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GROWTH PERFORMANCES AND BACTERIAL LOAD OF *HETEROPNEUSTES FOSSILIS* (BLOCH, 1794) USING CINNAMON AS FEED SUPPLEMENT

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Abstract: Cinnamon has different biological properties such as antioxidant, antimicrobial, antidiabetic and antiallergic. This study was carried out to investigate the effect of cinnamon as feed supplement on growth performances and bacterial load of *Heteropneustes fossilis*. Twenty fries were stocked in each 60 litre aquarium for a rearing period of 90 days. Commercial diet was used that contains 0.0% (control), 0.5%, and 1.0% cinnamon bark powder. The diet containing 1% cinnamon resulted in greater ADG, SGR, FCR and survival rate. Whereas, the diet containing 0.5% cinnamon resulted in greater condition factor and lower FCR. In most of the cases the diet containing 1% cinnamon showed lower amount of bacterial count than the controlled condition. Cinnamon could have an antibacterial activity antagonistic to *Vibrio* and *Aeromonas* as there was no count found in fish flesh samples after 90 days of culture. The findings of this study suggest that growth performances and bacterial load of *H. fossilis* were better in commercial feed containing cinnamon powder. It is recommended that fish farmers can use cinnamon as feed supplement to improve growth performance and reduce bacterial load during culture of *H. fossilis*.

Key words: *Heteropneustes fossilis,* cinnamon, growth performance, bacterial load

INTRODUCTION

Stinging catfish, *Heteropneustes fossilis* (Bloch 1794) is an air-breathing catfish and commonly known as *Shing machh* in Bangladesh (http://en.bdfish.org/2011/09/stinging-catfish-heteropneustes-fossilis-bloch-1794/ accessed on 13 October 2018). This species is very popular for its delicious taste and high nutritional and medicinal value (Chakraborty and Nur 2012). It remains high amount of iron and fairly high content of calcium compared to many other freshwater fishes (Saha and Guha 1939). It is considered as a valuable food fish species and recommended as diet for the sick and convalescents. Being a lean fish it is very suitable for people for whom animal fats are undesirable (Rahman *et al.* 1982). It is an indigenous fish species and inhabits open water system of floodplains, canals, beel and haors of Bangladesh. But due to over exploitation and ecological changes in its natural habitats; this species may be threatened (http://www.iucnredlist.org/details/166452/0 accessed on 13 October 2018).

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This fish has potential for aquaculture and it could be easily farmed in ponds and small ditches. In recent years, fish farming has been consolidating and occupying greater space in both national and international markets. Among freshwater fish species, *H. fossilis* stands out for its flesh quality, hardiness and faster growth during intensive farming. It is essential to develop suitable culture system for *H. fossilis* using best quality balanced diet for better production. Feed additives are edible substances that are added to animal feeds in small quantity to enhance the feed quality so that it enhances growth performance, feed utilization and reduces mortality in fish (Dada 2015). Several studies revealed that spices and medicinal plants can be used as feed additive in aquaculture for better growth and survival and microbial disease treatment as well (Immanuel *et al.* 2004). Usually spices are added to different types of food to impart flavor as well as to improve storage stability. Cinnamon has been using worldwide as a flavoring spice. Ahmad *et al.* (2011) reported that the diet containing 1% cinnamon powder resulted in significantly greater ($p < 0.05$) specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER), apparent protein utilization (APU), and energy utilization (EU). Setiawati *et al.* (2016) observed that the addition of cinnamon leaf extract and powder significantly increased the fish specific growth rate, feed efficiency, protein retention as compared to the control. Among its proven biological activities, several reports highlighted its antimicrobial activity (Singh *et al.* 2007 and Starliper *et al.* 2015), primarily attributable to cinnamaldehyde present in the bark of the plant (Wong *et al.* 2014). Cinnamon possesses various biological properties such as antioxidant, antimicrobial, antidiabetic and antiallergic. Main component of cinnamon bark oil is cinnamaldehyde which have strong antibacterial activity against nine strains of bacteria including *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia, Salmonella* species and *Vibrio parahaemolyticus* (Chang *et al.* 2001).

The overall objective of the proposed study was to investigate the effect of cinnamon on growth performances and to control bacterial load of *H. fossilis*. The specific objectives were: preparation of commercial feed with different percentage of cinnamon, culture of experimental fish, determination of growth performance parameters such as ADG, SGR, FCR, condition factor and survival rate and determination of bacterial load in experimental fish species.

MATERIAL AND METHODS

The cinnamon barks used were obtained from super market, then dried in an oven at 60°C. Dried barks were grinded and sieved to produce cinnamon powder.

Isocaloric commercial diet was obtained from fish feed industry. This feed was treated by the addition of cinnamon bark powder at 0, 0.5 and 1% (Ahmad *et al.* 2011). The experiment was performed as- treatment 1 (T1/Control): commercial feed + 0% cinnamon powder, treatment 2 (T2): commercial feed + 0.5% cinnamon powder and treatment 3 (T3): commercial feed + 1% cinnamon powder.

Fry of *H. fossilis* (9.91 ± 0.258 cm and 6.176 ± 0.218 g) were collected from Reliance Aquafarm, Mymensingh. The rearing of *H. fossilis* was conducted for 90 days (March to June, 2017). The experiment was designed as above mentioned three treatments in three replication of each. The fry were randomly distributed at a rate of 20 fry per aquarium. Feeding was done twice daily at 8% of the average body weight for first 30 days, then 5% for next 30 days, after that, 2% for remaining 30 days. Each ration was divided into two equal parts, one portion was offered at 9.00 a.m. while the other at 4.00 p.m. Water from each aquarium was exchanged at three days interval for the removal of uneaten feed and faeces. The aquarium was also cleaned per week and clean water was supplied in each of the aquariums. Physico-chemical parameters of water such as temperature, dissolve oxygen (DO) and pH were recorded twice in a week using mercury thermometer, DO meter (HANNA, HI-9146, USA), pH meter (HANNA, HI-8424, USA), respectively. Sampling was done each 15 days to determine the individual length and weight of the fish and data were recorded. Water samples of each treatment were tested for bacterial load at 15 days interval. At the end, the fish was fasted for 24 hrs, then counted and final length and weight was measured. At that time the bacterial loads of fish samples were determined.

Fish growth performance: Fish growth performance was calculated using the following formulae:

a. Average Daily Gain (ADG, g/day) = (Mean final weight – mean initial weight) /time interval.
b. Specific Growth Rate (SGR) = {(Log$_e$ final weight – Log$_e$ initial weight) /time interval} ×100.
c. Feed Conversion Ratio (FCR) = Feed consumed/weight gain.
d. Condition factor, K = W/L^3 × 100 where, K = Condition factor, W = Body weight in grams and L = Body length in cm.
e. Survival Rate (%) = Number of fry that survived/Total no. of fry stocked × 100.

According to Punom *et al.* (2016) bacterial load was determined for total bacterial count (TBC), total *Vibrio* count, total *Salmonella-Shigella* count, total *Staphylococcal* count and total *Aeromonas* count on nutrient agar, TCBS media, SS agar, Manitol Salt Agar (MSA) and *Aeromonas* agar plate, respectively. For bacterial counts l g of each sample was homogenized with 9 ml physiological saline. Then 100 µl of sample solution was diluted with 900 µl sterile physiological saline and further diluted up to 10^{-5} for inoculating on agar media. Then l00 µl of each homogenized diluted subsample was inoculated on agar plates and incubated at 37°C for 24 hrs in incubator (WTB Binder, Germany). After 24 h of incubation, the plates having well discrete colonies were selected for counting. The plates were counted as cfu/g of sampled fish.

Statistical analysis: Data were analyzed by using one-way ANOVA followed by Tukey's HSD post hoc for multiple comparisons. The data were presented as mean ± SEM and evaluated by using the statistical package of SPSS (version 20.0) with the level of significance at $p < 0.05$. Microsoft office excel (2013) was used to plot graph.

RESULTS AND DISCUSSION

The present study described the growth performance and the bacterial density of Stinging catfish fed with commercial feed with 0, 0.5 and 1% cinnamon powder.

Water quality parameters for culturing H. fossilis: In the study for rearing *H. fossilis* fry dissolved oxygen, pH and temperature that were recorded are provided in Table 1.

Table 1. Water quality parameters in three treatments during the study period

Parameter	Three treatments		
	Commercial feed (Control/T1)	Commercial feed+ 0.5% cinnamon (T2)	Commercial feed+1% cinnamon (T3)
Dissolved oxygen (DO) (mg/l)	2.31 ± 0.77	1.91 ± 0.48	1.27 ± 0.60
pH	7.31 ± 0.18	7.24 ± 0.1	7.29 ± 0.13
Temperature (°C)	28.05 ± 0.51	27.98 ± 0.3	28.08 ± 0.53

Water quality parameter in the rearing aquarium of *H. fossilis* in this experiment varied with the type of diet applied but did not show any negative effect on fish. No significant differences were observed on temperature, pH and dissolved oxygen (DO). Ahmad *et al.* (2011) evaluated the effect of cinnamon (*Cinnamomum zeylanicum*) on growth performance, feed utilization, whole-body composition and resistance to *Aeromonas hydrophila* in Nile Tilapia and they found, water temperature ranged between 26 - 29°C, dissolved oxygen (DO) ranged between 4.5 - 5.5 mg/l, pH ranged between 7.6 - 8.0.

Growth performances of *Heteropneustes fossilis*

The highest condition factor was found in T2 (0.66 ± 0.22%) fed with commercial feed with 0.5% cinnamon powder and the lowest was in T1 (0.65 ± 0.04%) fed with commercial feed (Fig. 1). On 90th day, comparatively higher value (0.66 ± 0.22%) was found in commercial feed with 0.5% cinnamon powder than commercial feed containing 0% cinnamon and 1% cinnamon powder.

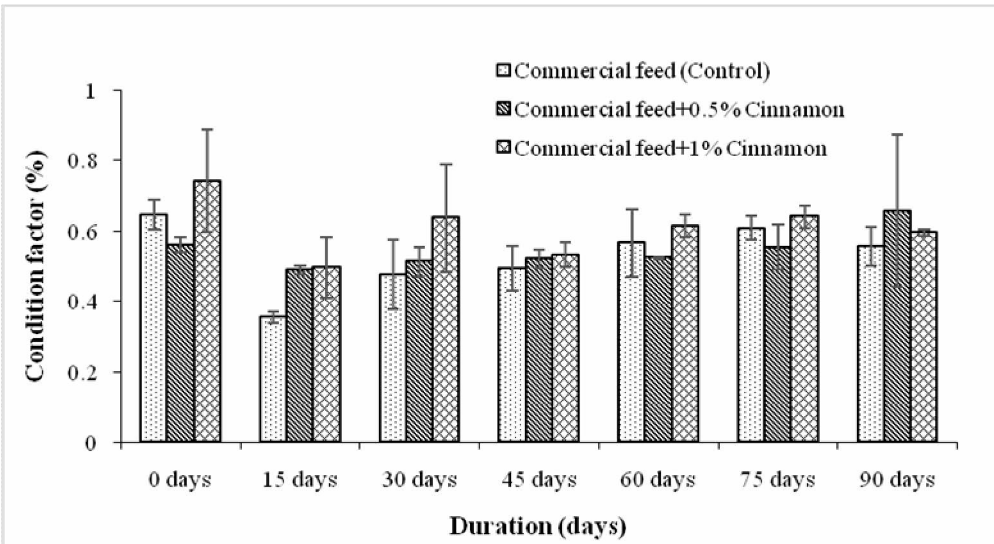

Fig. 1. Condition factor (%) of *H. fossilis* at 90 days rearing period. Bars (Mean ± SEM) with no superscripts are not significant within group (ANOVA, $p > 0.05$).

Rahman *et al.* (1997) in a study on the survival and growth of cat fish giving selected supplemental feeds got the values of condition factor between 0.81 and 0.87. Ahmad *et al.* (2011) evaluated the effect of cinnamon on growth performance in Nile Tilapia and they used 0% (control), 0.5, 1 and 1.5% of cinnamon powder and recorded K factor were 1.59 ± 0.02, 1.52 ± 0.05, 1.67 ± 0.01 and 1.47 ± 0.02, respectively.

Fig. 2 represented the highest ADG of shing fry which was found in T3 (0.097 ± 0.03 g/day) and the lowest was in T1 (0.079 ± 0.007 g/day). The ADG value of T3 was higher than T1. On 45th day, ADG of fish was higher in T2 (0.13 ± 0.038 g/day), however, on 60th day, the value was higher in T3 (0.11 ± 0.016 g/day) compared to T1 and T2. No significant difference was observed between the three different rearing conditions.

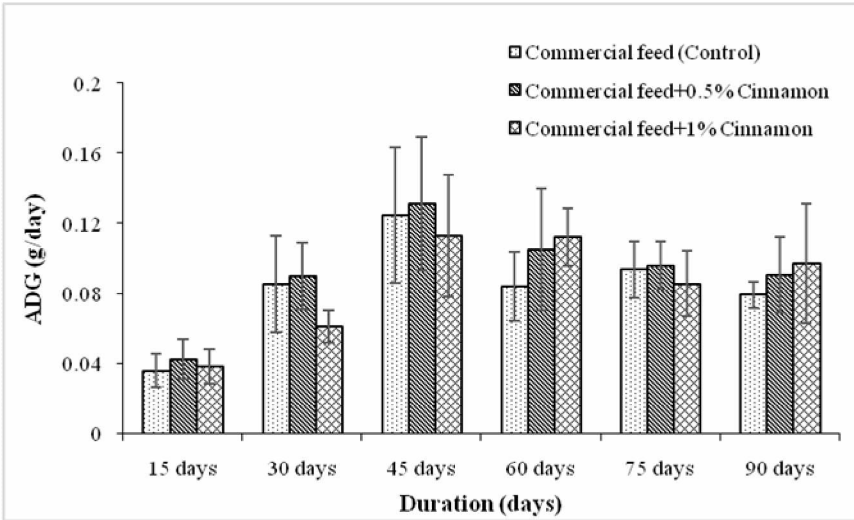

Fig. 2. Average daily gain (g/day) of *H. fossilis* at 90 days rearing period. Bars (Mean ± SEM) with no superscripts are not significant within group (ANOVA, $p > 0.05$).

The highest specific growth rate of shing fry was found in T3 (0.73 ± 0.25%) and the lowest was in T1 (0.63 ± 0.05%). On 90th day specific growth rate was not significantly different in three rearing conditions.

Setiawati *et al.* (2016) evaluated the growth performance of Asian catfish (*Pangasianodon hypophthalmus*) fed with diets containing *Cinnamomum burmannii* leaf powder and extract. They recorded highest SGR (1.18 ± 0.05%) in fish fed with diet containing 1% cinnamon leaf powder for 60 days. Ahmad *et al.* (2011) found that the fish fed the diet containing 1% cinnamon showed the highest average body weight, weight gain percentage, and SGR in comparison with other diets.

Fish, that were fed on commercial feed (3.07 ± 0.29) exhibited lowest feed conversion ratio (FCR) than those fed with commercial feed + 0.5% cinnamon powder (3.18 ± 0.63) and commercial feed+1% cinnamon powder (3.66 ± 1.59). On 90th day feed conversion ratio did not differ significantly (ANOVA, $p > 0.05$) (Fig. 4). For fish fed well prepared diets, FCR values below 1 have been reported, although generally it ranges between 1.2 and 1.5 (De Silva and Anderson 1995). Ahmad *et al.* (2011) used 0% (control), 0.5, 1 and 1.5% of cinnamon powder to rear Nile tilapia and recorded the FCR values 2.21 ± 0.022, 2.05 ± 0.015, 1.95 ± 0.01, 1.94 ± 0.01, respectively.

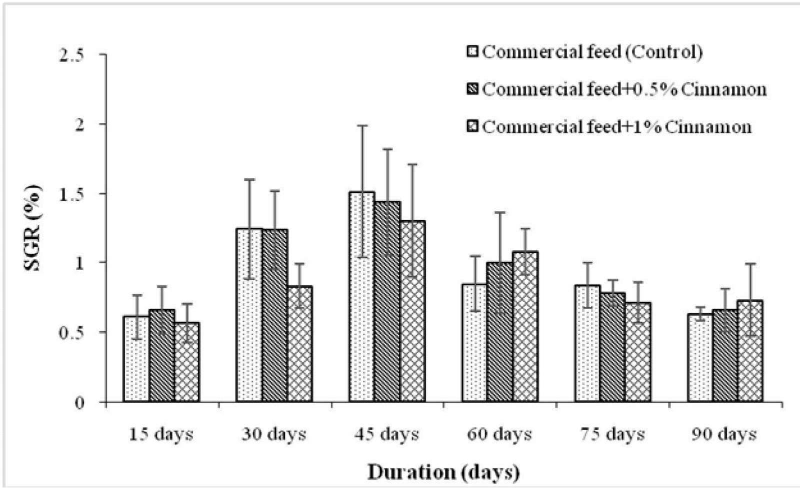

Fig. 3. Specific growth rate (%) of *H. fossilis* at 90 days rearing period. Bars (Mean ± SEM) with no superscripts are not significant within group (ANOVA, $p > 0.05$).

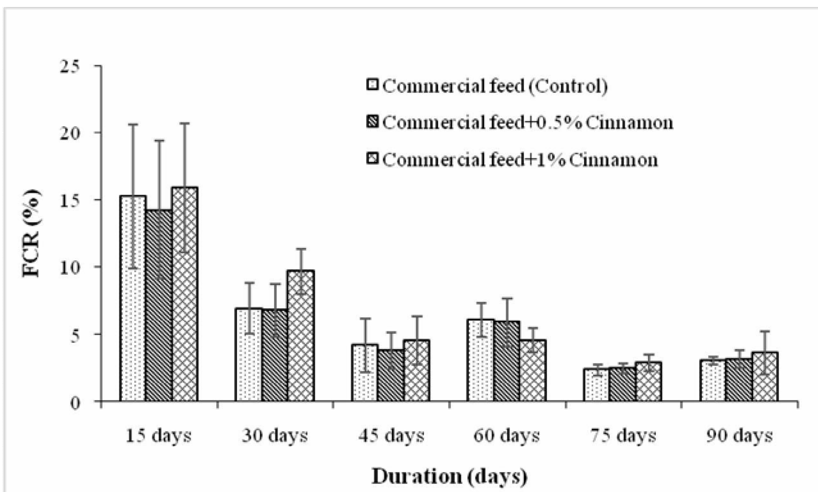

Fig. 4. Feed conversion ratio (FCR) (%) of *H. fossilis* at 90 days rearing period. Bars (Mean ± SEM) with no superscripts are not significant within group (ANOVA, $p > 0.05$).

After culture period, Fig. 5 presented here the survival rates of shing fry in T1, T2 and T3 were (92 ± 0.58%), (94.68 ± 0.33%) and (97.32 ± 0.33%), respectively.

Setiawati *et al.* (2016) recorded 100% survival rate for all treatments during 60 days of maintenance while studying the growth performance of *Pangasianodon hypophthalmus* fed diets containing *Cinnamomum burmannii* leaf either in powder or extract form at different doses like 0.1% cinnamon leaf extract and 1% cinnamon leaf powder.

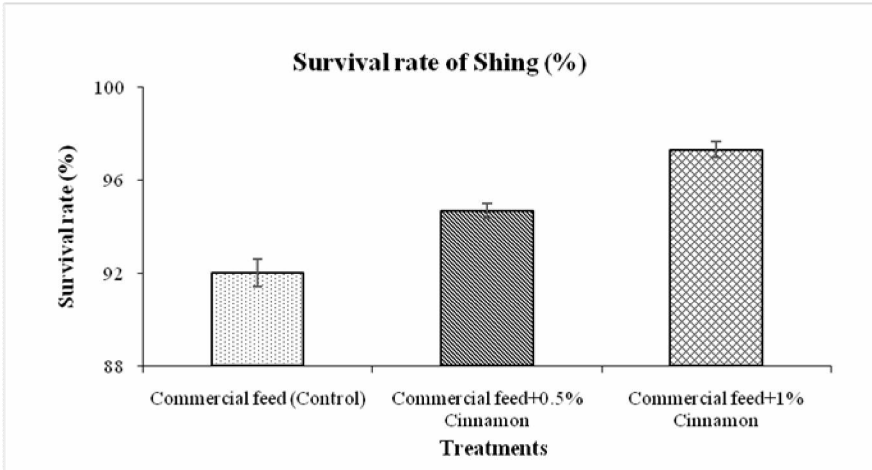

Fig. 5. Survival rate (%) of *H. fossilis* at 90 days rearing period. Bars (Mean ± SEM) with no superscripts are not significant within group (ANOVA, $p > 0.05$).

Bacterial density of water and shing sample on different rearing conditions

Bacterial count of water samples: Results of the quantitative estimation of total bacteria, total *Vibrio*, total *Salmonella-Shigella*, total *Aeromonas* and total Staphylococcal count for water samples of three different treatment at different rearing periods are demonstrated in Table 2. On 15th day, the highest total bacterial density of water samples was $1.67 \pm 0.41 \times 10^5$ cfu/g in T2. On 75th day, the lowest total bacterial density of water sample was found in T1 which was $2.75 \pm 1.61 \times 10^3$ cfu/g. Significant difference was found; in between T2 and T3 at 60 days of rearing period, between T1 and T2 at 75 days of rearing period. The highest *Salmonella-Shigella* count was $6.18 \pm 0.82 \times 10^4$ cfu/g in T2 on 15th day, however, on 90th day, the lowest value was $0.60 \pm 0.50 \times 10^2$ cfu/g in T3. On 45th day, the highest total Staphylococcal count was evaluated in T1 and that was $6.3 \pm 5.6 \times 10^4$ cfu/g but on the 60th day the lowest density was $0.03 \pm 0.03 \times 10^2$ in T3. However, for water samples, total *Aeromonas* count from 75th to 90th day rearing period was zero. On 45th day, *Vibrio* count was significantly different ($p < 0.05$) between T1 and T2, T1 and T3. But no *Vibrio* was observed at 90 days of rearing period. Therefore, it can be said that cinnamon might have antibacterial activity as in most of the cases the lowest bacterial load was found T3 which was treated with commercial feed with 1% cinnamon. It can also be said that the antibacterial activity is effective after a certain period of time since in this study it was observed that *Salmonella-Shigella* count was lowest on 90th day of rearing whereas at that time *Aeromonas* and *Vibrio* count was zero.

Table 2. Bacterial count (Mean ± SEM) for water samples of three different treatments in different rearing periods of culture. Different letters represent significant differences within group (ANOVA, $p < 0.05$)

Bacterial density (cfu/g)	Treatments	Rearing periods					
		15 days	30 days	45 days	60 days	75 days	90 days
Total bacterial count	Commercial feed	$1.32±0.92×10^5$	$6.13±3.04×10^4$	$1.24±0.38×10^5$	$3.57±1.12×10^{3ab}$	$2.75±1.61×10^{3b}$	$3.83±1.20×10^4$
	Commercial feed + 0.5% Cinnamon	$1.67±0.41×10^5$	$3.92±2.59×10^4$	$1.03±0.90×10^4$	$7.52±0.62×10^{3a}$	$1.19±0.37×10^{4a}$	$2.13±1.57×10^4$
	Commercial feed + 1% Cinnamon	$1.05±0.19×10^5$	$1.64±0.63×10^4$	$2.91±1.71×10^4$	$3.00±0.55×10^{3b}$	$3.68±0.61×10^{3ab}$	$8.88±6.12×10^3$
Total *Salmonella shigella* count	Commercial feed	$4.34±2.37×10^4$	$3.52±3.37×10^4$	$4.07±3.77×10^4$	$2.88±1.04×10^3$	$2.23±0.23×10^2$	$0.97±0.41×10^2$
	Commercial feed + 0.5% Cinnamon	$6.18±0.82×10^4$	$2.10±1.70×10^2$	$8.27±8.24×10^3$	$1.23±0.03×10^3$	$1.40±0.40×10^2$	$2.40±2.40×10^2$
	Commercial feed + 1% Cinnamon	$3.85±0.52×10^4$	$5.10±3.50×10^2$	$2.43±1.79×10^4$	$1.16±0.75×10^3$	$1.43±0.84×10^2$	$0.60±0.50×10^2$
Total Staphylo-coccal count	Commercial feed	$3.29±2.20×10^4$	$1.42±1.34×10^4$	$6.3±5.6×10^4$	$0.17±0.17×10^2$	$8.01±5.69×10^2$	$4.87±2.63×10^2$
	Commercial feed + 0.5% Cinnamon	$5.03±1.99×10^4$	$1.59±1.12×10^4$	$9.15±9.05×10^2$	$1.25±1.25×10^2$	$9.68±4.33×10^2$	$5.46±3.69×10^3$
	Commercial feed + 1% Cinnamon	$3.24±0.88×10^4$	$7.73±3.35×10^3$	$2.03±0.86×10^3$	$0.03±0.03×10^2$	$2.73±1.39×10^2$	$2.19±1.86×10^3$
Total *Aeromonas* count	Commercial feed	$1.41±1.07×10^4$	$1.34±1.33×10^3$	$8.34±7.35×10^3$	$2.27±1.88×10^2$	0	0
	Commercial feed + 0.5% Cinnamon	$2.29±0.75×10^4$	0	$0.85±0.75×10^2$	$6.30±5.30×10^2$	0	0
	Commercial feed + 1% Cinnamon	$8.25±3.11×10^3$	$3.77±3.61×10^2$	0	0	0	0
Total *Vibrio* count	Commercial feed	$3.54±0.17×10^3$	0	$3.00±0.00×10^{3a}$	0	$0.53±0.53×10^2$	0
	Commercial feed + 0.5% Cinnamon	$3.22±0.21×10^3$	0	$2.22±0.78×10^{2b}$	0	0	0
	Commercial feed + 1% Cinnamon	$2.89±0.22×10^3$	0	$3.00±0.00×10^{2b}$	0	$0.10±0.06×10^2$	0

Bacterial count of fish samples: The highest total bacterial density of fish samples was quantified on T2 ($1.14 \pm 1.05 \times 10^5$ cfu/g), where the lowest total bacterial count was defined from T1 ($8.39 \pm 3.96 \times 10^4$ cfu/g). Maximum total *Salmonella-Shigella* count ($1.2 \pm 1.2 \times 10^3$ cfu/g) was evaluated from T1 where fish were fed with commercial feed. The minimum total *Salmonella-Shigella* count was evaluated from T2 (commercial feed + 0.5% cinnamon powder) and counted density was $1.17 \pm 0.73 \times 10^2$ cfu/g.

Table 3. Bacterial count (Mean ± SEM) for fish samples of three different treatments after 90 days of culture (ANOVA, p > 0.05)

Bacterial density (cfu/g)	Treatments		
	Commercial feed (Control)	Commercial feed + 0.5% cinnamon	Commercial feed + 1% cinnamon
Total bacterial count	$8.39 \pm 3.96 \times 10^4$	$1.14 \pm 1.05 \times 10^5$	$1.1 \pm 0.32 \times 10^5$
Total *Salmonella-Shigella* count	$1.2 \pm 1.2 \times 10^3$	$1.17 \pm 0.73 \times 10^2$	$3.33 \pm 2.84 \times 10^2$
Total Staphylococcal count	$5.57 \pm 2.75 \times 10^4$	$3.12 \pm 2.78 \times 10^4$	$4.73 \pm 2.83 \times 10^4$
Total *Aeromonas* count	0	0	0
Total *Vibrio* count	0	0	0

Accordingly, the highest total Staphylococcal count was evaluated from T1 ($5.57 \pm 2.8 \times 10^4$ cfu/g) and lowest count was found from T2 ($3.12 \pm 2.8 \times 10^4$ cfu/g). No total *Aeromonas* and total *Vibrio* count was obtained from the fish muscle samples of any treatment. No significant difference was observed among all bacterial count.

According to ICMSF (1986) guideline, acceptable limit of total bacterial counts for giant prawns and white fish are 10^6 and 5×10^5 cfu/g, respectively. In this study, total bacterial count was within the acceptable limit. It was also determined from this study that total bacterial density was lower for cinnamon mixed diet than the commercial diet. The limit of harmful and pathogenic microorganisms, such as *Salmonella* spp., *Shigella* spp. is 0 cfu/g and 1.0×10^2 cfu/g, respectively (ICMSF 1986). *Salmonella, Shigella* are not usually found as these are not the normal flora of fishes or of their environment (Huss *et al.* 2003). In the present study the value is slightly higher than the limit. Therefore it can be said that the supplied water and supplied feed were not good enough to maintain the health of fish. A considerable amount of *Staphylococcus* spp. found in water and fish type of experimented sample. According to ICMSF (1986) the suggested limit for *Staphylococcus* spp. is $>10^3$ cfu/g which confirms the contagion of the experimental samples with *Staphylococcus*. In the present study, maximum total *Aeromonas* count was quantified from water samples but nothing found from the fish samples. The use of cinnamon could enhance the innate immune system of fish, allowing a better response to stressful environmental conditions (Rattanachaikunsopon and Phumkhachorn 2010). Cinnamon had an antibacterial activity antagonistic to pathogenic *A. hydrophila* (GMF 2007). Abdel-Wahab *et al.* (2007) found that 0.5% cinnamon level in the diet is enough to eliminate harmful microbes in the gut, improve food absorption, and control blood sugar to a certain extent. According to ICMSF (1986), fresh and frozen fish should be free of *Vibrio* (0 cfu/g). The present study revealed that microbial quality of fish were acceptable in order of absence of *Vibrio* in fish samples. Arancibia *et al.* (2014) observed the antibacterial effect of cinnamon essential oil on *Vibrio parahaemolyticus* and proved that the release of cinnamon essential oil from polysaccharide bilayer films was effective for *V. parahaemolyticus* inhibition in chilled shrimps.

CONCLUSION

Feed enrichment with 0.5 and 1% powder of cinnamon bark was efficient and applicable in improving the growth performance of stinging catfish with increasing specific growth rate, feed conversion ratio and survival rate. Fish feed supplied with cinnamon powder as a feed additive may improve the health status and growth performances of *H. fossilis* as this study recorded bacterial load was comparatively lower in the culture conditions supplied with cinnamon mixed commercial feed.

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