Dietary phosphorus levels affect snakehead (*Channa striata*) fish growth and feed utilisation

[1]Tran T. T. Hien, [1]Tran L. C. Tu, [1]Pham M. Duc, [1]Thach S. La, [2]Hillary Egna, [1]Tran. M. Phu

[1] College of Aquaculture and Fisheries, Can Tho University, Can Tho city, Vietnam; [2] Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon, USA. Corresponding author: T. L. C. Tu, tlctu@ctu.edu.vn

Abstract. This study aimed to determine phosphorus requirements and phosphorus apparent digestibility coefficients (ADC_P) of various ingredients in diets for snakehead (*Channa striata*) juveniles. Digestible phosphorus requirements were determined using seven iso-nitrogenous (45% crude protein) and iso-caloric (19.0 KJ g^{-1}) diets with different dietary phosphorus levels ranging from 0.64% (P-deficient control) to 2.15%. Phosphorus deficiency did not affect survival rate but reduced the specific growth rate by around 15%. The optimal dietary available phosphorus concentration for growth was 0.69%, which gave a specific growth rate of 2.46% day^{-1}. Fish fed the phosphorus deficient diet had significantly more body fat, a lower protein efficiency ratio, lower protein use and significantly lower calcium and phosphorus levels in their body, skeletal tissues and scales.
Key Words: *Channa striata*, mineral requirements, nutrition requirements, snakehead.

Introduction. Snakehead (*Channa striata*) has been farmed in various systems: small-scale for poverty reduction; intensive farming in earthen ponds; cage farming; and lined tank farming in Vietnam (Sinh & Chung 2009; Lam et al 2011). This fish was traditionally cultured using a diet based on chopped or minced "trash" fish, which was wasteful and led to deterioration in water quality and to the spread of diseases (Hien et al 2015a). Commercial pellet feed is now widely used in snakehead culture in Vietnam as a result of advanced research in snakehead nutrition requirements, for example, weaning methods using formulated feeds for snakehead larvae (Hien et al 2016, 2017a) and replacement of fishmeal by soybean meal in diets for snakehead (Hien et al 2015b, 2017b). Dietary phosphorus deficiencies in aquatic animals affect skeletal formation, growth, and feed conversion (Sugiura et al 2004). Food is the main source of phosphorus for fish, although fish can also absorb minerals from the surrounding water (NRC 2011). However, freshwater typically has a low phosphorus concentration (Lall 2003). Optimal dietary phosphorus produces maximum fish growth and reduces the quantity of phosphorus discharged into the environment (Bureau & Cho 1999; Prabhu et al 2016). Snakehead can grow well on a diet containing 30% soybean meal, but to increase the use of soybean meal up to 40%, phytase has to be added to improve the digestibility of phytate-bound phosphorus (Hien et al 2015b). Limited studies on phosphorus requirement of snakehead exist; thus, this study was carried out to determine the phosphorus requirement of snakehead fingerlings for optimal growth, which will contribute benefits to the environment along with improved commercial pellet efficacy.

Material and Method

***Fish growth experiment**.* The experiment included seven iso-nitrogenous (45% crude protein) and iso-caloric (19.0 KJ g^{-1}) practical diets that were formulated to contain graded phosphorus levels of 0.64, 0.9, 1.15, 1.4, 1.65, 1.9, and 2.15% (Table 1). We produced experimental feed following Hien et al (2017b).

Table 1
Formulation and chemical composition (% dry matter) of diets containing different levels of phosphorus

Ingredients (%)	% phosphorous in diets						
	0.64	*0.90*	*1.15*	*1.40*	*1.65*	*1.90*	*2.15*
Fish meal[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal[2]	38.0	38.0	38.0	38.0	38.0	38.0	38.0
Wheat gluten[3]	14.6	14.6	14.6	14.6	14.6	14.6	14.6
Squid oil	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Soybean oil	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Cr_2O_3	1	1	1	1	1	1	1
Premix vitamins and minerals[4]	2	2	2	2	2	2	2
Dextrin	16.3	16.3	16.3	16.3	16.3	16.3	16.3
CaCO	2.59	2.15	1.72	1.29	0.86	0.43	0
MCP[5]	0.0	1.2	2.37	3.53	4.7	5.86	7.0
CMC[6]	4.51	3.75	3.01	2.28	1.54	0.81	0.1
Gelatin	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Feed proximate compositions (% of dry matter)							
Protein	45.2	45.2	45.3	45.2	45.2	45.2	45.3
Lipid	5.84	5.72	5.62	5.69	5.75	5.59	5.68
Ash	9.40	9.80	11.1	11.6	12.5	12.9	13.6
NFE	34.0	34.6	34.0	34.3	34.0	34.5	34.4
Phosphorus	0.61	0.92	1.20	1.38	1.59	1.89	2.17
Calcium	1.59	1.54	1.58	1.57	1.54	1.57	1.56
Energy (KJ g^{-1})	18.9	19.0	19.0	18.9	18.9	18.9	19.0

[1] Fishmeal was obtained from Kien Giang Co., LTD, Kien Giang province, Vietnam; [2] Soybean meal was derived from Afiex company, An Giang province, Vietnam. [3] Wheat gluten by Hong Ha Ltd. Company, CanTho City, Vietnam; [4] Premix vitamins and minerals (unit kg^{-1}) (from Vemedim Co., Ltd, Can Tho, Vietnam): Vitamin A, 2,000,000 IU; Vitamin D, 400,000 IU; Vitamin E, 6 g; Vitamin B_1, 800 mg; Vitamin B_2, 800 mg; Vitamin B_{12}, 2 mg; Calcium D. Pantotenate, 2 g; Folic acid, 160 mg; Vitamin C, 15g; Choline Chloride, 100 mg; Iron (Fe^{2+}), 1g; Zinc (Zn^{2+}), 3 g; Manganese (Mn^{2+}), 2 g; Copper (Cu^{2+}), 100 mg; Iodine (I^-), 20 mg; Cobalt (Co^{2+}), 10 mg; [5] MCP: monocalcium phosphate (22.85% P; 16.92% Ca; moisture of 2.8%) obtained from Rotem, Turkey; [6] CMC: carboxymethyl cellulose: Xilong Chemical Industry Incorporated Co. Ltd (China).

Experimental fish (~10.0 g) were purchased from a hatchery in An Giang province, Vietnam, and transferred to the wet laboratory at Can Tho University. The experiment was set up at College of Aquaculture and Fisheries, Can Tho University from February to July 2017. The fish were acclimated in tanks (4 m^3) for 2 weeks. Fish (11.4±0.03 g initial weight) were then randomly assigned to 28 composite tanks (500 L $tank^{-1}$) with aeration, at a stocking density of 35 fish $tank^{-1}$. Each treatment had four replicates. Water was exchanged at a rate of 50% every 2 days. The experiment lasted for 8 weeks. Fish were fed to satiation twice a day (8:00 and 14:00), and the amount of feed given and excess uneaten feed were recorded daily. Dead fish were collected and weighed. During the experiment, temperature ranged from 26.8 to 28.7°C; dissolved oxygen remained above 6 mg L^{-1}; and pH was 7.24-7.46. All experiments were carried out in accordance with national guidelines on the protection and experimental animal welfare in Vietnam (Law of Animal Health 2015).

Each fish was weighed before and after the experiment to determine initial (Wi) and final weights (Wf). Specific growth rate (SGR; % d^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR, %), and hepatosomatic index (HSI) were calculated as:

SR = [(fish count at the end of experiment)/(fish count at the start of the experiment)] x 100

$$SGR = (\ln(W_f) - \ln(W_i)) \times 100/t \text{ (where t = time in days)}$$

FCR = (amount of consumed feed in dry matter)/(weight gain)

$$PER = (W_f - W_i)/(\text{protein intake})$$

HSI = (liver weight)/(total body weight)

The chemical composition of fish at the start and end of the experiment (10 fish tank^{-1}) and feed (100 g) were analyzed following the methods of AOAC (2016).

***Apparent digestibility of dietary available phosphorus*.** Dietary available phosphorus is defined as the quantity of phosphorus that can be digested by fish from the total phosphorus content in the diet. At the end of the growth experiment, fish from the same treatment were randomly re-stocked in 28 composite tanks (250 L) at a density of 20 fish tank^{-1}, with three replicate tanks for each treatment. Dietary phosphorus levels were the same as those used in the growth experiment, but chromic oxide (Cr_2O_3) was added to each diet at a concentration of 1% as an inert digestion marker. Feeding and feces collection were done as described by Hien et al (2017b). Feces and experimental feed were analyzed for moisture, crude protein, crude lipid, and total ash by standard AOAC (2016) methods. Cr_2O_3 was determined following Furukawa & Tsukahara (1966).

The apparent digestibility coefficient (ADC) of nutrients in feeds was calculated from the concentration of Cr_2O_3, an indirect marker, in feed and feces using the following equation (NRC 2011):

$$ADC = 1 - (Cr_2O_3 \text{ in feed}/Cr_2O_3 \text{ in feces})$$

The ADC of specific nutrient in feed were calculated as follows:

$$ADC = 1 - (Cr_2O_3 \text{ in feed}/Cr_2O_3 \text{ in feces}) \times (\text{phosphorus content of feces}/\text{phosphorus content of feed})$$

***Data analyses*.** The differences in mean of analyzed parameters among dietary phosphorus treatments were tested by one-way ANOVA followed by Duncan's multiple range tests (using SPSS 16.0, USA). The differences were considered significant at $p < 0.05$. Determination of the dietary available phosphorus requirement was carried out by the broken-line method (Zeitoun et al 1976). All experiments were carried out in accordance with Vietnam's national guidelines for the protection and welfare of experimental animals (Law of Animal Health 2015) (Report number: VM5068).

Results and Discussion

***Apparent digestibility experiment for determination of dietary available phosphorus*.** Apparent digestibility coefficients for dry matter (ADC_{DM}) ranged from 84.2 to 88.1% and decreased with increasing phosphorus levels in the diet (Table 2). ADC_{DM} of diets containing 0.64 and 0.9% phosphorus (88.0% and 88.1%, respectively) were significantly ($p < 0.05$) higher than those of other treatments. The diet containing 0.64% phosphorus had a significantly lower ADC_P (53.9%) than other diets (64.7 to 66.7%) (Table 2). From these results, the dietary available phosphorus was calculated as 0.34, 0.60, 0.75, 0.92, 1.09, 1.24, and 1.39% corresponding to phosphorus levels in diets of 0.64, 0.90, 1.15, 1.4, 1.65, 1.9, and 2.15%.

Table 2

Apparent digestibility coefficient (ADC) of feed for dry matter, phosphorous and dietary available phosphorus

Treatments (% P in diets)	*ADCDM (%)*	*ADCP (%)*	*Dietary available phosphorous (%)*
0.64	88.0 ± 0.44^{a}	53.9 ± 0.62^{c}	0.34
0.90	88.1 ± 0.60^{a}	66.7 ± 0.72^{a}	0.60
1.15	86.7 ± 0.55^{b}	65.6 ± 2.21^{ab}	0.75
1.40	86.4 ± 0.30^{b}	65.8 ± 0.46^{ab}	0.92
1.65	85.0 ± 0.38^{c}	66.2 ± 1.09^{ab}	1.09
1.90	86.2 ± 0.59^{b}	65.4 ± 1.25^{ab}	1.24
2.15	84.2 ± 0.80^{c}	64.7 ± 0.46^{b}	1.39

Values are mean±standard deviation of three replicates. Different superscript letters in the same column indicate the significant difference at $p < 0.05$. ADCDM = apparent digestibility coefficient of feed in dry matter; ADCP = apparent digestibility coefficient of phosphorus.

Growth performance, feed utilization efficiency and chemical body composition of snakehead fed diets with different dietary phosphorus levels. The SR ranged from 89.3 to 92.1% and there was no significant difference among treatments ($p > 0.05$). The SGR, PER in the diet containing 0.34% dietary available phosphorus were significantly lower than those of the other treatments ($p < 0.05$), whereas FCR was significantly higher (Table 3). There were no significant differences ($p > 0.05$) in SGR, PER and FCR for diets with 0.6% to 1.39% available phosphorus.

Table 3

Growth performance and feed utilization efficiency of snakehead fed different dietary available phosphorus

Dietary available P(%)	*W_i (g)*	*W_f (g)*	*SGR (% day^{-1})*	*FCR*	*PER*
0.34	11.4±0.04^a	54.3±3.70^b	2.26±0.27^b	1.71±0.29^b	1.40±0.24^b
0.60	11.4±0.03^a	61.4±1.91^a	2.62±0.13^a	1.38±0.06^a	1.69±0.05^a
0.75	11.4±0.04^a	62.3±2.64^a	2.70±0.06^a	1.30±0.06^a	1.80±0.09^a
0.92	11.4±0.02^a	58.8±1.07^a	2.65±0.12^a	1.34±0.09^a	1.73±0.12^a
1.09	11.4±0.04^a	58.9±3.07^a	2.63±0.03^a	1.29±0.08^a	1.79±0.06^a
1.24	11.4±0.03^a	58.7±2.59^a	2.70±0.12^a	1.29±0.06^a	1.84±0.10^a
1.39	11.4±0.03^a	61.1±2.36^a	2.75±0.21^a	1.25±0.05^a	1.92±0.22^a

Values are presented as mean ± standard deviation of four replicates. The same superscript letters in the same column indicate the non-significant difference at $p < 0.05$.

Based on the broken-line analysis between SGR and the dietary available phosphorus (Figure 1) the optimal SGR was 2.64% in the diet containing 0.69% available phosphorus.

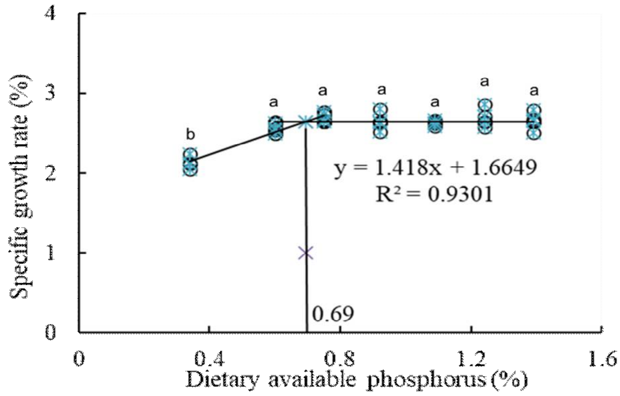

Figure 1. Multiple linear regression of specific growth rate and dietary available phosphorus to determine the optimal dietary available phosphorus for growth of snakehead juveniles.

The whole-body moisture and crude protein were not significantly different among treatments ($p > 0.05$) (Table 4). With increasing dietary available phosphorus, crude lipid content in the whole body decreased from 5.2 to 3.2%. The highest crude lipid content (5.2%) was found in the treatment with 0.34% dietary available phosphorus, significantly higher than the other treatments ($p < 0.05$). In contrast, total ash increased with increasing level of phosphorus in diets, ranging from 3.3 to 5.9%. The control treatment had the lowest total ash content of 3.3%, which was significantly different from the other treatments ($p < 0.05$). Calcium and phosphorus of the whole body increased from 2.9 to 6.3% and from 1.9 to 3.6%, respectively, with increasing levels of phosphorus in the diet. Calcium and phosphorus concentrations in the whole body were both significantly lower in the treatment with 0.34% dietary available phosphorus compared with other treatments ($p < 0.05$) (Table 4). The Ca/P ratio increased non-linearly with increasing phosphorus in the diet. The lowest Ca/P was found in the 0.6% dietary available phosphorus treatment, which was significantly different from other treatments ($p < 0.05$).

Table 4

Proximate moisture, crude protein, crude lipid, total ash, calcium and phosphorus content, hepatosomatic index (HSI), and rate of abnormalities in experimental fish after 8 weeks of growout

Dietary available P (%)	*Moisture (%)*	*Crude protein (%)*	*Crude lipid (%)*	*Total ash (%)*	*Ca (%)*	*P (%)*	*Ca/P*	*HSI*
0.34	73.0±0.73^{a}	15.8±0.21^{a}	5.2±0.13^{a}	3.3±0.22^{d}	2.9±0.07^{e}	1.9±0.13^{c}	1.5±0.13^{ab}	2.60±0.43^{a}
0.60	72.9±0.15^{a}	15.9±0.51^{a}	4.3±0.11^{b}	4.0±0.15^{c}	3.7±0.19^{d}	2.8±0.12^{c}	1.3±0.11^{c}	2.18±0.26^{ab}
0.75	72.8±0.82^{a}	15.5±0.56^{a}	4.3±0.04^{b}	4.1±0.17^{c}	4.7±0.25^{c}	2.9±0.13^{c}	1.6±0.08^{ab}	2.09±0.07^{abc}
0.92	72.5±0.38^{a}	15.2±0.19^{a}	3.9±0.17^{c}	4.9±0.12^{b}	5.1±0.28^{bc}	3.3±0.05^{b}	1.5±0.09^{b}	2.08±0.12^{abc}
1.09	72.6±0.28^{a}	15.3±0.40^{a}	3.5±0.14^{d}	5.5±0.18^{a}	5.5±0.55^{b}	3.4±0.20^{ab}	1.6±0.22^{ab}	1.87±0.12^{bc}
1.24	72.6±0.46^{a}	15.7±0.63^{a}	3.3±0.05^{de}	5.5±0.20^{a}	6.2±0.38^{a}	3.5±0.21^{ab}	1.7±0.14^{a}	1.89±0.80^{bc}
1.39	72.8±0.11^{a}	15.8±0.12^{a}	3.2±0.15^{e}	5.9±0.07^{a}	6.3±0.24^{a}	3.6±0.23^{a}	1.7±0.07^{a}	1.60±0.10^{c}

Values are presented as mean±standard deviation of four replicates. The same superscript letters in the same column indicate the non-significant difference at $p < 0.05$. The values of Ca and P were expressed as the percentage in dry matter.

While there was an overall trend of increasing phosphorus concentrations in the whole body, vertebrae and scales with increasing dietary available phosphorus, the increase was most significant between the treatments with 0.34% and 0.6% dietary available phosphorus, and phosphorus concentrations in all three body parts tended to plateau out at dietary available phosphorus concentrations of 0.6% and above, and in most cases were not statistically different (Figure 2).

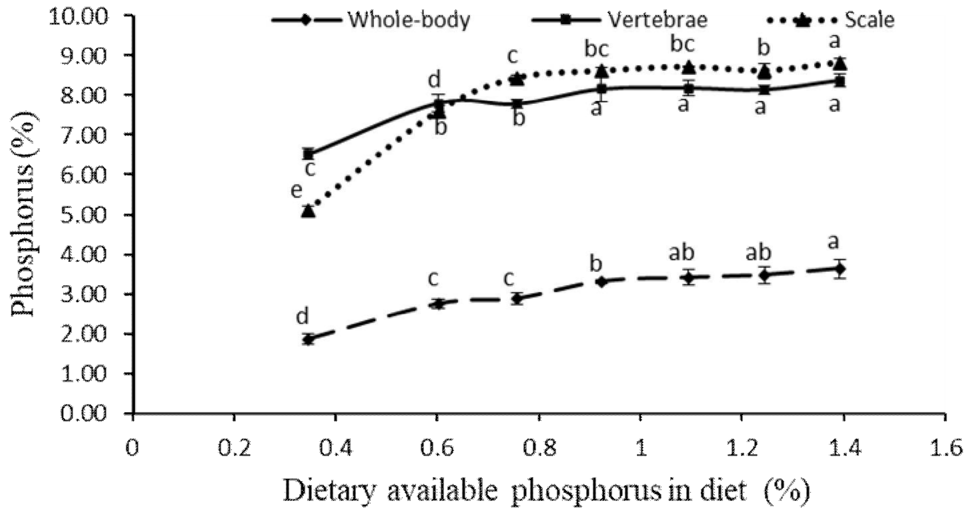

Figure 2. Phosphorus contents (%) in vertebrae, scales, and whole-body at different dietary available phosphorus.

Discussion. Phosphorus is an essential major mineral in animal feed because it is a key constituent of skeletal tissue and is involved in many metabolic processes including, for example, energy transformations, cellular membrane formation, and genetic coding (Lovell 1989). While there is no evidence that a deficiency of phosphorus in the diet affects survival rate (Phromkunthong & Udom 2008; Shao et al 2008; Yuan et al 2011; Tang et al 2012), a deficiency in dietary available phosphorus can reduce weight gain and, in some cases, can lead to skeletal deformities because of the competing demands of growth, skeleton formation, and other physiological processes for the limited supply of available phosphorus (Brown et al 1993). In terms of weight gain, we found the optimal dietary available phosphorus concentration to be 0.69%, which gave a SGR of 2.46% day^{-1}. SGR dropped sharply at dietary available phosphorus concentrations below 0.69%, while above this concentration, SGR plateaued out, and snakehead juveniles were unable to utilize all of the dietary available phosphorus efficiently, which in turn, could lead to the release of more phosphorus into the environment. The optimum level of 0.69% dietary available phosphorus for maximum growth of snakehead is comparable to that reported for Japanese seabass *Lateolabrax japonicus* (0.68%; Zhang et al 2006), large yellow croaker *Pseudosciaena crocea* (0.70%; Mai et al 2006) and haddock *Melanogrammus aeglefinus* (0.72%; Roy & Lall 2003). These differences between species could be due to the composition of the diet and its sources of phosphorus, to genetic or environmentally induced changes in metabolism, or to differences in the mineral composition of skeletal tissues.

In addition to improving growth rate, supplementing the diet with highly digestible monocalcium phosphate also improved the PER and net protein use (NPU), but reduced the FCR. Similar results have been reported for rainbow trout *Oncorhynchus mykiss* (Hernández et al 2005), black seabream *Sparus macrocephalus* (Shao et al 2008), sex-reversed red tilapia *Oreochromis* sp. (Phromkunthong & Udom 2008), African catfish *Clarias gariepinus* (Nwanna et al 2009), and Chinese sucker *Myxocyprinus asiaticus* (Yuan et al 2011).

Fish fed the phosphorus-deficient basal diet had significantly higher crude lipid content in the whole body, enhanced carcass fat and reduced body water content compared to those fed diets containing with sufficient dietary available phosphorus. Similar effects on lipid content and body fats have been reported in several studies (Takeuchi & Nakazoe 1981; Roy & Lall 2003; Mai et al 2006; Zhang et al 2006; Phromkunthong & Udom 2008; Shao et al 2008; Xu et al 2011). These effects could be due to inhibition of the tricarboxylic acid (TCA) cycle, so that the acetyl-CoA production from carbohydrate, lipid and other energy-providing compounds is used to synthesize lipid accumulated in internal organs and muscle tissues (Skonberg et al 1997).

As observed in previous studies Roy & Lall (2003), Cheng et al (2005), Ye et al (2006), Phromkunthong & Udom (2008), Shao et al (2008), and Xu et al (2011), phosphorus deficiency in the basal diet reduced accumulation of calcium and phosphorus leading to poor bone mineralization. Supplementing the diet with monocalcium phosphate significantly increased the calcium, phosphorus and mineral content in whole body, and the phosphorus content in vertebrae and scales, most likely by increasing the level of calcium hydroyapatite, which is reported to be the major component in fish vertebrae and scales in rainbow trout (Skonberg et al 1997).

Notwithstanding the lower calcium and phosphorus levels in the skeleton and scales of the snakehead fingerling stage in the phosphorus deficient diet, the incidence of skeletal abnormalities was very low at 1% or less for all diets, and there was no evidence of an increase in skeletal abnormalities as a consequence of phosphorus deficiency. Fish deformities can also involve deficiency of several vitamins (A, D, E, K and C) and minerals (zinc, manganese, phosphorus, calcium and selenium) (Lall & Lewis-McCrea 2007). In our study, the phosphorus-deficient diet contained about 50% of the phosphorus requirement of snakehead, whereas redlip mullet *Liza haematocheila* showed deficiency signs when phosphorus levels were only slightly below their requirement level (El-Zibdeh et al 1995), and gilthead sea bream *Sparus aurata* exhibited signs of phosphorus deficiency when the phosphorus level in the test diet was 20-30% below the dietary requirement (Pimentel-Rodrigues & Oliva-Teles 2001). These differences might be species-specific, or they might be associated with a deficiency in vitamins or other essential minerals.

Conclusions. The available phosphorus requirement for optimal growth of snakehead was 0.69% and 0.89% for optimum bone mineralization. Deficiency of phosphorus led to increased lipid content in fish body and decreased calcium and phosphorus accumulation in vertebrae and scale. The rate of abnormalities snakehead fingerling stage was not affected by dietary available phosphorus. Thus, the study on Ca:P ratio in diet should be done to figure it out. Moreover, the available phosphorus requirement should be trialed at commercial snakehead size to confirm the abnormalities of snakehead.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Tran Thi Thanh Hien, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Can Tho, Vietnam, e-mail: ttthien@ctu.edu.vn
Tran Le Cam Tu, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Can Tho, Vietnam, e-mail: tlctu@ctu.edu.vn
Pham Minh Duc, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Can Tho, Vietnam, e-mail: pmduc@ctu.edu.vn
Thach Sa La, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Can Tho, Vietnam, e-mail: thachlansa87@gmail.com
Hillary Egna, Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon, USA, e-mail: egnahs@gmail.com
Tran Minh Phu, College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 Street, 94000 Can Tho, Vietnam, e-mail: tmphu@ctu.edu.vn

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