

การใช้แบคทีเรีย *Bacillus* spp. เป็นโปรไบโอติกผสมในอาหารเลี้ยงปลาช่อนนัยนา เสนาศรี^{1, #} จิตรา สิมาวาน¹ และสุกัญญา คำหล้า¹¹คณะทรัพยากรธรรมชาติ มหาวิทยาลัยเทคโนโลยีราชมงคลธัญบุรี วิทยาเขตสกลนคร 47160

บทคัดย่อ: การทดลองใช้แบคทีเรียกลุ่ม *Bacillus* spp. เป็นโปรไบโอติกในการเลี้ยงปลาช่อน มีวัตถุประสงค์เพื่อศึกษา อัตราการเจริญเติบโต อัตราการรอดตาย และปริมาณที่เหมาะสมในการเลี้ยงปลาช่อน โดยทำการเลี้ยงปลาช่อนขนาดน้ำหนักเริ่มต้นที่ 2.50-3.50 กรัมต่อตัว ในถังไฟเบอร์ขนาด 250 ลิตร จำนวน 3 ซ้ำ ใช้แบคทีเรีย *Bacillus* spp. ในระดับประมาณเชื้อ 3.0×10^7 CFU/g ผสมกับอาหารเม็ดสำเร็จรูปในปริมาณที่แตกต่างกัน คือ 0 2 4 และ 6 กรัมต่ออาหาร 1 กิโลกรัม ทำการเลี้ยงเป็นระยะเวลา 60 วัน ผลการทดลองพบว่า น้ำหนักที่เพิ่มขึ้น ความยาวที่เพิ่มขึ้น และอัตราการเจริญเติบโตของปลาช่อนในชุดทดลองที่ได้รับแบคทีเรีย *Bacillus* spp. ปริมาณ 6 กรัมต่ออาหาร 1 กิโลกรัม ให้ผลดีที่สุด โดยน้ำหนักที่เพิ่มขึ้นเท่ากับ 6.62 ± 0.44 กรัมต่อตัว ความยาวที่เพิ่มขึ้น 3.13 ± 0.45 เซนติเมตรต่อตัว และอัตราการเจริญเติบโตเท่ากับ 2.06 ± 0.02 เปอร์เซ็นต์ต่อวัน ซึ่งมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) กับชุดทดลองอื่นๆ ส่วนอัตราการรอดตายไม่มีความแตกต่างทางสถิติในแต่ละชุดทดลอง สำหรับการวิเคราะห์ค่าคุณภาพน้ำในการเลี้ยงปลาช่อน พบว่า การใช้แบคทีเรีย *Bacillus* spp. กับกลุ่มควบคุมไม่แตกต่างกันทางสถิติ ($P > 0.05$) จากผลการศึกษาดังกล่าวการใช้แบคทีเรีย *Bacillus* spp. ที่ระดับ 6 กรัมต่ออาหาร 1 กิโลกรัม เป็นปริมาณที่เหมาะสมที่สุดในการนำมาใช้เป็นโปรไบโอติกผสมกับอาหารเลี้ยงปลาช่อน เนื่องจากทำให้ปลาช่อนมีอัตราการเจริญเติบโต ทั้งน้ำหนักเฉลี่ย ความยาวเฉลี่ยดีที่สุด

คำสำคัญ: แบคทีเรีย เชื้อบาซิลลัส โปรไบโอติก ปลาช่อน

#ผู้รับผิดชอบบทความ

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The Use of *Bacillus* spp. as Probiotic in Feed on Snake-head Fish (*Channa striata*) Culture

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Abstract: The experiment was conducted to determine the optimum level of concentration of probiotic supplement, *Bacillus* spp., in feed on growth rate and survival rate of snake-head fish (*Channa striata*). Snake-head fishes with the range of initial weights during 2.50-3.50 g were raised in 250-liter fiberglass tanks, 3 replications per treatment. *Bacillus* spp. (3.0×10^7 CFU/g) in different levels as 0, 2, 4 and 6 g were added to 1 kg of commercial diet to feed fish and then he fished were offered he diet for 60 days. The result showed that using 6 g/kg feed of *Bacillus* spp. was the best treatment for increasing the average of weight gain, length gain and growth rate of the studied fishes as 6.62 ± 0.44 g/fish, 3.13 ± 0.45 cm/fish and 2.06 ± 0.02 %/day, respectively, with a statistically significant difference ($P < 0.05$). However, it was not found the difference of the survival rate. The result of water quality analysis exhibited no any statistically significant difference among the studied groups ($P > 0.05$). In conclusion, using 6 g/kg feed of *Bacillus* spp. as feed additive on snake-head fish culture was the best choice for increasing weight gain, length gain, and growth rate of snake-head fishes.

Keywords: Bacteria, *Bacillus* spp., Probiotic, Snake-head fish (*Channa striata*)

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Introduction

Snake-head fish (*Channa striata*) is one of economic aquatic animals of Thailand. The fish were cooked for several dishes such as sour paste with tamarind, hot and spicy

soup (Tom yum), fried and grilled. Moreover, they can be salt dried or fermented for food preservation (Rojanapitag, 2006). For this reason, they are very expensive and nowadays the consumer demand is still

increasing. Hence, to increase fish productivity, aquaculture technology should be applied for fish culture. One of the technologies is the use of probiotic for fish feeding. Using probiotic mixed with animal feed can enhance nutrient digestibility, reduce stress and improve their reproduction, as well as be tolerant to diseases of livestock (Villamil et al., 2003).

Therefore, *Bacillus* spp. as probiotic in feed was conducted to determine the optimal level to be used as snake-head fish probiotic supplementation. Fish were also evaluated for their weight gain, length gain and growth rate. In addition, water qualities were also determined.

Materials and Methods

This experiment was assigned according to completely randomized design (CRD) with 3 replications (80 snakehead fish/ replication). Powder of *Bacillus* spp. containing of *Bacillus subtilis* and *Bacillus licheniformis* with 3.0×10^7 CFU/g was mixed with 5% of fish oil before being dried at 80°C for 30 minutes. It was blended with feed

pellets and then it was fed to animals. (Kongkrajang and Laohavisuti, 2011)

Fish were raised in 250 liters of 12 fiberglass tanks. The tanks were cleaned and dried for 2 days then they were added of 150 liters of tap water. Oxygen was provided to the tanks by the oxygen pump for 3-4 days prior starting the experiments.

Fish with initial weight of 2.50-3.50 g were fed with feed twice a day (8.30 am and 4.00 pm) for 60 days. The conditions were described below:

Group 1 feeding with fish feed without *Bacillus* spp. (control group)

Group 2 feeding with fish feed and 2 g of *Bacillus* spp. per 1 kg of fish feed

Group 3 feeding with fish feed and 4 g of *Bacillus* spp. per 1 kg of fish feed

Group 4 feeding with fish feed and 6 g of *Bacillus* spp. per 1 kg of fish feed

Fish were measured for weight gain, length gain and growth rate before the experiments was started and once a week after the experiment was begun unit 60 days by using the equation as below:

Increasing weight (gram/fish) = the average weight at the end - the average weight at the beginning

Increasing length (centimeter/fish) = the average length at the end - the average length at the beginning

Specific growth rate (SGR; %/day) = $\frac{(\text{Log}_{10} \text{ average weight at the end} - \text{Log}_{10} \text{ average weight at the beginning}) \times 100}{\text{The time for experiment}}$

$$\text{Survival rate (\%)} = \frac{\text{The amount of fish at the end of experiment} \times 100}{\text{The amount of fish at the beginning}}$$

In addition, to control water quality, water temperature ($^{\circ}\text{C}$), pH, dissolved oxygen (mg/L), ammonia (mg/L), nitrite (mg/L) and nitrate (mg/L) were routine evaluated.

One-Way ANOVA was selected for statistical analysis for specific growth rate, survival rate, water quality. The significantly difference is at 95% by Duncan's new multiple range test (MRT).

Results and Discussion

In this study, the snake-head fishes fed with 6 g/kg of *Bacillus* spp. exhibited average increased weight (6.62 ± 0.44 g/fish) had higher value than those in the control group (3.76 ± 0.41 g/fish) with statistically significant difference ($P < 0.05$) and also showed higher average length (3.13 ± 0.45 cm/fish) than those in the control group (1.94 ± 0.30 cm/fish) with statistically significant difference ($P < 0.05$) (Table 1 and 2). These results agreed with those reported of Auerbim (2005) who studied the use of *Bacillus subtilis* in snake-head fish and found the use of *Bacillus subtilis* in snake-head fishes culture could increase average weight (32.89 g/fish) when compared with the snake-head fishes offered the diet without

Bacillus subtilis (14.96 g/fish). These results agreed with those reported of Wang (2007) that the dietary supplementation of *Bacillus* spp. to white shrimps (*Litopenaeus vannamei*) for 28 days, could increase weight gain of the shrimps. Moreover, the mixture of photosynthetic bacteria and *Bacillus* spp. from fancy Carp (*Cyprinus carpio*) culture, could increase final weight of white shrimps (1.71 ± 0.06 g) when compared the shrimps in the control group (1.57 ± 0.05 g). From the current study, the specific growth rate of the snake-head fishes supplemented with *Bacillus* spp. at level of 6 g/kg feed (2.06 ± 0.02 %/day) was higher than those in the control group (1.63 ± 0.04 %/day) with statistically significant difference ($P < 0.05$) (Table 3).

The snake-head fishes are natural carnivores. They can scavenge for food over a wide area, such as small fishes and water insects. Thus, when there was lack of food, the smaller snake-head fishes could not be survived from eating of the bigger ones. Hence, their survival rate was decreased (Wiwatnachaichat, 1998) and fluctuated in this study (Table 3).

Table 1 Average weight gain (Mean±SD) of the snake-head fishes in the current study

<i>Bacillus</i> spp. (g/kg feed)	Initial body weight (g/fish)	Final weight (g/fish)	Increased weight (g/fish)
0	3.41±0.30	7.18±0.15 ^c	3.76±0.41 ^c
2	2.96±0.47	8.51±0.67 ^b	5.55±0.77 ^b
4	2.93±0.62	8.04±0.88 ^{bc}	5.11±0.29 ^b
6	3.23±0.40	9.85±0.07 ^a	6.62±0.44 ^a

^{abc}Mean values with different superscripts in the same column were significantly different ($P<0.05$)

Table 2 Average length gain (Mean±SD) of the snake-head fishes in the current study

<i>Bacillus</i> spp. (g/kg feed)	Initial length (cm/fish)	Final length (cm/fish)	Increased length (cm/fish)
0	7.52±0.30	9.47±0.13 ^c	1.94±0.30 ^b
2	7.31±0.31	10.33±0.22 ^{ab}	3.02±0.30 ^a
4	7.20±0.49	10.13±0.35 ^b	2.92±0.19 ^a
6	7.57±0.29	10.71±0.19 ^a	3.13±0.45 ^a

^{abc}Mean values with different superscripts in the same column were significantly different ($P<0.05$)

Table 3 Specific growth rate and survival rate (Mean±SD) of the snake-head fishes in the current study

<i>Bacillus</i> spp. (g/kg feed)	Specific growth rate (%/day)	Survival rate (%)
0	1.63±0.04 ^c	47.00±9.16
2	1.89±0.10 ^b	41.66±10.59
4	1.83±0.08 ^b	43.33±11.93
6	2.06±0.02 ^a	53.00±4.35

^{abc}Mean values with different superscripts in the same column were significantly different ($P<0.05$)

The results of water analysis have shown that no statistically significant difference ($P>0.05$) among the experiment groups (table 4). The average of dissolved oxygen, water temperature, water pH, nitrite level in water, nitrate level in water and ammonia level in water were ranged from 4.55 to 5.26 mg/L, 26.25 to 26.52 °C, 6.18 to 6.70, 0.02 to 0.02 mg/L, 14.44 to 17.22 mg/L and 1.06 to 1.48 mg/L, respectively. Thus,

Table 4 Water quality (Mean±SD) in fiber tanks cultured the snake-head fishes in the current study

Parameters	<i>Bacillus</i> spp. (g/kg feed)			
	0	2	4	6
Temperature (C°)	26.29±0.32	26.36±0.06	26.25±0.12	26.52±0.17
pH	6.18±0.37	6.57±0.41	6.70±0.08	6.59±0.37
O ₂ (mg/L)	4.94±0.53	5.26±0.53	5.04±0.86	4.55±0.33
Nitrite (mg/L)	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Nitrate (mg/L)	15.55±3.09	14.81±3.69	17.22±1.11	14.44±0.55
Ammonia (mg/L)	1.18±0.35	1.48±0.91	1.06±0.63	1.14±0.14

our study exhibited no effect of water quality on fish feeding and responses.

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Conclusion

The results of present study showed that using 6 g of 3.0×10^7 CFU/g *Bacillus* spp. in 1 kg of feed is appropriate for feeding snake-head fishes. The dietary supplementation of *Bacillus* spp. would improve specific growth rate, weight gain and length gain in snake-head fishes. Moreover, their survival rates were increased. An abstract version of this research study was published in the abstract proceedings of the 5th International Conference on Sustainable Animal Agricultural for Developing Countries (SAADC 2015).

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