, initial weight (g); FW, final weight (g); WG, weight gain (g); SGR, specific growth rate (%/day); ADG, average daily gain (g/day); FCR, feed conversion ratio; K-factor, condition factor; SR, survival rate (%).

*3.2 Intestinal morphology*

The effects of dietary BP on intestinal morphology of catfish are illustrated in Figure 1 and the data are demonstrated in Table 2. The results indicated that dietary supplementation of $1\times10^7$ CFU/g significantly improved villi width and villi height in all parts of the intestines ($P<0.05$). In addition, total muscular thicknesses of middle and distal parts of the intestines were significantly enhanced in fish fed the diets containing $1\times10^6$ and $1\times10^7$ CFU/g ($P<0.05$). Moreover, goblet cell number observed in anterior and posterior parts of intestines of fish fed the diet incorporated with $1\times10^7$ CFU/g were significantly greater than those of fish fed the basal diet ($P<0.05$). Interestingly, microvillus height was significantly increased in the middle intestine of fish fed $1\times10^7$ CFU/g when compared to the control ($P<0.05$).

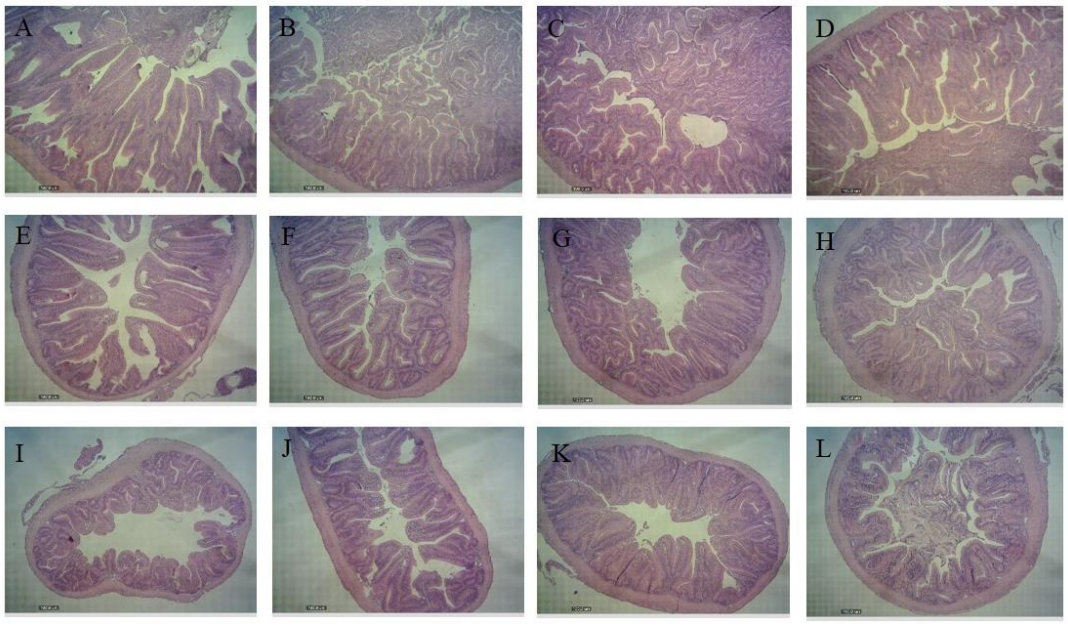


**Figure 1** Histological sections of anterior (A-D), middle (E-H) and posterior (I-L) intestines of catfish fed the diets supplemented with 0 (A, E, I), $1\times10^6$ (B, F, J), $1\times10^7$ (C, G, K) and $1\times10^8$ (D, H, L) CFU/g for 8 weeks (Scale bar = 100 μm).

**Table 2** Intestinal morphology of catfish fed the diets supplemented with BP for 8 weeks.

| Parameters | BP levels in the experimental diets (CFU/g) | | | |
|---|---|---|---|---|
| | 0 | $1\times10^6$ | $1\times10^7$ | $1\times10^8$ |
| Anterior part of the intestines | | | | |
| Villi height (μm) | 430.70±7.36$^{a}$ | 510.85±6.53$^{ab}$ | 630.14±5.23$^{b}$ | 519.87±4.31$^{ab}$ |
| Villi width (μm) | 110.73±6.52$^{a}$ | 117.50±5.26$^{b}$ | 125.80±4.56$^{b}$ | 98.03±6.37$^{a}$ |
| Total muscular thickness (μm) | 92.52±8.56$^{ab}$ | 105.32±7.88$^{b}$ | 87.10±5.16$^{a}$ | 71.65±4.63$^{a}$ |
| Inner circulatory smooth muscle thickness (μm) | 54.69±5.39 | 66.53±6.10 | 58.97±4.50 | 47.262±2.62 |
| Outer longitudinal smooth muscle thickness (μm) | 30.31±2.16 | 38.52±5.22 | 32.46±3.48 | 28.89±0.85 |
| Microvilli height (μm) | 1.11±0.04 | 1.25±0.13 | 1.87±0.08 | 1.56±0.21 |
| Goblet cell number | 7.73±0.56$^{a}$ | 8.24±0.47$^{a}$ | 10.11±0.43$^{b}$ | 9.70±0.41$^{b}$ |
| Middle part of the intestines | | | | |
| Villi height (μm) | 349.06±4.23$^{a}$ | 420.62±5.68$^{ab}$ | 498.79±6.89$^{b}$ | 451.41±7.23$^{b}$ |
| Villi width (μm) | 88.01±6.90$^{a}$ | 89.17±8.16$^{a}$ | 111.08±6.89$^{b}$ | 110.01±8.16$^{ab}$ |
| Total muscular thickness (μm) | 73.02±5.75$^{a}$ | 86.21±6.07$^{b}$ | 90.62±4.56$^{b}$ | 89.65±6.42$^{b}$ |
| Inner circulatory smooth muscle thickness (μm) | 37.33±3.12$^{ab}$ | 49.97±4.61$^{b}$ | 50.51±2.72$^{b}$ | 61.97±7.76$^{c}$ |
| Outer longitudinal smooth muscle thickness (μm) | 35.89±4.05 | 36.18±2.63 | 35.90±4.67 | 37.23±3.97 |
| Microvilli height (μm) | 1.09±0.10$^{a}$ | 1.41±0.15$^{a}$ | 2.04±0.11$^{b}$ | 1.11±0.14$^{a}$ |
| Goblet cell number | 9.15±0.70 | 9.86±0.40 | 10.30±0.62 | 9.65±0.48 |
| Posterior part of the intestines | | | | |
| Villi height (μm) | 345.60±6.29$^{a}$ | 385.69±7.26$^{a}$ | 470.23±5.26$^{c}$ | 380.19±7.22$^{b}$ |
| Villi width (μm) | 89.81±7.24$^{a}$ | 113.77±7.87$^{b}$ | 112.69±7.89$^{b}$ | 110.18±5.81$^{b}$ |
| Total muscular thickness (μm) | 80.11±11.90$^{a}$ | 104.23±6.71$^{b}$ | 110.62±3.90$^{b}$ | 76.96±5.33$^{a}$ |
| Inner circulatory smooth muscle thickness (μm) | 47.95±6.20$^{a}$ | 67.07±2.55$^{b}$ | 68.44±6.79$^{b}$ | 42.94±2.97$^{a}$ |
| Outer longitudinal smooth muscle thickness (μm) | 39.88±3.99 | 37.22±4.51 | 46.85±5.02 | 34.40±3.27 |
| Microvilli height (μm) | 1.37±0.14 | 1.32±0.09 | 1.98±0.11 | 1.41±0.18 |
| Goblet cell number | 7.80±0.08$^{a}$ | 8.60±0.07$^{ab}$ | 9.61±0.04$^{b}$ | 9.44±0.01$^{b}$ |

**Remarks:** Values are mean±SEM ($n$=3). Different superscripts ($^{a-c}$) are significant differences between the control and experimental groups (P<0.05).

*3.3 Hematology*

The effect of dietary BP on some hematological indices of catfish is showed in Table 3. Hematological evaluation showed supplementation of $1\times10^7$ and $1\times10^8$ CFU/g in the fish diets significantly increased red blood cell, white blood cell, lymphocyte and neutrophil compared to the control diet ($P<0.05$). Additionally, monocyte, eosinophil and basophil in fish fed the experimental diets were tended to increase but did not reach to statistical significant when compared to the basal diet ($P>0.05$).

**Table 3** Some hematological indices of catfish fed the diets supplemented with BP for 8 weeks.

| Parameters | BP levels in the experimental diets (CFU/g) | | | |
|---|---|---|---|---|
| | 0 | $1\times10^6$ | $1\times10^7$ | $1\times10^8$ |
| RBC ($\times10^{12}$/L) | $1.26\pm0.18^a$ | $1.42\pm0.23^{ab}$ | $1.52\pm0.22^b$ | $1.96\pm0.25^b$ |
| WBC ($\times10^5$cells/mm$^3$) | $8.00\pm0.06^a$ | $10.2\pm0.52^{ab}$ | $11.1\pm0.01^b$ | $8.10\pm0.07^a$ |
| Neutrophil (%) | $0.55\pm0.23^a$ | $1.22\pm0.36^b$ | $1.00\pm0.37^b$ | $1.11\pm0.17^b$ |
| Lymphocyte (%) | $60.11\pm2.35^a$ | $61.00\pm2.14^a$ | $66.23\pm2.67^b$ | $67.66\pm2.38^b$ |
| Monocyte (%) | 16.44±1.31 | 19.66±2.32 | 19.44±2.72 | 19.88±1.42 |
| Eosinophil (%) | 0.22±0.14 | 0.88±0.30 | 0.44±0.17 | 0.77±0.36 |
| Basophil (%) | 3.44±0.66 | 4.66±1.50 | 3.87±0.66 | 4.66±0.47 |

**Remarks:** Values are mean±SEM ($n$=3). Different superscripts ($^{a-c}$) are significant differences between the control and experimental groups ($P<0.05$).
RBC, red blood cell ($\times10^{12}$/L); WBC, white blood cell ($\times10^5$cells/mm$^3$).

**4. Discussion**

The results of this present study showed that fish fed the diets supplemented with BP for 8 weeks significantly improved growth parameters, intestinal histology and some hematological indices when compared to the basal diet. The data also suggested the potential use of BP as a probiotic in fish cultivation.

*4.1 Growth performance*

Growth promoting properties produced several types of probiotics in aquaculture production have been reported [3-5]. There is evidence that striped catfish received the diets mixed with *B. amyloliquefaciens* 54A and *B. pumilus* 47B for 90 days significantly increased final weight and WG compared to the control, but both strains did not affect FCR and SGR values [6]. Also, WG, final weight and SGR of Nile tilapia fed the diet containing *B. licheniformis* significantly improved when compared to the basal diet [15]. It has been exhibited that SGR of catfish fed dietary *B. megaterium* PTB 1.4 was higher than fish fed the control diet [7]. However, not all probiotics affect growth of fish [9]. The mechanisms of stimulatory effect of probiotics on growth of fish are poorly understood. Previous report indicated that *B. pumilus* can activate the activity of certain digestive enzymes such as protease, amylase and cellulase in order to enhance digestion and absorption of essential nutrients in the digestive tract of Rohu fingerlings [8]. Furthermore, Afrilasari et al. [7] noticed that catfish fed the diets containing probiotic *B. megaterium* PTB 1.4 showed a significant increase in digestive enzyme activity of protease and amylase. These reasons could lead to support the growth promoting effects of probiotic in this present study. It has been revealed that probiotic supplementation could provide numerous growth factors like vitamins, fatty acids and amino acids to support the growth of fish [4 & 5]. It is possible to hypothesize that other modes of action of probiotics may possibly be due to the competition for nutrients, adhesion sites and colonization against such opportunistic pathogens in the alimentary canal, causing a decrease in the prevalence of gastrointestinal diseases [4 & 5]. It is indicated that probiotics can produce antimicrobial agents such as bacteriocins or activate intestinal cells to produce modullins to modulate host immune responses [20]. Thus, these could be the reasons why the application of probiotic in the diet can improve growth and general well-beings of cultured fish.

*4.2 Intestinal morphology*

In this research, villi heights, villi width and muscular thickness of fish fed the diets supplemented with probiotic were significantly higher than that of the untreated fish. Similar results were demonstrated that Nile tilapia fed the diets incorporated with *B. amyloliquefaciens* for 60 days increased intestinal villi height in all parts of the intestines [21]. The modes of action of probiotic on villi height and thickness of the intestine are still unclear. It is well established that increased intestinal villi heights and widths might be attributed to the propagation of probiotics and the attachment of probiotics on the apical surface of epithelial cells in the digestive tract to improve carbohydrate utilization and short chain fatty acid production [21-24]. Such reports revealed that short chain fatty acids produced by probiotics can modulate the metabolism of intestinal epithelial cells [21-24].

Also, short chain fatty acids can stimulate cell proliferation and cell division in the gastrointestinal tracts through the production of some growth factor or certain peptides, leading to an increase in villus height [21-24]. It is generally accepted that increased intestinal villi height and width provide greater surface areas for nutrient absorption [21-24]. Moreover, the functions of muscular layers in the intestines are associated with nutrient absorption, defecation and moisture reabsorption [21]. Thus, these reasons would lead to support the potential use of BP to enhance growth.

Goblet cells were significantly increased in the experimental groups in comparison with the control group. These results indicated that dietary BP could affect the growth performance of fish through the modulation of goblet cell proliferation in the digestive tract [4,14,21&22]. Reda and Selim [21] described that the highest goblet cell number was found in Nile tilapia fed the diet containing probiotic *B. amyloliquefaciens*. Furthermore, Silva et al. [25] demonstrated that Nile tilapia reared in the cage fed the diets supplemented with *B. amyloliquefaciens* for 90 days showed a significant increase in the number of goblet cell per villi in comparison with the control. It is well-known that mucus contents produced by goblet cells play a key role in the modulation of digestive enzyme functions, the prevention of intestinal cell surface from physical and chemical digestions as well as the protection of the gastrointestinal tract from several pathogenic microorganisms [22,25&26]. It is assumed that increased goblet cell number displayed by fish fed the diet containing BP in this present study could be due to the effects of such growth factors, cytokines or other products produced by this probiotic on cell division and cell differentiation [21 & 22,25]. However, further investigations are required to fully understand the effects of probiotics on goblet cell number in the intestine of fish.

Microvilli are found on the apical surface of intestinal epithelium cell to provide the surface area of digestion and absorption of essential nutrients [14,27&28]. Additionally, the activities of digestive enzymes are also active in these areas [14,27]. However, few reports have focused on the effects of probiotics on intestinal microvilli of fish [14,27&28]. Merrifield et al. [27] presented that a significant increase in microvilli height in the proximal region of the intestines was observed in rainbow trout (*Oncorhynchus mykiss*) fed the diet mixed with *Pediococcus acidilactici* for 5 weeks. It was suggested that such ingredients produced by probiotics could affect cell proliferation, migration and turnover in the digestive tract, resulting in an increase in microvilli height [28]. For this reason, it is possible to hypothesize that BP may secret some active compounds or fermented products that consequently modulate cell proliferation or cell division directly or indirectly, resulting in increased microvilli height as observed in this present investigation [28].

*4.3 Hematology*

The functions of RBC are to transport oxygen to target tissues. The numbers and types of WBC are considered as good indicators of fish health [21,25]. It was reported that dietary supplementation with probiotics can enhance RBC and WBC counts in many species of cultured fish, indicating that probiotics possess immunostimulant effects on fish [18,29&30]. Similar results were indicated that oscar (*Astronotus ocellatus*) fingerlings fed the diet mixed with probiotic for 60 days significantly improved RBC, WBC, lymphocyte, monocyte and neutrophil when compared to the control [18]. Moreover, juvenile Nile tilapia cultured in cages fed the diets containing probiotics for 127 days significantly increased hemoglobin, hematocrit and neutrophil and tended to enhance RBC and WBC, but not statistically significant in comparison with the basal diet [31]. Channel catfish fed the diet containing 2% yeast polysaccharide significantly elevated monocyte when compared to control [19]. The mechanisms whereby probiotics improve RBC and the immune functions as well as disease resistance are not fully understood. There is evidence that an increase in the number of RBC in fish fed the diet mixed with probiotic might be due to the improvement of fish metabolism by probiotic and subsequently a rise in oxygen consumption [18]. Thus, increased RBC count would lead to the elevation of hemoglobin levels to support a high oxygen carrying capacity in fish fed the probiotic-supplemented diet [18]. Previous reports have shown that probiotic supplementations induce peripheral immune responses by raising the expression of some regulatory cytokines in fish such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) [2-5,20]. Additionally, probiotics could interact with gut-associated lymphoid tissue (GALT) to induce the enhancement of Ig+ cells, acidophilic granulocytes and T-cell counts [20,22]. However, it is informed that not all probiotics affect immune responses in fish [21,25]. Thus, further work is necessary to examine the effects of probiotics on fish immunity. This present study demonstrated that the application of BP to the fish diets significantly increased the levels of RBC, WBC, neutrophil and lymphocyte compared to the control fish diet. Thus, these results supported the potential immunostimulant property of BP in fish.

## 5. Conclusion

To the best of our knowledge, this present study is the first report to support the use of BP in diets as a probiotic in order to enhance growth, intestinal morphology and hematology of catfish. The result also suggested that the optimum dietary level of BP for catfish cultivation was $1\times10^7$ CFU/g.

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