

RESEARCH ARTICLE

# Effect of phytase on the water quality of Vietnamese pangasius ponds

## Ảnh hưởng của enzyme phytase lên môi trường nước của ao nuôi cá tra Việt Nam

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Striped catfish (*Pangasianodon hypophthalmus*) is freshwater fish that is raising mainly in the Mekong Delta. The research was implemented at the Model Farm (College of Aquaculture and Fisheries, Can Tho University during 6 months from May to November of 2016 and aimed to evaluate the water quality between two treatments of feed in the pangasius production for proposing a better environmental management method. The treatment 1 (control treatment, CT) has two ponds used feed without adding phytase and treatment 2 (experiment treatment, ET) includes two ponds used feed with adding phytase. The results showed that the temperature, pH, DO, TSS, COD, BOD, N-NO<sub>2</sub><sup>-</sup>, N-NO<sub>3</sub><sup>-</sup>, TKN, TAN were not significantly different ( $p > 0.05$ ) between the CT and ET ponds. At the experiment pond (EP), the factors (TP: 2.28 mg/L; P-PO<sub>4</sub><sup>3-</sup>: 1.24 mg/L) which has P release to the environment are less than control pond (CP) (TP: 2.62 mg/L; P-PO<sub>4</sub><sup>3-</sup>: 2.13 mg/L). These results suggested that the addition of phytase in feed could contribute to reducing the nutrient to the water body of pangasius pond.

Cá tra (*Pangasianodon hypophthalmus*) là loài thủy sản nước ngọt được nuôi chủ yếu ở các tỉnh Đồng bằng sông Cửu Long. Nghiên cứu được thực hiện trong 6 tháng từ tháng 05 – 11/2016 tại Trang trại mẫu đặt tại Khoa Thủy sản, Trường Đại học Cần Thơ nhằm đánh giá chất lượng nước giữa 2 nghiệm thức thức ăn trong giai đoạn nuôi cá thịt để đề xuất biện pháp quản lý chất lượng nước ao nuôi tốt hơn. Nghiệm thức 1 (nghiệm thức đối chứng, CT) gồm 2 ao nuôi sử dụng thức ăn không bổ sung enzyme phytase, nghiệm thức 2 (nghiệm thức thí nghiệm, ET) gồm 2 ao nuôi sử dụng thức ăn có bổ sung enzyme phytase. Kết quả thí nghiệm cho thấy giá trị nhiệt độ, pH, DO, TSS, COD, BOD, H<sub>2</sub>S, N-NO<sub>2</sub><sup>-</sup>, N-NO<sub>3</sub><sup>-</sup>, TKN, TAN ở mức phù hợp cho ao đối chứng (CT). Tại ao thí nghiệm (ET) (TP: 2,28 mg/L; P-PO<sub>4</sub><sup>3-</sup>: 1,24 mg/L) lượng P thải ra ít hơn so với ao CT (TP: 2,62 mg/L; P-PO<sub>4</sub><sup>3-</sup>: 2,13 mg/L) chứng tỏ việc bổ sung enzyme phytase trong thức ăn đã góp phần làm giảm lượng lân thải ra môi trường nước trong nuôi cá tra.

**Keywords:** catfish, water quality, enzyme phytase

## 1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*) is a main production sector and high exporting value of Mekong Delta with a yield of 734,880 tons annually and 1.55 billion USD (VASEP, 2016). In recent years, the water quality of pangasius production is warned on pollution due to the huge supply of feed but only part of it was consumed by fish, the rest was discharged to the water environment. According to Cao Van Thich (2008), the striped catfish absorbed only about 43.6% N, 30.1% P, the remain N, P released to the environment. Nguyen Thi Thanh Truc *et al.* (2012) also researched on the addition of phytase to feed which helped to increase the growth rate and reduced the Phosphorous (P) in outlet water. However, the research was implemented on a small scale and short time. Therefore, the investigation of water quality between two types of feed was done to propose a good management method for water environment of pangasius production ponds. The

research was done at Model Farm in Cai Rang district, Can Tho city under the framework of SUPA project.

## 2. Research methodology

### 2.1 Experiment

The research was implemented from May to November of 2016 in four striped catfish ponds with an average area of 200 m<sup>2</sup> for each and 2.5 m deep. Water was exchanged every day about 30% each time by pumping from sedimentation pond (3000 m<sup>2</sup>).

Stock fish was bought at Phu Thuan A commune, Hong Ngu district, Dong Thap province with an average weight is 18 g/fish and density is about 40 fish/m<sup>2</sup>. The experiment has two treatments with two replications of each. Control treatment (CT): without using phytase in feed and with using phytase 0.01% in feed at experiment treatment (ET).

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Phosphorous in the phytase or phytic acid form was not digested by aquatic animals and consequently discharged through faeces caused an environmental pollution. These phosphorous were also the feed sources for bacteria growing and dispersing in water led to an algae bloom and adversely affected to the aquatic species (Sharpley *et al.*, 1995, Mullaney *et al.*, 2000, Satyanarayana, 2001). It needs a phytase enzyme to decompose phytase because aquatic animals cannot synthesize phytase enzyme by themselves. Therefore, this enzyme was added to feed to enhance the digestion effect and adsorption of phosphorous as well as other available nutrients. Adding phytase to feed was not only increasing the assimilation of phosphorous component existing in the feed and adsorption of protein but also reducing the environmental impact (Igbasan *et al.*, 2001).

During the first three months, striped catfish was fed two times per day by feeds size 2 mm, 30% protein with food portion was about 3-6% fish weight/day. From the fourth month, it was fed only one time per day with feed contains 26% protein at size 4-6 mm.

## 2.2 Sample collection and analysis method

Samples were collected at the same point under the feeding bridge where fish were fed at every time. Temperature, pH, DO were directly measured at the ponds. COD: collected to 125 ml glass bottle and pushed under water surface about 10–20 cm, sealed cap under water to avoid air bubble. Fixation by the H<sub>2</sub>SO<sub>4</sub> 4M solution. BOD, TSS, H<sub>2</sub>S, TAN, P-PO<sub>4</sub><sup>3-</sup>, TN, TP, N-NO<sub>2</sub><sup>-</sup>, N-NO<sub>3</sub><sup>-</sup>: collected in a 1-litre plastic bottle and kept stored in cold preservation box. H<sub>2</sub>S: collected to 125 ml brown glass bottle and pushed under water surface about 20–30 cm. pH, temperature, and DO were measured every day (at 8 am and 4 pm) by HANNA analyser. Samples of other factors such as COD, BOD, TSS, H<sub>2</sub>S, TAN, P-PO<sub>4</sub><sup>3-</sup>, TKN, TP, N-NO<sub>2</sub><sup>-</sup>, N-NO<sub>3</sub><sup>-</sup> were collected before raising (L1) and every two weeks in the morning time (total 13 times). Water was analysed at Water Quality Laboratory – College of Aquaculture and Fisheries, Can Tho University. Sample reserve and analysis method followed the instruction and standard (APHA *et al.*, 1995).

## 2.3 Data analysis method

Data were analysed and treated in Excel and using ANOVA analysis method by SPSS software with statistic significant  $p \leq 0.05$ .

## 3. Results and discussion

### 3.1 Temperature, pH, DO between control and experiment

In the control pond (CP), the parameters in morning and afternoon were fluctuated in ranges of temperature 29.3-33.3°C, pH 6.8-8.5, and DO 1.6-8.3 mg/L. In the experiment pond (EP), parameters were also fluctuated in ranges of temperature 29.5-33.1°C, pH 7.0-8.0 and DO 2.0-8.5 mg/L.

Generally, the investigated temperature in the ponds was not significantly fluctuated during the production period, the temperature in the mornings was lower than in the afternoons but not exceeded 2°C. The pH at the beginning period was always high and reduced at the end of the period, and dissolved oxygen was low in the morning and high at noon due to the photosynthesis of algae. These results were similar to other studies from different authors. Duong Thuy Yen (2003) showed that striped catfish lived in a large range of temperature with maximum 40.8°C and minimum 16.7°C. They can survive in the environment that pH was lower than 4 and DO was lower than 2 mg/L. Boyd (1998) also mentioned that the optimum conditions for striped catfish development were temperature 28-32°C, pH 6.0-9.0 and DO 5-10 mg/L. Therefore, temperature, pH, and DO in this research could be the suitable conditions for striped catfish production.

### 3.2 Fluctuation of BOD and COD

The COD value fluctuated at every collected sample in both control pond (CP) and experiment pond (EP) with adding phytase. COD before raising at EP was:  $35.5 \pm 1.36$  mg/L and at CP  $23.5 \pm 2.04$  mg/L ( $P > 0.05$ ) and COD after harvesting at EP was  $57.6 \pm 4.53$  and at CP was  $20.8 \pm 2.26$  mg/L ( $P < 0.05$ ). At EP, COD fluctuated in 28.8-156 mg/L, the average values of COD in whole period were  $62.4 \pm 33.2$  mg/L which were higher than CP (COD 20.8-119 mg/L, the average value was  $47.5 \pm 30.6$  mg/L). As late to the end of the production period, as more increasing of biomass in the pond because of waste increased from fish which caused a strong oxidation of organics. It led to increasing COD at the end of the period and exceeded the suitable threshold (COD < 150 mg/L (Vietnamese Standard QCVN 02-20:2014/BNNPTNT)) (Figure 1).

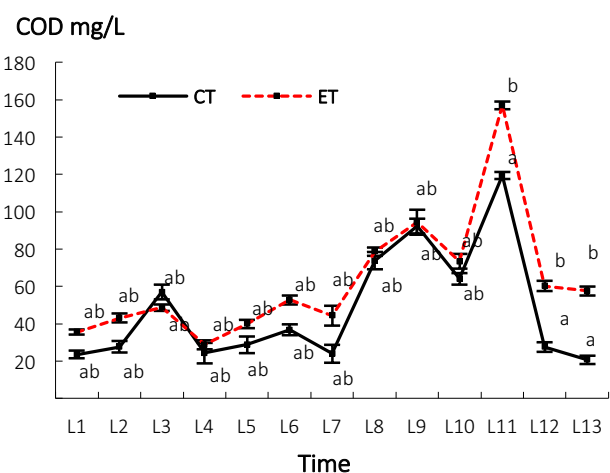


Figure 1. Fluctuation of COD at different times

COD in the EP with adding enzyme phytase had values at different collection samples L11, L12 and L13 are:  $157 \pm 1.99^b$  mg/L;  $60.2 \pm 2.76^b$  mg/L and  $57.6 \pm 2.26^b$  mg/L, higher ( $p < 0.05$ ) than the CP with values such as:  $119 \pm 1.81^a$  mg/L (L11);  $27.6 \pm 2.47^a$  mg/L (L12) and  $20.8 \pm 2.26^a$  mg/L (L13). Fish weight in the EP at sample L13 was 4,948

kg and feed consumed till this period was 6,014 kg, meanwhile at the CP fish weight was only 3,283 kg and feed was 4,110 kg. Therefore, wastes in EP higher than CP and COD in EP was also higher than CP.

According to Nguyen Thanh Phuong *et al.* (2012), the suitable COD value for striped catfish was 15-30 mg/L, and the maximum was 15-40 mg/L. Other authors also gave the same results as a suitable range of COD 10-20 mg/L (Duong Nhut Long, 2005) and 9.6-11.4 mg/L (Cao Van Thich, 2008). Similarly, BOD value (Figure 2) in CP was about 6.26-16.5 mg/L, average  $10.8 \pm 2.96$  mg/L; BOD in EP was about 9.0-18.0 mg/L, average  $13.7 \pm 2.61$  mg/L. The suitable condition of BOD was about 10-20 mg/L (Boyd, 1998). Huynh Truong Giang *et al.* (2008) asserted that the BOD value at striped catfish production ponds in An Giang was high around 1.9-23 mg/L. Therefore, BOD values in these ponds are still in the suitable range condition for growing of striped catfish.

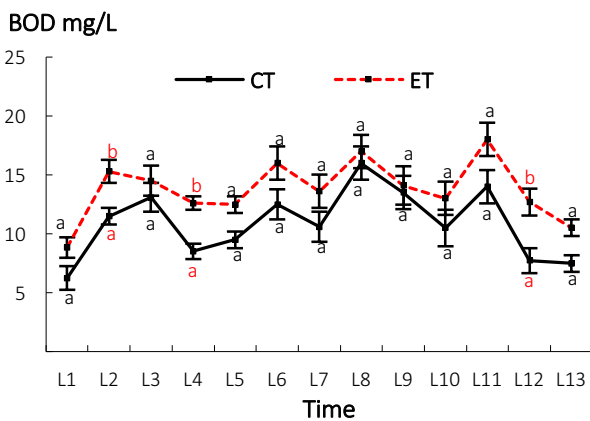


Figure 2. Fluctuation of BOD at different times

### 3.3 Fluctuation of TSS

TSS values have no significant differences between control ponds (CP) and experiment ponds (EP) with adding phytase ( $p > 0.05$ ). In CP, TSS values were 26.5-222 mg/L, the average was  $118 \pm 60.5$  mg/L. In experiment pond, TSS was 35.0-208 mg/L, average  $116 \pm 67.0$  mg/L. TSS values in this research have a trend of increasing by production time (L1 – L9) (Figure 3).

TSS values were high leading to low purity and vice versa, the suitable value for aquaculture production is lower than 50 mg/L (Boyd, 1998). In the pond, the surplus feed caused an increase in TSS in the pond and always exceeding the warning threshold value of striped catfish production. These results were similar to the investigation of Dang Van Quoi (2014) with value is  $72.3 \pm 33.4$  mg/L, in the range 40-132 mg/L.

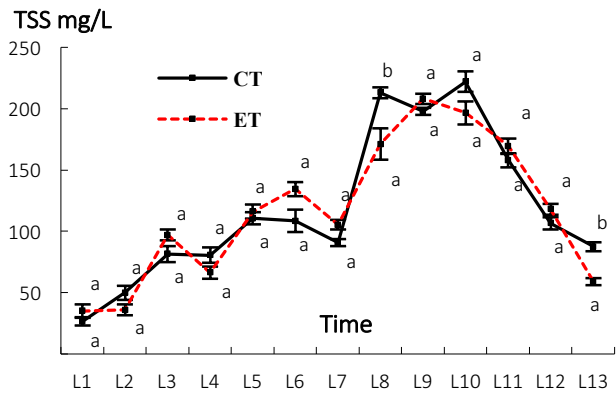


Figure 3. Fluctuation of TSS at different times

### 3.4 Fluctuation of TAN, NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup>

TAN values of ponds were shown in Figure 4. In CP, TAN average was  $1.78 \pm 0.035$  mg/L (0.172-3.1 mg/L), and the average value for experiment pond was  $1.83 \pm 0.047$  mg/L (0.16-3.04 mg/L). TAN was rapidly increasing from the first to the fifth sample and reducing at the end of the period. These results were also similar to other researches such as Nguyen Thanh Phuong *et al.* (2012) with TAN in the pond was about 2.86-4.06 mg/L.

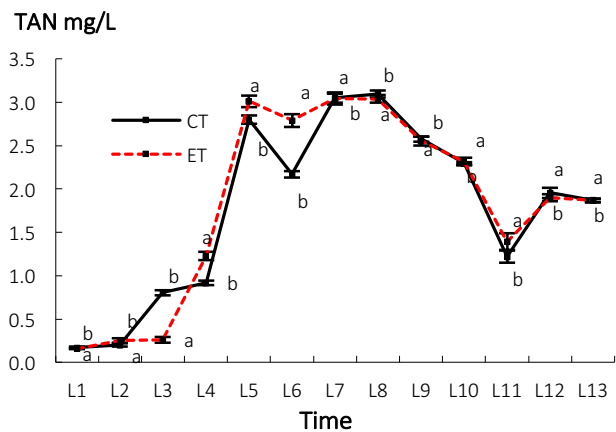


Figure 4. Fluctuation of TAN at different time

TAN is the total value of NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup>, in which NH<sub>3</sub> is very toxic gas for fish. NH<sub>3</sub> in CP was around 0.005-0.25 mg/L, average  $0.043 \pm 0.067$  mg/L; in EP was 0.002-0.172 mg/L, average  $0.042 \pm 0.050$  mg/L. NH<sub>3</sub> values were not significantly different ( $p > 0.05$ ) in all samples. According to Huynh Truong Giang *et al.* (2008), NH<sub>3</sub> < 0.02 mg/L was suitable for aquaculture production. In the pond, the presence of NH<sub>4</sub><sup>+</sup> will not affect to fish, but at high concentration will cause an algae bloom and poison to fish. In this research, NH<sub>4</sub><sup>+</sup> was in the range of 0.138-2.97 mg/L; average in the control pond was 1.74 mg/L and in the experiment pond: 1.84 mg/L (Figure 5b).

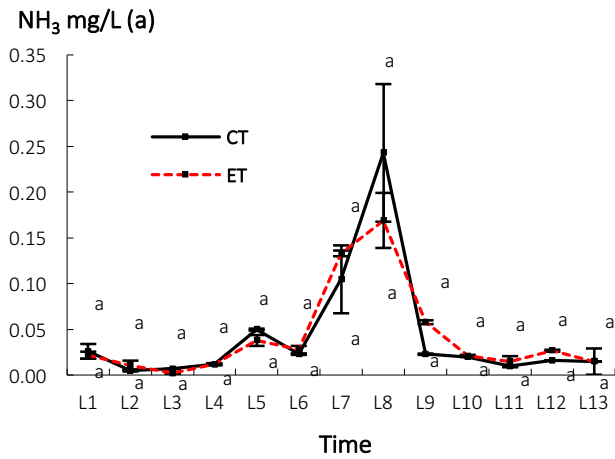


Figure 5a. Fluctuation of  $\text{NH}_3$  at different times

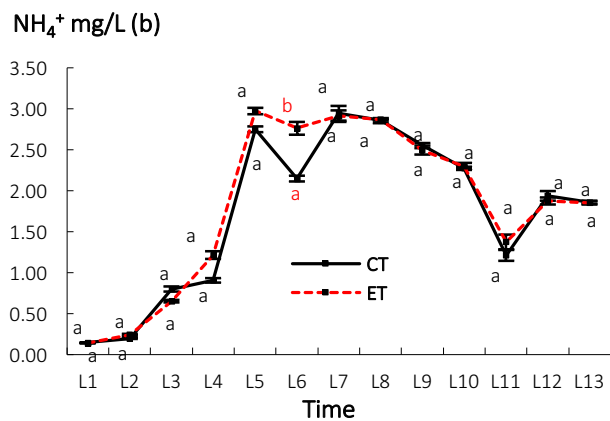


Figure 5b. Fluctuation of  $\text{NH}_4^+$  at different times

### 3.5 Fluctuation of $\text{N-NO}_2^-$ , $\text{N-NO}_3^-$ and TKN

The results showed that  $\text{N-NO}_2^-$  concentration in the CP was around 0.009-0.315 mg/L (average value:  $0.108 \pm 0.085$  mg/L), in the EP was 0.012-0.397 mg/L (average  $0.144 \pm 0.120$  mg/L). From the first to eight samples (L1-L8),  $\text{N-NO}_2^-$  concentration in the CP and the EP were low and increased to get the highest value in the ninth sample. It was decreased after that to the end of the period. This can be explained that the nitrification needs oxygen for oxidation  $\text{NH}_3$  to  $\text{N-NO}_2^-$  and from  $\text{N-NO}_2^-$  to  $\text{N-NO}_3^-$  by bacteria (Truong Quoc Phu, 2006) then at the end of the period the low dissolved oxygen led to decreasing of  $\text{N-NO}_2^-$ . However, it shows that the  $\text{N-NO}_2^-$  concentrations are still safe for catfish.

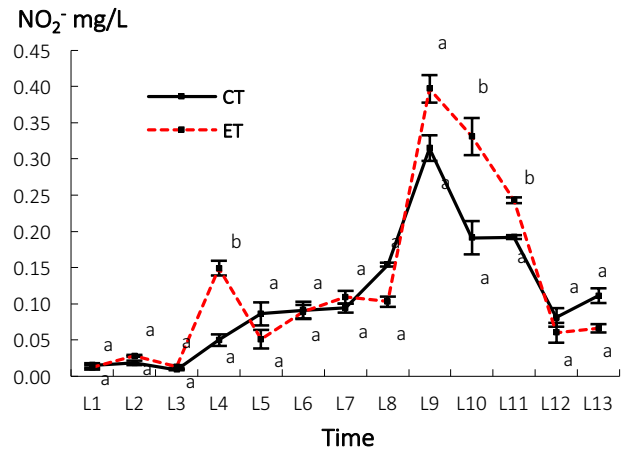


Figure 6. Fluctuation of  $\text{N-NO}_2^-$  at different times

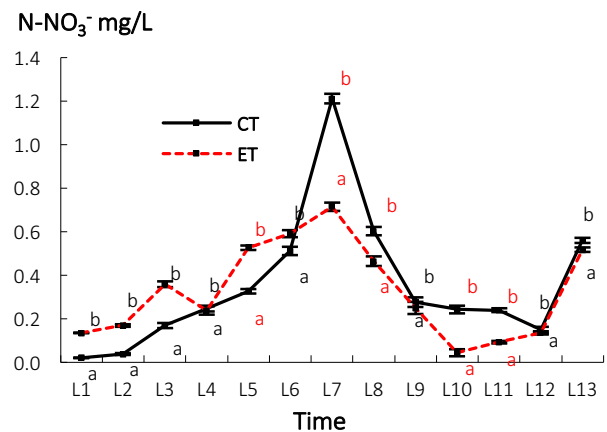


Figure 7. Fluctuation of  $\text{N-NO}_3^-$  at different times

$\text{N-NO}_3^-$  was produced from the oxidation of  $\text{N-NO}_2^-$  by bacteria,  $\text{N-NO}_3^-$  in the ponds was not toxic but it will affect at high concentration ( $> 10$  mg/L) (Boyd, 1998; Le Van Cat *et al*, 2006). Concentration of  $\text{N-NO}_3^-$  in the CP was around 0.019-1.21 mg/L ( $0.352 \pm 0.309$  mg/L), and in the EP was 0.043-0.715 mg/L ( $0.325 \pm 0.213$  mg/L).  $\text{N-NO}_3^-$  concentration was increasing from the first to the seventh and it was decreasing at the end of period (Figure 7). Therefore,  $\text{N-NO}_3^-$  concentration has low value and was safe to catfish. In addition, the water exchange and algae adsorption also helped to decrease the nitrate concentration.

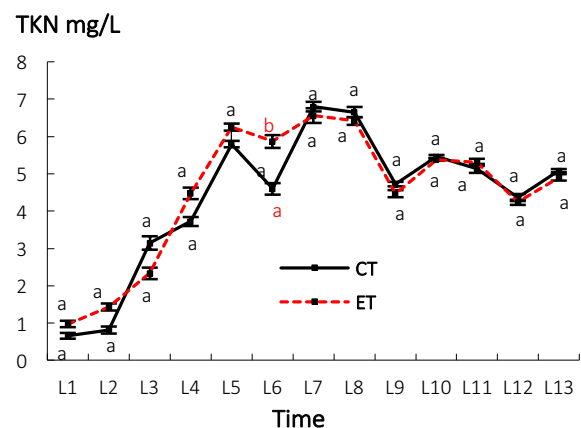


Figure 8. Fluctuation of TKN at different times

TKN investigation at ponds was shown in Fig 8. In the control pond, the average value TKN was  $4.38 \pm 1.88$  mg/L (0.659-6.79 mg/L) and in the experiment pond was  $4.48 \pm 1.84$  (0.975-6.55 mg/L). TKN was slightly increasing from the beginning of the period and reached the highest values in the 7<sup>th</sup> and 8<sup>th</sup> sample, it then decreased to the end period. These results are the same as the research of Chau Minh Khoi *et al.* (2012).

### 3.6 Fluctuation of P-PO<sub>4</sub><sup>3-</sup> and TP

Concentration P-PO<sub>4</sub><sup>3-</sup> (Fig.7) shown that it increased from the beginning to the end period in both control and experiment pond. The average value of P-PO<sub>4</sub><sup>3-</sup> at the control pond:  $1.15 \pm 1.07$  mg/L (fluctuated from 0.045-3.16 mg/L) higher than the experiment pond:  $0.816 \pm 0.68$  mg/L (fluctuated from 0.07-1.88 mg/L) ( $p > 0.05$ ). At the first 7 samples, the concentration of P-PO<sub>4</sub><sup>3-</sup> in the CP and EP were not significantly different ( $p > 0.05$ ). However, from the 7<sup>th</sup> sample, the concentration of P-PO<sub>4</sub><sup>3-</sup> in the EP was much lower than the CP ( $p < 0.05$ ), especially at the last sample, the concentration P-PO<sub>4</sub><sup>3-</sup> ( $1.64 \pm 0.13^a$  mg/L) in the EP was much lower than the CP ( $2.82 \pm 0.14^b$  mg/L). These results showed that adding enzyme phytase to feed of the EP could cause reducing of phosphate content in comparison with the CP. According to Truong Quoc Phu (2006), concentration P-PO<sub>4</sub><sup>3-</sup> recommended for aquaculture was 0.005-2 mg/L. Concentration P-PO<sub>4</sub><sup>3-</sup> investigated at these ponds were higher than recommended but in the striped catfish production, the feed consumed was very high, which caused a high accumulation of waste. Chau Minh Khoi (2012) also given that in An Giang, P-PO<sub>4</sub><sup>3-</sup> in the pond had value at 8 mg/L, higher than these research values at CP ( $3.16 \pm 0.12$  mg/L) and EP ( $2.02 \pm 0.09$  mg/L) but the fish was still alive.

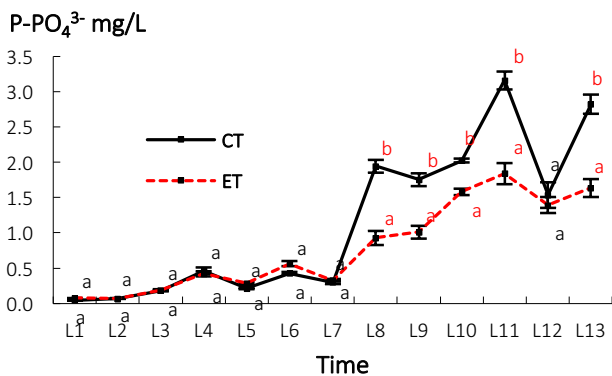


Figure 9. Fluctuation of PO<sub>4</sub><sup>3-</sup> at different times

The value TP in the control and experiment ponds was increasing, especially from the 8<sup>th</sup> sample the value of TP in the experiment ponds was significantly lower ( $p < 0.05$ ) than the control ponds. This was clearly shown in the 9<sup>th</sup> and 13<sup>th</sup> sample, the concentration of TP at the experiment pond with adding phytase ( $1.82 \pm 0.12^a$  mg/L and  $3.14 \pm 0.294^a$  mg/L) was much lower than the control pond ( $3.49 \pm 0.156^b$  mg/L and  $3.47 \pm 0.229^b$  mg/L) ( $p < 0.05$ ) (Fig 10). Therefore, the addition of phytase to feed created a better

result, reduced TP and improved the environmental condition in the production ponds.

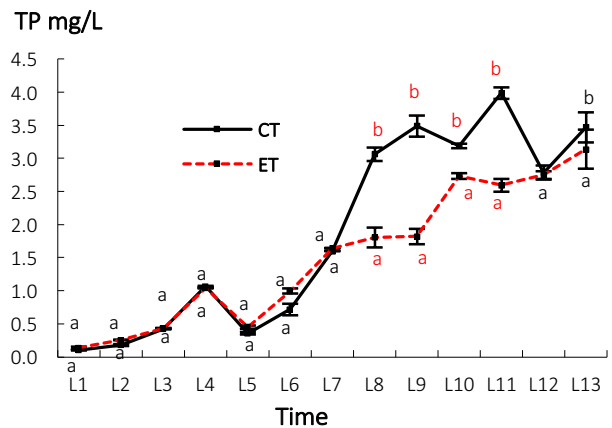


Figure 10. Fluctuation of TP at different times

## 4. Conclusion and recommendation

### 4.1 Conclusion

The physical factors of the water environment (pH, temperature, TSS) and chemical factors (DO, COD, BOD, TAN, H<sub>2</sub>S, N-NO<sub>2</sub><sup>-</sup>, N-NO<sub>3</sub><sup>-</sup>, TKN) was suitable to striped catfish production and there were no significant differences between the control and the experiment pond.

The average values of P-PO<sub>4</sub><sup>3-</sup>, TP were 0.816 mg/L; 1.52 mg/L at the experiment pond and 1.15 mg/L; 1.88 at the control pond. These values at the experiment pond were lower than the control pond but still in the suitable range for striped catfish production. The addition of phytase enzyme helped to reduce the phosphate in the striped catfish production ponds.

### 4.2 Recommendation

The research needs to replicate more at each experiment for clear recognition of the effect of phytase enzyme to the environmental water factors in the striped catfish production ponds.

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