



## Supplementation of spirulina (*Arthrospira platensis*) improves physical activeness level of the catfish (*Clarias gariepinus*)

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### Abstract

This study determines the effects of spirulina supplementation on the physical activeness level in cultured catfish. Five experimental diets containing different percent of spirulina (0% (control), 1%, 3%, 5% and 7%) were fed daily to a total of 30 catfish fry/cage (in triplicate) at 5% body weight, for a period of 105 days. The level of physical activeness was determined by giving a score during daily feeding for 90 consecutive days, fortnight handling for total body weight and length measurement, and feeding during *Aeromonas hydrophila* challenge experiments. At daily feeding and handling for body weight and length measurement, groups of catfish supplemented with spirulina scored very active level of physical activeness, while the control group catfish score was normal. The physical activeness level during *A. hydrophila* challenge showed that the catfish with spirulina supplementation scored better physical activeness compared to catfish with control diet. This study concludes that supplementation of spirulina as low as 1% in feed will improve physical activeness level of the catfish. Moreover, spirulina helps in the motivation for feeding during non-infection and infection by bacterial pathogen.

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## Introduction

Spirulina (*Arthrospira platensis*) is a natural feed ingredient (Radhakrishnan *et al.*, 2014). There are various applications of spirulina, including cooperated cosmetic ingredients, biodiesel, food supplement and also as animal feeds (Becker, 2007). Previous studies revealed that supplementation of spirulina in feeds were known to improve nutritional composition (Brown *et al.*, 1997; Natrah *et al.*, 2007), giving probiotic effects and stimulates the immunity of the cultured aquatic animals (Muller-Feuga, 2000; Sirakov *et al.*, 2012). Spirulina is rich in protein, essential amino acids, vitamins, minerals and anti-oxidant pigments that make it a suitable candidate for supplement in aquaculture (Certik and Shimizu, 1999; Radhakrishnan *et al.*, 2014).

A study showed a strong relationship between the health of fish with high level of physical activeness (Martins *et al.*, 2006). In fact, the physical activeness level is one of the cheapest and fast recognition on the health of the reared fish (Fatihah *et al.*, 2017). However, less was known on the effect of spirulina on the physical activeness level of the fish. Understanding on this aspect will helps the researchers improving the formulated feeds. This study determines the effects of spirulina supplementation on the physical activeness level of the cultured catfish. It is hope that the results from this study would promote the utilization of spirulina in aquatic animal farming.

## Materials and methods

### *Catfish's acclimatization*

A total of 450 healthy catfish (*Clarias gariepinus*) fry were purchased from local fish supplier and acclimatized for two weeks. The fish were fed *ad libitum* with control diet (Cargill, Malaysia) daily. At the end of the acclimatization period, the catfish fry were randomly placed into the five cages with 30 catfish fry/cage and in triplicate for each diet treatment.

### *Experimental diets*

The experimental diets preparation was conducted as

previously described (Fatihah *et al.*, 2017). Briefly, five experimental diets were produced and used in the study by manipulating the percentage of spirulina at 0% (control), 1%, 3%, 5% and 7% in the commercial fish feed (Cargill, Malaysia). The feeds were then given to the fish throughout the 104 days of study period, at 5% of body weight daily.

### *Challenge of catfish with A. hydrophila*

The bacterial challenged procedures on catfish were conducted as previously described (Fatihah *et al.*, 2017). Briefly, *Aeromonas hydrophila* culture stock was obtained from Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, UPM Serdang, Selangor. The isolate code is AhSa5 and was previously isolated from diseased catfish during *A. hydrophila* outbreak. At 91 days of experimental feeding period, all of the experimental catfish were intraperitoneally challenged with 10<sup>7</sup> CFU/ml of virulent *A. hydrophila*. The catfish aggressiveness level was then observed for the following 14 days, as described below.

### *Monitoring of catfish's aggressiveness level*

The catfish's aggressiveness level was monitored and scored at three different sampling times. The first observation was conducted daily for 90 consecutive days, during daily experimental feedings (8:00 am and 5:00 pm). The second observation were conducted at fortnight interval until 90 days of experimental period, specifically during handling for body weight and length measurement (data not shown; n = 45 for each group), while the third observation was conducted during daily feeding (8:00 am and 5:00 pm) following *A. hydrophila* infection (day 91 to 105), for a total of 10 minutes observation for each group.

In each observation, the scoring for physical activeness level of the catfish was scored by two people for better accuracy of the results and the data were recorded as mean from both observers. The physical activeness level of the catfish was scored from 0 (not active) to 10 (most active), as modified from Martins *et al.* (2008) (Table 1).

### Statistical analysis

Statistical analysis was conducted by analyzing the mean and SEM of the data. Significant differences ( $p < 0.05$ ) for the overall aggressiveness level between each treatment groups were determined using the one-way ANOVA with Turkey Post Hoc Multiple Comparison.

### Results

The physical activeness score of the catfish based on

different spirulina supplementation in the feed are presented in Table 2. Catfish with 0% inclusion of spirulina showed a normal level of behavior during daily experimental feeding ( $5.98 \pm 0.04$ ) and handling for body mass and length measurement ( $5.78 \pm 0.04$ ). However, there were significant different ( $p < 0.05$ ) between the scores of both stated sampling time in control group compared to other experimental diet groups.

**Table 1.** Scoring guidelines for the physical activeness of the catfish following supplementation with different percentage of spirulina.

Score	Definition of the score	Sampling time		
		Daily experimental feeding	Handling for body mass and length measurement	<i>A. hydrophila</i> infection challenge
0	Non active/dead	Feeding process complete within or more than ten minutes	Measurement process complete less than one minutes	Feeding process complete within or more than ten minutes
1	Critical	Feeding process complete within nine minutes	Measurement process complete within one minutes	Feeding process complete within nine minutes
2	Barely active	Feeding process complete within eight minutes	Measurement process complete within two minutes	Feeding process complete within eight minutes
3	Weak	Feeding process complete within seven minutes	Measurement process complete within three minutes	Feeding process complete within seven minutes
4	Less active	Feeding process complete within six minutes	Measurement process complete within four minutes	Feeding process complete within six minutes
5	Normal	Feeding process complete within five minutes	Measurement process complete within five minutes	Feeding process complete within five minutes
6	Normal-very active	Feeding process complete within four minutes	Measurement process complete within six minutes	Feeding process complete within four minutes
7	Very active	Feeding process complete within three minutes	Measurement process complete within seven minutes	Feeding process complete within three minutes
8	Very-most-active	Feeding process complete within two minutes	Measurement process complete within eight minutes	Feeding process complete within two minutes
9	Most active	Feeding process complete within one minute	Measurement process complete within nine minutes	Feeding process complete within one minute
10	Non-control activeness	Feeding process complete less than one minute	Measurement process complete within or more than ten minutes	Feeding process complete less than one minute

The catfish supplemented with 1% to 7% spirulina scored very high physical activeness level ( $7.04 \pm 0.06$  to  $7.16 \pm 0.06$ ) during daily experimental feeding, and scored from normal to very active ( $6.52 \pm 0.05$  to  $6.71 \pm 0.06$ ) during handling for body mass and length measurement, without any significant different ( $p > 0.05$ ) between the spirulina supplementation groups.

The physical activeness score during the feeding after *A. hydrophila* challenge showed that the catfish were

barely active. Catfish supplemented with 0% of spirulina scored with lowest physical activeness ( $1.18 \pm 0.48$ ), and significantly low ( $p < 0.05$ ) compared to other spirulina supplemented groups ( $1.64 \pm 0.08$  to  $2.21 \pm 0.13$ ).

### Discussion

This study showed that the catfish supplemented with spirulina were very active in two different observation periods, which were during daily experimental feeding and during handling for body mass and

length measurement when compared to catfish which not supplemented with spirulina. As suggested by Harel *et al.* (2002) and Palmegiano *et al.* (2005), these observations might be due to high protein and energy source in spirulina, which make the catfish

more active and energetic. Study by Martins *et al.*, (2008) found that the aggressiveness in term of activeness of catfish influence by its efficiency in term of nutrient use, and the healthy fish usually associated with higher activeness level.

**Table 2.** Catfish physical activity score during different feeding observation times.

Percentage of spirulina	Sampling time		
	Daily experimental feeding	Handling for body mass and length measurement	<i>A. hydrophila</i> infection challenge
0	5.98±0.04 <sup>a</sup>	5.78±0.04 <sup>a</sup>	1.18±0.48 <sup>a</sup>
1	7.16±0.06 <sup>b</sup>	6.61±0.06 <sup>b</sup>	2.20±0.12 <sup>c</sup>
3	7.11±0.06 <sup>b</sup>	6.59±0.07 <sup>b</sup>	2.19±0.12 <sup>c</sup>
5	7.04±0.06 <sup>b</sup>	6.52±0.05 <sup>b</sup>	1.64±0.08 <sup>b</sup>
7	7.14±0.06 <sup>b</sup>	6.71±0.06 <sup>b</sup>	2.21±0.13 <sup>c</sup>

<sup>a,b</sup>Different superscripts indicate significant difference ( $p < 0.05$ ) of the same column. Values are presented in mean ±SEM.

Martins *et al.* (2008) suggested that when a fish is efficient in the nutrient use, it will allow faster and shorter term of metabolic responses in fulfilling the energetic demands imposed by aggression. This statement explained that spirulina helps in the efficiency of the nutrient use by the catfish, thus exhibited high activeness level compared to non-spirulina supplemented catfish. Besides, Martins *et al.* (2008) stated that individual fish that are highly motivated to eat shown to be highly efficient and more active. As a result, catfish supplemented with spirulina in this study showed high level of activeness during the feeding which indicates that they are highly motivated for feeding.

The physical activity score of the catfish during feeding in the period of *A. hydrophila* challenge showed that all the catfish were categories as barely active, which the activity of the catfish were greatly reduced due to the infection of the bacteria. Even so, the level of physical activity of catfish supplemented with spirulina was higher compared to the control group. This observation related to the probiotic property of spirulina and the presence of phycoerythrin in stimulating the immunity of catfish against the bacteria challenge (Vonshak, 1997; Bhowmik *et al.*, 2009; Promya and Chitmanat, 2011).

## Conclusion

This study concludes that supplementation of spirulina as low as 1% in feed will improve physical activeness level of the catfish. Moreover, spirulina helps in the efficiency of nutrient use by the catfish and increases the motivation for feeding during non-infection and infection by bacterial pathogen.

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