International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 40 No. 2 Aug. 2023, pp. 412-420

http://www.ijias.issr-journals.org/

Use of Phytase in Aquaculture and Possibilities of Incorporation into Trout (*Oncorhynchus Mykiss*) Feed

Niang Mamadou Sileye[1] and Jean Fall[2]

[1]Cheikh Anta Diop University of Dakar, Faculty of Sciences and Techniques, ED-SEV, ED-SHA, Senegal

[2]University Institute of Fisheries and Aquaculture, UCADII Educational Building, First floor, P.O Box: 5005, Senegal

ABSTRACT: The study focuses on the use of phytase in aquaculture, its zootechnical benefits and the possibilities of incorporating it into feed. The aim is to reduce phosphorus waste in fish feed, with no loss of zootechnical performance and lower feed costs. We studied the data in the bibliography, in order to better valorize raw materials (total phosphorus, phytic phosphorus and available phosphorus); to determine phosphorus requirements for aquaculture species; to determine signs of phosphorus deficiency in fish; to study antagonism between phosphorus and calcium and also to study the different forms of discharge by rainbow trout. The results found in the bibliography enable us to test several hypotheses for the formulation of feeds for rainbow trout with different raw materials. This simulation and waste calculation enabled us to validate two feeds: a control feed A1 (0.5% monocalcium phosphate) and a test feed A2 (supplementation with 0.002% phytase and phosphate). The feeds were produced and sent to an experimental facility (Lycée agricole de Bréhoulou). The results of the formulation showed a 28% reduction in phosphorus loss for the test feed compared with control feed. Supplementation resulted in a gain of 2.3 euros per tonne. The results show no significant difference in zootechnical parameters (growth rate, mortality, weight gain and obvious conversion) between the control and trial feeds. Waste measurements also show no significant difference between the control feed and the test feed, but the difference would enable waste to be reduced by 35.6% through the use of phytase.

KEYWORDS: Phosphorus, phytic acid, phytase, requirement, digestibility, formulation, feed, rejection.

1 INTRODUCTION

Aquaculture is the world fastest-growing source of animal protein, currently supplying more than half of the fish products consumed worldwide (FAO, 2022). The growth and intensification of aquaculture has raised a number of discharge and chemical pollution issues related to phosphorus and nitrogen that need to be addressed to ensure the sustainability of the aquaculture industry (Asha *et al*., 2021).

Inland aquaculture has been criticized for the discharges generated by fish farming. Phosphorus (P) is one of the elements targeted. Phosphorus is essential for the growth and development of aquaculture species (Sugiura, 2018; Verlhac *et al*., 2007; Kaushik, 2005; Hardy and Gatlin, 2002).

The main source of P for farmed fish in aquaculture feeds is fishmeal. Moreover, aquaculture today uses more than 80% of P. Fishmeal is a source of proteins of high biological value (amino acids); fish oil is rich in essential fatty acids (EPA, DHA) (Toshihiro, 2022).

Today, fishmeal and fish oil prices are rising sharply on the markets, due to constant supply and growing demand. To remain competitive, the aquaculture industry has been striving to replace these marine raw materials. This explains the current trend to incorporate vegetable raw materials instead of fishmeal in fish feeds.

The use of fishmeal in fish feed is due to its high content of bioavailable P, inorganic phosphate. Plant raw materials contain phytic P, which is poorly assimilated by fish. The use of phytase makes this phytic P more assimilable, thus limiting the addition of inorganic phosphate to farmed fish feed. This technique would limit P discharges in fish farms and reduce the cost of fish feed.

The objectives of this study on the use of phytase in aquaculture are:

- To enrich scientific knowledge of P requirements and the use of the enzyme in rainbow trout;
- To propose a formulation using phytase to improve the environmental impact of feeds used in fish farming;
- To test the effectiveness of phytase coating (homogeneity of phytase concentrations, measurement of phytase stability during feed storage).

2 Material And Methods

2.1 Material

In this trial 1080 animals were used. Hundred and eighty (180) individuals were stocked per tank, in order to achieve a maximum density of 18kg/m3 by the end of the experiment.

The system used was open-circuit with a renewal rate of 100%/d. Water temperature was between 13 and 14°C. The average initial test weight was 55g and the average final weight was 200g. Test duration was 10 weeks. Estimated weight variation factor: 2. Air diffusers were be added permanently.

2.2 Test Methods

2.2.1 Parameters Studied For Compound Feed Formulation Requirements

The following parameters evaluated: fish P requirements, P deficiency, P/Ca antagonism, P release and phytase. These are the main elements involved in fish feed formulation in fish farming.

2.2.2 Feed Formulation

2.2.2.1 Valorization Of Raw Materials

A feed formulation matrix was drawn up. For each type of raw material, the total P, phytic P and available P content were determined. These elements were then analyzed to verify their exact composition.

2.2.2.2 Simulation Formulation

Five (05) feeds were formulated (A1, A2, A3, A4 and A5): diet 1 with phytase (A1), diet 2 with monocalcium phosphate (A2), diet 3 with dicalcium phosphate (A3), diet 4 with fish bone meal (A4) and diet 5 without inorganic phosphate or phytase (A5). This approach allows to select the most interesting raw materials (base on available P content and total P) and to check which of the different formulated feeds gives the minimum rejection at the lowest cost. The available P content/raw material price ratio determine the most profitable raw materials.

Two of the five formulas evaluated in the simulations were chosen for feed manufacture:

- A 4 mm diameter control, in which 0.5% monocalcium phosphate is incorporated into the formulation
- A 4 mm diameter test, in which monocalcium phosphate is replaced by 0.02% phytase.

Phytase is heat-sensitive. It is destroyed at 70°C. We must therefore ensure that it remains effective after prolonged storage on the farm (silo temperatures can reach 65°C in midsummer). It is produced in water-soluble form, i.e. not very soluble in oil. This means that when mixed with oil, a stable emulsion is required. The "oils + phytase" mixture must therefore be carefully monitored (duration and quality of mixing).

As phytase is thermostable, it must be incorporated after the extrusion and drying stages. The simplest solution is to incorporate it into the mixture of different oils after the feed has been dried.

2.2.3 DIET ANALYSES AND EXPERIMENTAL PROTOCOL

Table 1 shows the results of the analyses carried out on each feed. Moisture, protein, fat, ash, calcium, total P and phytic content were analyzed in each sample. For the test batch, 7 samples were taken after the oil and pellet coating stage (under vacuum). Phytic activity is measured on these samples to assess the homogeneity of the coating. Analytical methods and references for each analysis are presented in Table 1

Table 1. Feed analysis methods and references

Measured or desired characteristic	Analysis methods	References Analysis
Moisture	Desiccation, Gravimetry	NF V 18-109
Crude Protein	Kjeldahl: Mineralization, Distillation, Titrimetry	NF V 18-120
Crude Fat	Distillation, gravimetry	NF V18-117
Crude ash	Incineration, gravimetry	NF V 18-101
Calcium	Spectrometry	NF V 18-108
Phosphorus	Spectrometry	NF V 18-106

In our formulation matrix, total P, phytic P and P digestibility coefficient (PUD) have been valorized from the bibliography (Maga, 1982; Tyagi et al., 1998), (Singh et al., 2011).

2.2.4 EXPERIMENTAL SET-UP

The aim of the trial was to compare two batches in 3 replicates with different feeds:

The trial took place at the Centre Technologique Aquacole de Bréhoulou, in the salmon farming hall. Facing north-west, the salmon farming hall is open-air, but enclosed on three of its four sides, and half of it is also covered. The 6 x 2 m^3 tanks involved in this experiment are located under the canopy.

To avoid any environmental factors affecting the results, the elementary batches were staggered and each tank was fitted with a lid.

It was decided not to duplicate the batch in the middle of the experiment; the trial therefore involved 1080 animals, i.e. 180 individuals per tank, in order to achieve a maximum density of 18kg/m3 at the end of the experiment.

- Batch design

The batches were made up of individuals of average weight 55g from the same rearing band. The batches were homogeneous, i.e. the same number of individuals in each tank, a rate of variation between average weights of less than 5% and a coefficient of variation of less than 15% in each tank.

- Feeding

The fish were fed every day of the week, except Sunday, i.e. 6 days out of 7, early in the morning. Feeds were spread over ten hours, using automatic belt feeders. The feed ration is adjusted weekly according to average weight. As the overall site temperature is constant, the rationing rate, identical for each tank, is 1.67, and feed grain size is unchanged. Rationing is adjusted weekly according to biomass.

-Mortality

The number of dead fish was recorded daily. Where possible, reasons for death are given. During the trial, the mortality rate was calculated weekly.

- Water quality

Oxygen (O_2) levels are measured every hour.

Temperature is taken at a fixed time every day, with a single value for all pools.

Water P content is monitored by spectrophotometry (once a fortnight). Water samples are taken using an automated system, spectrophotometric analysis is carried out and the necessary equipment (including consumables) is supplied by Lycée de Bréhoulou.

On the day of measurement, water flow is reduced to increase P concentration. Oxygen levels are maintained during this period.

-Health status

Any pathology occurring in one of the tanks was recorded and dealt with accordingly.

The quantity of feed distributed and any events or remarks likely to influence the analysis are recorded in the trial monitoring register.

-Fish analysis

To measure the total P content of the fish, fish samples (batches of 10 fish) were taken at the start and end of the experiment. These fish were analyzed in the laboratory (whole fish grinding), to determine total P content.

- Analysis of fish content at start and end of trial**.**

-Statistical analysis

Statistical analyses were performed at the 5% confidence level, and results are presented as mean (±) standard deviation. Using R software, we performed a one-way analysis of variance (ANOVA) and a comparison of means test (Student).

3 Results And Discussion

3.1 Valorization Of Raw Materials Used In Animal Feed Production

The results of the valorization of raw materials used in feed production. In the formulation matrix, total P, phytic P and the P digestibility coefficient (PUD) were valorized from the bibliography (Singh et al., 2011; Tyagi et al., 1998; Maga, 1982).

The results of the analyses on the total P content of the formulated feed are shown in figure 1. The variations observed between the different values are relatively small (around 0.01%), with the exception of fishmeal and wheat gluten, for which a variation of 0.357 and 0.104 is observed for fishmeal and wheat gluten respectively. For fishmeal, as for wheat gluten, variations in phosphorus content can be measured as a function of variety, origin, season, processing conditions, and so on. In fish, feed is the main source of P, as the concentration in water is naturally very low (Akeem et al., 2019; Vandenberg, 2001; NRC, 1993). Fishmeal is very rich in P, which is supplied in the form of hydroxyapathy. The availability of P in fish feed is influenced by many factors, including chemical form, digestibility of the diet, interaction with other nutrients, feeding method and water chemistry (Kaushik, 2005; Hardy and Gatlin, 2002; Ravindran et al., 1994; Lall, 1991).

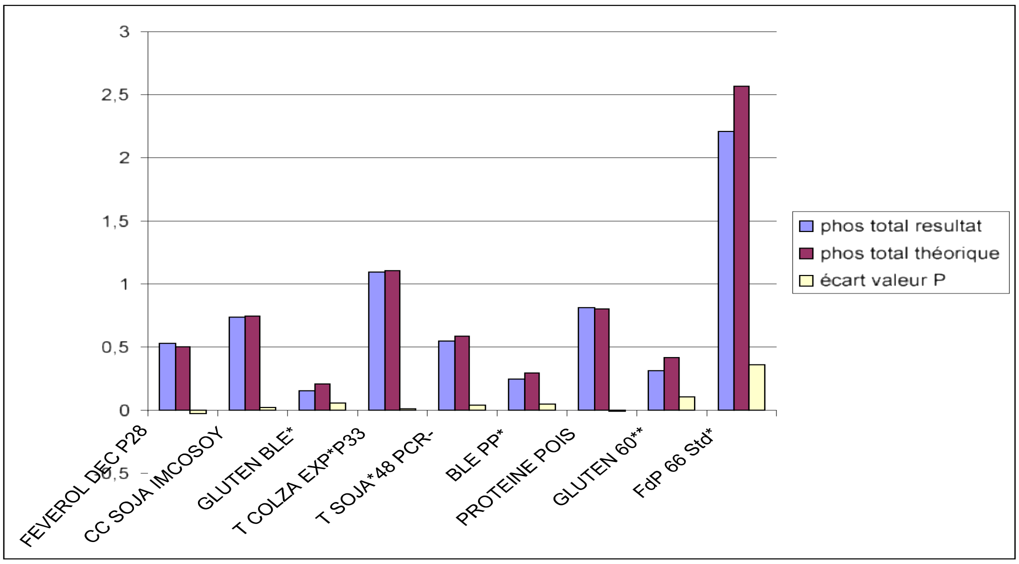

Fig. 1. Raw material analysis and comparison with theoretical values

3.2 Simulation Of Feed Formulas

All simulated feeds are formulated with a minimum of 0.5% available phosphorus, which is slightly higher than the literature recommendation (0.39-0.45) (Kaushik, 2005). Using Libra feed formulation software, we calculated the price and rejects of each feed. Price and rejection calculations are used to select the most appropriate formulas (one with phytase (A1), one with monocalcium phosphate (A2)).

The inorganic phosphate and phytase-free feed was first formulated using Libra. This simulation is used to check the need for phosphorus or phytase supplementation. To meet the minimum available P requirement of 0.5%, 19.6% fishmeal must be incorporated. This fishmeal will therefore provide over 77% of the formula's available P. The increase in (expensive) fishmeal translates into an increase in the price of the feed. This formula proves that supplementation is economically attractive and reduces the impact of farming on fishmeal resources.

The second feed tested was formulated with fishmeal (Phoscal). Phoscal, at an incorporation rate of 1.4%, enables fishmeal intakes to be substituted for available P (fishmeal now accounts for only 57.5% of total available P intakes).

P releases increase and the price decreases. Calcium levels also increase (0.98%), which, according to some authors such as Calvo-R (2020), Lellis (2002), could lead to phosphorus deficiency. Reducing the calcium level to a maximum of 0.98%, or approaching the Ca/P = 1 ratio, is equivalent to formulating a feed with fishmeal (like the first simulated feed). P deficiency leads to anorexia, reduced growth and skeletal weight, bone demineralization and skeletal deformities (Sugiura et al., 2004; Roy and Lall, 2003; Mazziotti, 2022; Lall, 2002; Vandenberg, 2001; Kaushik 1999; Ketaren et al., 1993).

After Phoscal, dicalcium phosphate feed was formulated (Table 2), followed by monocalcium phosphate feed (Table 2). The dicalcium phosphate feed is more expensive and generates higher rejects than the monocalcium phosphate feed.

Monocalcium phosphate is more digestible than dicalcium phosphate (table 2). Monocalcium phosphate rejects less P (4.1 kg P/ton feed) than dicalcium phosphate (4.8 kg P/ton feed). (Kaushik, 2005; Ouellet 1999; Lall 1991; Ogino and Takeda, 1978).

Finally, phytase feed is formulated. This feed is less costly (1,239 euros) and P emission calculations give the lowest values (2.8 kg P/tonne feed).

The phytase feed (Table 2) and the monocalcium phosphate feed were chosen, as they are both more profitable from an economic point of view, and more interesting from an environmental point of view.

Table 2. Feed formulation result

Formulation	Inorganic phosphate-free, phytase-free	with Phoscal	with dicalcium phosphate	with monocalcium phosphate	with phytase
Feed1	0	POSCAL 1.4	P BICA 0.74	PH. MOCA 0.45	RONO P L POISS 0.02
Feed2	PHOS.TOTAL % 0.818	CALCIUM % 0.983	CALCIUM 0,914	CALCIUM % 0.914	CALCIUM % 0.725
Feed3	CALCIUM % 0.904	PHOSTOTAL % 0.843	PHOS.TOT % 0,84	PHOS.TOTAL % 0.84	PHOS, TOTAL % 0.709

Among the 5 feed formulas, we have chosen the two most interesting (price).

Table 3. Comparative details of the two formulas

INGREDIENTS	Control	Test
MONOCALCIC P	0.5	0
PHYTASE	0	0.02
CALCIUM%	0.81	0.73
P TOTAL% /Brute	0.82	0.71
P. PHYTICS /Brute	0.21	0.21
P. AVAILPHOS FISH /BRUTE	0.52	0.51
DUC P	63.4	71.8
MOISTURE	7.9	7.8
DIGESTIBLE ENERGY	19.2	19.2
AVAILPHOS %/ SEC	0.565	0.554
AVAILPHOS G /MJ	0.27	0.265

Estimated rejects and feed costs

The use of phytase in the test feed reduces the selling price by 1.6 euros/t for diameter3mm, 3.57 euros for diameter 4mm and 3.4 euros for diameter 5mm (Table 5). Rejections are expressed in kg of P / 1 ton of weight gain (Table 4). From an environmental point of view, if we compare test and control feeds, the use of phytase in feeds would enable an annual reduction in P emissions of 30% (Table 5).

These results (Table 4) seem to corroborate those found in the literature (Verlhac et al., 2007, Hardy and Gatlin, 2002), which show that phytase supplementation in the diet of rainbow trout compared with fish fed a phytase-free diet (with inorganic phosphate), shows no significant difference on zootechnical performance (weight gain, growth and mortality rate). But other studies show that P deficiency in fish may not affect zootechnical performance such as growth, weight gain and mortality rate.

For P rejects, the statistical results show (Table 4). that there is no significant difference (pc = 0, 101) between control and trial. However, there is a 35.6% difference in releases between the control and the trial diet. If we examine the P release results graphically, we can see that for the control, we have two tanks with relatively high concentrations (above 0.07) and one with low concentrations. Conversely, for the test, two tanks have low concentrations (0.041 on average) and one tank has a slightly high concentration (0.057 on average

The statistical difference in rejection between the control and the trial is not significant, but if this trend continues, as shown in the literature, this reduction in rejection is very important for farmers.

Phytase supplementation of aquaculture feeds significantly reduces P excretion by fish. Lanari et al (1998) showed that fish fed a 33% soy diet supplemented with 1000 units of phytase per kg of feed significantly reduced P excretion compared with an unsupplemented diet. The work of Verlhac et al. (2007), Robinson (1997) and Biswas et al. (2007), confirms this hypothesis.

Setting up a production line for incorporating phytase into aqua feeds requires heavy investment (automation, stable emulsifier, phytase activity control plan, etc.). The investment in equipment needs to be amortized over four years to be profitable. This requires an economic study to calculate the return on investment. These results still need to be confirmed in practice (experimental structure and field rearing).

Fish use the P available in their diet to satisfy their energy metabolism. Residual P is then eliminated in the urine via the kidneys and gills (Ouellet, 1999; Hardy et al. 2002; Flimlin et al. 2003). According to Bureau and Cho (1999), Cho and Bureau (2001) and de Lellis (2004), it is the excess digestible P that is eliminated in the urine and through the fish's gills.

However, regulations on P discharges in livestock effluent are currently being applied in certain countries (USA, EU). It is therefore important to anticipate the problem of phosphorus discharges. Verlhac et al. (2007) confirm this idea. According to these authors, it is in the context of sustainable development of aquaculture, the increasing use of plant-based ingredients in aquaculture, environmental concerns, and the presence of phytate as an anti-nutritional factor in plant raw materials that justifies the use of phytase in aquaculture.

Table 4. Experimental test results

Parameters	A1	A2	P (Critical probability)
Daily growth rate (%)	15.3±0.47[a]	14.6±0.5[a]	0.23
Apparent conversion rate (%)	1.5±0.08[a]	1.4±0.2[a]	0.85
Mortality rate (%)	8.3±2.4[a]	5.7±1.1[a]	0.53
Weight gain	25.35±2.34[a]	22.94±1.4[a]	0.26
Quantity of feed distributed (g)	2202.133±32.16[a]	2094.8±84.19[a]	0.10
Phosphorus (rejects mg/l)	0.073±0.019[a]	0.047±0.008[a]	0.10

Table 5. Calculation of rejects and costs for formulated feeds

	Diameter 3mm	Diameter 4mm	Diameter 5mm
Control test	**witeness test**	**witeness test**	**Témoin Essai**
Sales price (euros/tonne)	1241.1 1239.5	1267.07 1263.5	1287.7 1284.3
Difference (euros/tonne)	1.6	3.57	3.4
monocalcique PH %	0.5	0.55	0.55 -
Phytase %	0 0.02	0 0.02	0 0.02
Power supply features			
Calcium %	0.81 0.73	0.667 0.619	0.65 0.609
total P %	0.82 0.71	0.71 0.625	0.693 0.615
Available P %	0.52 0.51	0.449 0.45	0.449 0.455
phytic P %	0.21 0.21	0.179 0.202	0.168 0.2
Phosphorus emissions			
PI (dissolved P) kg/tonne	0.82 0.65	1.31 1.68	1.38 1.47
Total P discharge kg/tonne	4.11 2.95	2.6 1.75	2.43 1.65
Rejection difference P (compared with control)) %	28.2	32.6	32
Difference in dissolved P rejection (compared with control) %	20.7	28.2	65.2

4 CONCLUSION

The results of the simulation study show that phytase represents a real opportunity for the aquaculture industry, from an environmental point of view, by reducing phosphorus discharges into the natural environment and lowering production costs (feed costs). At the industrial level, the aim is to estimate the cost of logistical investments, as well as the cost of analytical control, which is a prerequisite for any future development. The aim of the analytical control plan (total P and phytate on raw materials) is to optimize the value of the raw materials used in the composition of fish feed, but above all to ensure that phytic phosphorus is sufficient in formulas including phytase. Finally, we have found that without phytic phosphorus, phytase loses its interest and phosphorus deficiency problems may arise.

REFERENCES

[1] Akeem B. D. Abdullateef A. Adenike S. Tola-F. Ayoola O. A (2019), Waste production in aquaculture: Sources, components and managements in different culture systems. Aquaculture and Fisheries Volume 4, Issue 3, May 2019, Pages 81-88.

[2] Asha P. T. Jayaleksmi S. J. Minnu B. Jithin S (2021), Aquaculture waste water treatment technologies and their sustainability: A review. Energy Nexus 4 (2021) 100022.

[3] Bureau D. P. et Cho C. Y (1999), Phosphorus utilization by rainbow trout (Oncorhynchus mykiss): estimation of dissolved phosphorus waste output. Aquaculture 179 (1-4): 127-140.

[4] Calvo.R M. Hou S.S. Snyder A.C (2020), Increased mitochondrial calcium levels associated with neuronal death in a mouse model of Alzheimer’s disease. Nat Commun 11, 2146 (2020). https://doi.org/10.1038/s41467-020-16074-2.

[5] Cho C. Y.et Bureau et al. D. P (2001), A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquaculture Research 32: 349-360.

[6] Denstadli V. Storebakken T. Svihus B. and Skrede A (2007), A comparison of online phytase pre-treatment of vegetable feed ingredients and phytase coating in diets for Atlantic salmon Salmo salar L. reared in cold water. Aquaculture. in press.

[7] Flimlin G. Sugiura S. et Ferraris. R. P (2003), examining phosphorus in effluents from rainbow trout (Oncorhynchus mykiss) Aquaculture. 6 p.

[8] Hardy R.W (2002), Rainbow trout Oncorhynchus mykiss -14. In Webster. C.D et Lim. C. Nutrient requirements and feeding of finfish for aquaculture. CAB international: 184-202.

[9] Hardy R. W. Gatlin III. D. M (2002), Nutritional strategies to reduce nutrient losses in intensive aquaculture. In: Cruz-Suarez. L. E., Ricque-Marie. D., Tapia-Salaza. M., Gaxiola Cortés. M. G. Simoes. N. (Eds).

[10] Kaushik S.-J (2005), P requirements and a Port in fish. INRA Prod. Anim. 18. 203-208.

[11] KAUSHIK, S. (2005). Phosphorus requirements and intake in fish. INRAE Productions Animales, 18 (3), 203-208. https://doi.org/10.20870/productions-animales.2005.18.3.3525.

[12] Kaushik S. Guillaume. J. Bergot. P. Métailler. R (1999). Nutrition and feeding of fish and shellfish. INRA Editions, 489p.

[13] Ketaren P.P. Batterham E.S. Dettmann E.B. and Farrell D. J (1993), Phosphorus studies in pigs. 3. Effect of phytase suplementation on the digestibility and availability of phosphorus in soya-bean meal for grower pigs. Br. J. Nutr. 70: 289-311.

[14] Lall S. P (2002), The Minerals. Pages 259-308 in J.E. Halver and R.W. Hardy. editors. Fish Nutrition. 3rd Edition. Academic Press. New York.

[15] Lall S.P (1991), Digestibility, metabolism and excretion of dietary phosphorus in fish. In: Cowey. C.B. Cho et al, C.Y. (Eds.), Nutritional Strategies and Aquaculture Wastes. Proceedings of the First International Symposium on Nutritional Strategies in Aquaculture Waste Management. University of Guelph. Guelph. Ontario. Ontario. Canada. P. 21- 26.

[16] Lellis W.A. Barrows F.T. Hardy. R.W (2004), Effects of phase-feeding dietary phosphorus on survival. growth and processing characteristics of rainbow trout Oncorhynchus mykiss. Aquaculture 242. Issues 1-4: 607-616.

[17] Li M. H. et E H. Robinson (1997), Microbial phytase can replace inorganic phosphorus suplements in channel catfish Ictalurus punctatus diets. J. World Aquaculture. Soc. 4: 402-406.

[18] Maga J.A (1982), Phytate: Its chemistry. Occurrence. Food interactions. nutritional significance. and methods of analysis. Crit. Rev. Food Sci. Nutr. 16: 1-48.

[19] National Research Council (1994), Nutrient requirements of poultry. Neuvième édition révisée. National Academy Press Washignton. DC.

[20] Mazziotti G. Lania A.G. & Canalis E (2022), Skeletal disorders associated with the growth hormone–insulin-like growth factor 1 axis. *Nat Rev Endocrinol* 18, 353–365 (2022). https://doi.org/10.1038/s41574-022-00649-8.

[21] Ogino C. et Takeda H (1978), Requirements of rainbow trout for dietary calcium and phosphorus. Bull. Jpn. Soc. Sci. Fish. 44: 1019-1022. Piper. R. G. ed. (1982) Fish Hatchery Management. U.S. Dept. of the Interior. Fish and Wildlife Service. Washington. DC

[22] Ouellet G (1999), Fish farm discharges and their environmental impact. Québec. MAP AQ. 42p.

[23] Ravindran V. Ravindran G. et Sivalogan. S (1994), Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chemistry 50 (2): 133-136.

[24] Roy P.K. Lall S.P (2003), Dietary phosphorus requirement of juvenile haddock (Melanogrammus aeglefinus L.) Aquaculture. 221. 451- 468.

[25] Singh B. Kunze G et Satyanarayana T (2011), Developments in biochemical aspects and biotechnological aplications of microbial phytases. Biotechnology and Molecular Biology Reviews Vol. 6 (3), P. 69-87.

[26] Sugiura S.H (2018), Phosphorus, Aquaculture, and the Environment, Reviews in Fisheries Science & Aquaculture, 26: 4, 515-521, DOI: 10.1080/23308249.2018.1471040.
[27] Sugiura S.H. Hardy R.W. Roberts R.J (2004), The pathology of phosphorus deficiency in fish - a review. J. Fish Diseases. 27. 255-265.
[28] Toshihiro W (2022), Fishing Technolog Division National Research Institute of Fisheries Engineering Fisheries Research Agency, Ebidai, HasakiK, ashfrnaI, baraki314-0421 Japan (tosihiro@af fc.go).
[29] Tyagi P.K. Tyagi P.K. Verma SVS (1998), Phytate phosphorus content of some common poultry feed stuffs. Indian J. Poult. Sci. 33: 86-88.
[30] Vandenberg W. G (2001), Encapsulation of microbial phytase: the influence on nutrient availability in rainbow trout. PhD thesis. Université Laval. 255 p.
[31] Verlhac V. Vielma J et Gabaudan J (2007), the use of phytase in aquatic feeds. DSM Nutritional Products Ltd. Animal nutrition and health Rand D France - 2. FGFRI. Finlande - 3. DSM Nutritional Products. Thaïlande.
[32] http://www.aquafeed.com/docs/ah2007/VERLHAC.doc).
[33] https://www.fao.org/3/cc0461en/cc0461en.pdf (2022).
[34] http://www.fao.org/news/story/fr/item/94233/icode.