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Original Research Articles

Effects of two phosphorous sources in the diet on the growth performance, digestibility, and plasma physiological parameters of *Pelodiscus sinensis* juveniles

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Phosphorus is an essential mineral for aquatic animals to maintain the health of the skeletal system and many physiological functions. This study assessed the effects of two inorganic phosphorus sources on growth performance, apparent phosphorus digestibility, whole-body proximate composition, and physiological status in juvenile *Pelodiscus sinensis*. Two experimental diets were supplemented with 4% calcium phosphate monobasic (MCP) and 5.47% calcium phosphate dibasic (DCP), respectively, to obtain equal total dietary phosphorus (2.20%). 96 turtles (initial body weight: 5.40±0.03g) were randomly distributed into 12 tanks and fed the corresponding diets for 60d. Results showed that phosphorus sources have not significantly influenced the growth parameters, including the specific growth rate, feeding rate, and feed conversion ratio (P>0.05). No significant differences were observed in the hepatosomatic index and whole-body proximate compositions between MCP and DCP groups (P>0.05). The apparent digestibility coefficients of dry matter and phosphorus in MCP group (53.22%) are slightly higher than that in DCP group (48.98%) but did not reach the statistically significant level (P > 0.05). Turtles in MCP and DCP groups are the same in plasma physiological parameters and have equal alkaline phosphatase activities in plasma and liver (P>0.05). In conclusion, calcium phosphate monobasic and calcium phosphate dibasic had the same biological phosphorus availability in diet for juvenile *Pelodiscus sinensis*.

INTRODUCTION

Phosphorus plays a prominent role in the development and maintenance of the skeletal system and performs many physiological functions, including energy metabolism and the maintenance of acid–base equilibrium in vertebrates.[1] Inadequate phosphorus will lead to lower mineralization of bone, lower feed efficiency ratio, higher lipid deposition, and retarded growth in aquatic animals.[2-4] The origins of phosphorus for aquatic animals stem from surrounding water and diet. In contrast, the quantity of phosphorus absorbed from surrounding water by aquatic animals is extremely remote. They mainly depend on the phosphorus in diets.[5] The contents of available phosphorus in feed ingredients cannot meet the requirements of aquatic animals. The dietary supplementations of exogenous inorganic phosphates have become the effective phosphorus supply in aqua-feed.[1]

Inorganic phosphates include calcium phosphate, sodium phosphate, and potassium phosphate.[6] Calcium phosphate is usually the common phosphorus source because it can simultaneously provide both phosphorus and calcium. Previous studies in many aquatic species found that different calcium phosphates had largely significant variations in phosphorus availability, it is observed that MCP had higher phosphorus availability and higher growth performance than DCP in Carp (*Cyprinus carpio*),[7] European sea bass (*Dicentrarchus labrax* L.),[8] *Hemibarbus maculatus*[9]; yellowtail (*Seriola quinqueradiata*).[6] However, the same availabilities of MCP and DCP were observed in *Oreochromis niloticus*.[10] Hence, the availabilities of phosphorus sources varied with the different phosphorus sources and species cultured. Moreover, public concern has increased

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about the effect of phosphorus discharge from aquaculture systems on the water environment.[11,12] Phosphorus is also a vital nutrient for phytoplankton in aquatic ecosystems. The unavailable dietary phosphorus will be discharged into the surrounding water, initiating excessive phytoplankton growth and eutrophication.[13,14] Therefore, it is vital to ascertain the appropriate phosphorous source for each animal to guarantee the health of aquatic animals and the environment.

Soft-shelled turtle (*Pelodiscus sinensis*) is an important aquatic species in Asia. Its production is beyond 300 thousand tonnes per year in China, and it is also a member of aquatic reptiles with a carapace and plastron as a skeleton instead of a spinal column. Few researches were conducted on the requirement of dietary phosphorus in reptiles. Recently our study found that the optimal available phosphorus in the diet for *Pelodiscus sinensis* is 1.041% (total phosphorus is 1.80%),[4] which is slightly higher than fish. However, its suitable phosphorus sources supplemented in diet are still open. Thereby, the objective of this study is to evaluate the effects of two calcium phosphates (MCP and DCP) on the growth performance, feed conversion efficiency, apparent digestibility of phosphorus, whole body proximate composition, and physiological status in juvenile *Pelodiscus sinensis*.

MATERIALS AND METHODS

DIETS PREPARATION

Two inorganic phosphorus sources were purchased from Shanghai Yien Chemical Technology Ltd. (Shanghai, China): Calcium phosphate monobasic ($Ca(H_2PO_4)_2 \cdot H_2O$, 92% purity, named as MCP), calcium phosphate dibasic ($CaHPO_4 \cdot 2H_2O$, 99% purity, named as DCP). Two isonitrogenous (41% crude protein), isoenergetic (19.5MJ/kg, gross energy), iso-calcium (2.5%), and iso-phosphorus (2.2%, total phosphorus) experimental diets were designed with MCP and DCP, the contents of MCP and DCP in diets were added at doses of 4% and 5.47% respectively. The consistent dietary calcium levels were obtained by adjusting limestone and microcrystal cellulose contents in two experimental diets. Fishmeal, chicken meal, casein, and wheat gluten were used as dietary major protein sources, α-starch as the carbohydrate source, and fish oil as the lipid source. Yttrium oxide (Y_2O_3) was added at 0.1% as an inert marker to determine the apparent digestibility coefficient. The available phosphorus contents in MCP and DCP diets were 1.18% and 1.08%, respectively, calculated by the total phosphorus content multiplied by the apparent digestibility coefficients of phosphorus. The feed ingredients and chemical composition of the two experimental diets are presented in **Table 1**. All ingredients were mixed and ground through 175-μm mesh. The 2.0 mm soft pellets were made by extruded pelletizer (EL-260, Youyi Machinery Factory, Weihai, China) and stored at -20°C until use.

Table 1. Formulation and proximate composition of experimental diets

	Groups	
Ingredients (%)	*MCP*[1]	*DCP*[2]
Fish meal	17.00	17.00
Chicken meal	18.00	18.00
Wheat gluten	7.00	7.00
Squid liver powder	4.00	4.00
Yeast powder	4.00	4.00
Casein	12.00	12.00
Gelatin	3.00	3.00
Yeast extract	2.00	2.00
α-starch	16.00	16.00
Fish oil	4.00	4.00
Vitamin & mineral premix[3]	2.00	2.00
Yttrium oxide (Y_2O_3)	0.10	0.10
Others[4]	1.30	1.30
Limestone	2.72	1.07
Calcium phosphate monobasic	4.00	0
Calcium phosphate dibasic	0	5.47
Microcrystal cellulose	2.88	3.06
Proximate composition (%)		
Moisture	16.74	16.06
Crude protein	40.87	41.58
Crude lipid	4.99	4.62
Crude ash	11.65	11.51
Gross energy (MJ/kg)	19.47	19.56
Calcium	2.46	2.55
Total phosphorus	2.22	2.20
Available phosphorus	1.18	1.08

[1]MCP: Calcium phosphate monobasic; [2]DCP: Calcium phosphate dibasic;[3]Vitamin & mineral premix: according to Zhang et al.[15]; [4]Others: methionine 0.17, lysine 0.20, sodium chloride 0.10, betaine 0.10, potassium chloride 0.20, taurine 0.40, choline chloride 0.10, mildew inhibitor 0.03.

TURTLES AND CULTURE CONDITIONS

Juvenile soft-shelled turtles were obtained from Yutian Farm (Tangshan, China). After 10 days' acclimation, turtles were deprived of diets for 24 h, and 96 turtles (initial body weight: 5.40±0.03g) were randomly assigned to 12 cylindrical fiberglass tanks (0.6m in diameter and 0.7m in height) with 6 tanks per group. Each tank was stocked with 8 turtles. All turtles were manually fed with the corresponding experimental diets to apparent satiation three times daily (8:00, 12:30, and 17:30) for 60 d. Feces and leftover diet were siphoned out 30 min post-feeding to calculate the feeding rate and apparent digestibility coefficients. The conditions during the experiment were as follows: water temperature is 30°C ±0.5°C, pH was 7.5±0.1, and the dissolved oxygen was more than 5 mg/L, ammonia-N was less than 0.11 mg/L.

SAMPLE COLLECTION AND CHEMICAL ANALYSIS

At the end of the trial, all turtles were fasted for 24h and anaesthetized with MS-222 (1000mg/L) before sampling. Turtles were counted and batch-weighted in each tank. Five turtles in each tank were randomly selected and pooled into one sample to analyze the body's proximate composition. Another three turtles per tank were weighted and decapitated individually, the blood was collected into heparinized tubes and centrifuged at 4000 g, 4℃ for 15 min, and plasma was stored in a -80°C freezer before analysis. Then, the turtles were dissected out the liver to weight and stored in the freezer for further alkaline phosphatase activity measure.

The proximate composition analysis of diets, feces, and whole body (moisture, crude protein, crude lipid, crude ash, and gross energy) was conducted following Zhang et al.[16] Phosphorus and calcium contents were determined according to the method of Wang et al.[4] The plasma contents of triglyceride, total cholesterol, glucose, and alkaline phosphatase activities in plasma and liver were measured with commercial kits according to the manufacturer's protocols (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) by spectrophotometry (TU-1901; Purkinje General Ltd., Beijing, China). Total protein concentration in the liver was determined with bovine serum albumin as the standard. The Y_2O_3 contents in feed and feces were analyzed by inductively coupled plasma source mass spectrophotometer (X Series 2 ICP-MS) (Thermo Electron Corporation, Waltham, MA, USA).

DATA CALCULATION

Survival rate (SR, %) = 100 × (final number of turtles/initial number of turtles).

Weight gain rate (WGR, %) = 100 × [FBW (g) - IBW (g)]/ IBW (g);

Specific growth rate (SGR, %/d) =100 × (LnFBW - LnIBW)/days;

Feed conversion ratio (FCR) =dry feed intake (g)/ wet weight gain (g);

Feeding rate (FR, %/d) = 100×dry feed intake (g)/ [(IBW (g) + FBW (g))/2]/days;

Hepatosomatic index (HSI, %) = 100× (liver weight, g) / (body weight, g);

Apparent digestibility coefficient of nutrient (ADC) =100 × (1- nutrient content in feces /nutrient content in diet × Y_2O_3 content in diet/ Y_2O_3 content in feces);

STATISTICAL ANALYSIS

All Data were expressed as means ± SD and analyzed using STATISTICA 10.0 software (Statsoft, Inc., Tulsa, OK, USA). Before analysis, the normality of data distribution and the homogeneity of variance were assessed by the Shapiro-Wilk and Levene's tests, respectively. An Independent T-test was employed between MCP and DCP treatments in this study. The significant level was set at P <0.05.

Table 2. Effects of two phosphorus sources on the growth performance of *Pelodiscus sinensis*

	Groups	
Parameters	*MCP*	*DCP*
Survival rate (%)	100	100
Final body weight (g)	40.42±5.15	40.24±6.11
Feeding rate (%/d)	2.06±0.13	2.03±0.12
Weight gain rate (%)	649.92±0.96	646.63±1.13
Specific growth rate (%/d)	3.29±0.21	3.28±0.24
Feed conversion ratio	0.82±0.08	0.82±0.08
Hepatosomatic index (%)	3.94±0.62	4.32±0.87

Note: Values within the same row with different letters are significantly different (P<0.05).

Table 3. Apparent digestibility coefficient (ADC) of *Pelodiscus sinensis* fed with two experimental diets

	Groups	
Parameters (%)	*MCP*	*DCP*
ADC of Dry matter	83.03±0.01	81.95±0.01
ADC of phosphorus	53.22±0.04	48.98±0.02

Note: Values within the same row with different letters are significantly different (P<0.05).

RESULTS

GROWTH PERFORMANCE AND FEED CONVERSION EFFICIENCY

The growth performance of turtles fed with different experimental diets is displayed in **Table 2**. No dead turtles were observed throughout the whole trial period in this study. Different phosphorus sources have not significantly influenced the growth parameters, including final body weight, weight gain rate, and specific growth rate (P > 0.05). The feeding rate and conversion ratio were unaffected (P > 0.05). No significant difference was observed in the hepatosomatic index between MCP and DCP groups (P > 0.05).

APPARENT DIGESTIBILITY AND PROXIMATE BODY COMPOSITION

The apparent digestibility coefficients of dry matter and phosphorus in MCP group are slightly higher than that in DCP group but did not reach the statistically significant level (P > 0.05) (**Table 3**). T-test showed no significant differences in body proximate composition, including moisture, crude protein, crude lipid, crude ash, gross energy, calcium, and phosphorus between the two treatments (**Table 4**).

PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

No significant differences were observed for plasma triglyceride, total cholesterol, glucose contents, and alkaline

Table 4. Whole-body proximate composition in *Pelodiscus sinensis* fed with two experimental diets (wet weight basis)

	Groups	
Parameters (%)	*MCP*	*DCP*
Moisture	72.42±0.01	72.15±0.01
Crude protein	17.24±0.01	17.50±0.01
Crude lipid	5.11±0.02	5.14±0.01
Crude ash	4.99±0.01	4.99±0.01
Gross energy (MJ/kg)	21.56±1.20	21.31±0.29
Calcium	1.18±0.03	1.19±0.02
Phosphorus	0.82±0.02	0.85±0.01

Note: Values within the same row with different letters are significantly different (P<0.05).

Table 5. Plasma physiological and biochemical parameters and hepatic alkaline phosphatase activity in *Pelodiscus sinensis* fed with two experimental diets

	Groups	
Parameters (%)	*MCP*	*DCP*
Triglyceride (mmol/L)	4.88±0.44	3.21±0.87
Total cholesterol (mmol/L)	6.42±0.85	6.27±0.80
Glucose (mmol/L)	5.44±0.35	5.92±0.56
Plasma Alkaline phosphatase (U/L)	8.13±2.12	7.30±1.33
Hepatic Alkaline phosphatase (U/g prot)	0.37±0.09	0.32±0.08

Note: Values within the same row with different letters are significantly different (P<0.05).

phosphatase activity between MCP and DCP groups, and the alkaline phosphatase activity of turtle liver in MCP treatment was similar to that in DCP treatment (P > 0.05) (**Table 5**).

DISCUSSION

In the present study, the survival rate of turtles was 100%, and the growth rate increased six-fold during the 60d feeding period, demonstrating that all turtles in this experiment were healthy under these experimental surroundings. Furthermore, the dietary calcium contents in two experimental groups of this study were adjusted with limestone to equal levels. Hence, there's a reasonable prospect that the different responses between the two treatments are all deserved from the only variable of dietary phosphorus in this study.

The apparent digestibility coefficients of dietary phosphorus and dry matter were not significantly affected by MCP and DCP in the current study, similar to the results in *Oreochromis niloticus*.[10] However, this result disagreed with most of fish, such as Carp (*Cyprinus carpio*),[7] European sea bass (*Dicentrarchus labrax* L.),[8] *Hemibarbus maculatus*,[9] and yellowtail (*Seriola quinqueradiata*).[6] This may be because of the solubility of calcium phosphates and the animal species. Phosphorus absorption in the gastrointestinal tract is positively correlated with the solubility. The more soluble the salt, the higher the phosphorus availability.[1] It is reported that MCP has higher water solubility (1.8g/100mL) than DCP (0.136g/100mL) at room temperature,[17] which is the main reason that the availability of MCP is more than DCP for some animals, especially in stomachless animals.

On the other hand, the animal species cultured is another factor influencing the phosphorus availability. The stomach-containing species could effectively secret acid gastric juice to absorb dietary phosphorus better than those stomachless species; salmonids utilize phosphorus in fish meals more efficiently than carp, tilapia, and channel catfish.[1] Generally, in commercial feed formulation, DCP is usually supplemented in the feed of terrestrial animals except for piglets, and MCP is added to the diet of fish and piglets. The piglet's stomach is underdeveloped in function compared with adult swine, and most fish, especially cyprinid fish (stomachless fish), had a relatively weaker secretion in gastric juice. So, piglets and most fish utilized dietary MCP better than DCP. *Pelodiscus sinensis*, a kind of aquatic reptile, has a relatively developed stomach and lives in a warm environment (its optimal growth temperature is 30℃), which promotes turtles to secrete the acid gastric juice abundantly for digesting and absorbing phosphorus in their diet. Therefore, *Pelodiscus sinensis* utilized MCP and DCP at the same apparent digestibility coefficient in this study.

Although the digestibility of phosphorus is considered as the direct evidence for the availability of phosphorus sources, the growth performance, whole-body proximate compositions, the plasma physiological and biochemical parameters are regarded as the comprehensive and reliable indicators to assess the requirements of phosphorus.[18-21] Dietary phosphorus levels have significantly influenced aquatic animals' growth performance; inadequate dietary available phosphorus and excessive phosphorus have given rise to decelerated growth and inferior feed utilization.[1,22] This phenomenon was also observed in our previous phosphorus research of *Pelodiscus sinensis*.[4] The dietary optimal available phosphorus level for *Pelodiscus sinensis* is 1.041%. According to this, this study's dietary available phosphorus levels (1.18% and 1.08% in MCP and DCP groups, respectively) are adequate for *Pelodiscus sinensis*.

Meanwhile, the MCP group and DCP group have the same growth response in this study, which is consistent with the results of the digestibility in this study and similar to the results in *Oreochromis niloticus*,[10] and *Hemibarbus maculatus*.[9] However, it is not similar to the results in *Leptobotia elongata*.[23] The reason is mainly from the different digestibility of phosphorus resources between MCP and DCP in diet, although they are the same in total dietary phosphorus.[21] In the present study, all ingredients in each experimental diet are the same except for the phosphorus sources, and the nutritional levels are equal in the two groups. There's a reasonable prospect that the different responses of growth performance in turtles will come from

the phosphorus sources. However, the same growth performance and feed coefficient ratio between MCP and DCP groups were observed in this study, which illustrated that different phosphorus sources did not influence the growth performance of turtles and further indirectly verified that *Pelodiscus sinensis* obtained equivalent phosphorus from both phosphorus sources.

Alkaline phosphatase (AKP) is a membrane-bound ectoenzyme and plays an important role in biomineralization. It hydrolyzes pyrophosphate and provides inorganic phosphate to promote mineralization and appears to be a sensitive and reliable indicator for dietary phosphorus requirement.[20,24] Generally, plasma AKP activities of aquatic animals were significantly decreased when fed with a high-phosphorus diet.[19,25] In the study of *Oreochromis niloticus*, the serum AKP activities in the MCP diet were significantly higher than in the DCP diet.[10] However, no other AKP results were reported in the studies of phosphorus sources. In the present experiment, the AKP activities in turtles' plasma and liver were unaffected by MCP and DCP supplementation. This may be because the available phosphorus contents in both MCP and DCP groups equally satisfied the needs of skeleton mineralization in turtles.

Moreover, dietary phosphorus levels often influence the body proximate composition of aquatic animals. The body's phosphorus content is usually positively connected with dietary phosphorus level, and the body's lipid content will increase when fed with an inadequate phosphorus diet.[1,19,20] Our prior study in *Pelodiscus sinensis* also demonstrated that insufficient dietary phosphorus decreased the body phosphorus level and increased the body lipid level of turtles.[4] In the present study, the body proximate composition, including crude protein, crude lipid, calcium, and phosphorus, was unaffected by MCP and DCP, which again illustrated that MCP and DCP had an equal effect on *Pelodiscus sinensis*.

In summary, dietary supplementation with MCP and DCP did not markedly influence the growth performance, digestibilities of dry matter and phosphorus, body proximate compositions, plasma physiological parameters, and the activities of alkaline phosphate in plasma and liver. The present study suggested that MCP and DCP had the same biological phosphorus availability in juvenile *Pelodiscus sinensis*.

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REFERENCES

1. Lall SP, Kaushik SJ. Nutrition and Metabolism of Minerals in Fish. *Animals*. 2021;11(9):2711. doi:10.3390/ani11092711

2. Fjelldal PG, Hansen T, Albrektsen S. Inadequate phosphorus nutrition in juvenile Atlantic salmon has a negative effect on long-term bone health. *Aquaculture*. 2012;334-337:117-123. doi:10.1016/j.aquaculture.2011.12.043

3. Costa JM, Sartori MMP, Nascimento NFD, et al. Inadequate dietary phosphorus levels cause skeletal anomalies and alter osteocalcin gene expression in zebrafish. *International Journal of Molecular Sciences*. 2018;19(2):364. doi:10.3390/ijms19020364

4. Wang Y, Geng Y, Shi X, et al. Effects of dietary phosphorus levels on growth performance, phosphorus utilization and intestinal calcium and phosphorus transport-related genes expression of juvenile Chinese soft-shelled turtle (*Pelodiscus sinensis*). *Animals*. 2022;12(22):3101. doi:10.3390/ani12223101

5. Vielma J, Lall SP. Control of phosphorus homeostasis of Atlantic salmon (*Salmo salar*) in fresh water. *Fish Physiology and Biochemistry*. 1998;19(1):83-93. doi:10.1023/a:1007757321695

6. Sarker PK, Fukada H, Masumoto T. Phosphorus availability from inorganic phosphorus sources in yellowtail (*Seriola quinqueradiata* temminck and schlegel). *Aquaculture*. 2009;289(1-2):113-117. doi:10.1016/j.aquaculture.2009.01.006

7. Yang YH, Guo Q, Han Y, Fan ZT. The apparent digestibility of phosphorus in different phosphorus in different phosphorus sources on carp. *Journal of Northeast Agricultural University*. 2005;36:762-766. doi:10.3969/j.issn.1005-9369.2005.06.014

8. Pimentel-Rodrigues A, Oliva-Teles A. Phosphorus availability of inorganic phosphates and fish meals in European sea bass (*Dicentrarchus labrax* L.) juveniles. *Aquaculture*. 2007;267(1-4):300-307. doi:10.1016/j.aquaculture.2007.01.018

9. Zhao CY, Zhou HQ, Xu P, Chen JM, Ye JY, Li HX. Availability of phosphorus in various inorganic phosphorus sources in dietary of *Hemibarbus maculatus* Bleeker. *Journal of Shanghai Ocean University*. 2008;2:199-203.

10. Bai FJ, Luo L, Chen RX, Chen YJ, Lin K. A comparison of Application effect among three kinds of inorganic phosphorus sources on generic improvement of farmed tilapia (*Oreochromis niloticus*). *Chinese Journal of Animal nutrition*. 2016;28:2969-2976. doi:10.3969/j.issn.1006-267x.2016.09.037

11. Bureau DP, Hua K. Towards effective nutritional management of waste outputs in aquaculture, with particular reference to salmonid aquaculture operations. *Aquaculture Research*. 2010;41(5):777-792. doi:10.1111/j.1365-2109.2009.02431.x

12. Gamble MM, Sarker PK, Kapuscinski AR, et al. Toward environmentally sustainable aquafeeds: Managing phosphorus discharge from Nile tilapia (*Oreochromis niloticus*) aquaculture with microalgaesupplemented diets. *Elementa: Science of the Anthropocene*. 2021;9(1):00170. doi:10.1525/elementa.2020.00170

13. Daniel TC, Sharpley AN, Lemunyon JL. Agricultural Phosphorus and Eutrophication: A Symposium Overview. *Journal of Environmental Quality*. 1999;27(2):251-257. doi:10.2134/jeq1998.00472425002700020002x

14. Sarker PK, Pilote A, Auffret M, et al. Reducing geosmin off-flavor compounds and waste outputs through dietary phosphorus management in rainbow trout aquaculture. *Aquaculture Environment Interactions*. 2014;6(1):105-117. doi:10.3354/aei00119

15. Zhang X, Yang Z, Deng D, et al. Effects of dietary starch levels on the growth, plasma metabolites and expression of genes involved in glucose transport and metabolism in *Pelodiscus sinensis* juveniles. *Aquaculture Research*. 2020;51(2):738-750. doi:10.1111/are.14424

16. Zhang HJ, Sun JW, Liu HY, Zhao WQ, Yang ZC. The effect of dietary lipid levels on growth performance, lipid deposition, and antioxidant status of juvenile turbot, *scophthalmus maximus*, fed isonitrogenous and isoenergetics diets. *Israeli Journal of Aquaculture - Bamidgeh*. 2015;67:1142. doi:10.46989/001c.20719

17. Tang AJ, Zhang LS. Feed additive: calcium dihydrogen phosphate. *Guizhou Chemical Industry*. 1997;4:20-23.

18. Ji K, Liang H, Mi H, et al. Effects of dietary phosphorus levels on growth performance, plasma biochemical parameters and relative gene expression of lipogenesis of bighead carp, *Aristichthys nobilis*. *Israeli Journal of Aquaculture - Bamidgeh*. 2017;69. doi:10.46989/001c.20842

19. Xu HG, Zhang X, Wei YL, Sun B, Jia LL, Liang MQ. Effects of dietary phosphorus level and stocking density on tiger puffer *Takifugu rubripes*: Growth performance, body composition, lipid metabolism, deposition of phosphorus and calcium, serum biochemical parameters, and phosphorus excretion. *Aquaculture*. 2020;529:735709. doi:10.1016/j.aquaculture.2020.735709

20. Lei Y, Sun Y, Wang X, et al. Effect of dietary phosphorus on growth performance, body composition, antioxidant activities and lipid metabolism of juvenile Chinese mitten crab (*Eriocheir sinensis*). *Aquaculture*. 2021;531:735856. doi:10.1016/j.aquaculture.2020.735856

21. Lall SP. Chapter 6 — The minerals. In: Hardy RW, Kaushik SJ, eds. *Fish Nutrition*. 4th ed. Academic Press; 2022:469-554. doi:10.1016/b978-0-12-819587-1.00005-7

22. NRC (National Research Council). *Nutrient Requirements of Fish and Shrimp*. The National Academies Press; 2011.

23. Yuan W, Liu B. Study on the application of calcium phosphate dibasic in aquaculture of *Leptobotia elongate*. *Feed Industry*. 2000;21(9):23-24. doi:10.3969/j.issn.1001-991X.2000.09.010

24. Orimo H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. *Journal of Nippon Medical School*. 2010;77(1):4-12. doi:10.1272/jnms.77.4

25. Zhao M, Luo J, Zhou Q, et al. Influence of dietary phosphorus on growth performance, phosphorus accumulation in tissue and energy metabolism of juvenile swimming crab (*Portunus trituberculatus*). *Aquaculture Reports*. 2021;20:100654. doi:10.1016/j.aqrep.2021.100654