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Phosphorus: problems and solutions

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Abstract

Phosphorus (P) has been increasingly recognized as a key nutrient in aquafeeds and the aquatic environment. In this review, five major issues concerning P are highlighted and their solutions are given. **(1) Diagnostic P indicators**: Among numerous P indicators studied in fish and animal species, no sensitive and stable indicator has been identified. For the diagnosis of fish P status, gauge indicators are most reliable and practical. **(2) Fish growth and N:P stoichiometry**: Body N and P ratio is constant irrespective of the body size, except early ontogeny and under malnutrition. The P requirement, therefore, can be accurately expressed as per body N accretion. In practice, the dietary requirements of P can be and should be expressed accurately by normalizing the measured P requirement based on its feed efficiency; i.e., standard requirements. **(3) Prevention of bone deformity**: Larval-early juvenile fish are most vulnerable to bone deformity because their collagen matrix is only partially calcified and stabilized. Collagens have species-specific low melting temperatures. Thus, only a slight increase in temperature results in bone malformation, especially upon under-mineralized collagen. Hence, high temperature, low-P, and low-vitamin C cause bone deformity. Since small fish contribute little to effluent P, reducing dietary P during this vulnerable period should be avoided. **(4) Digestibility and absorption**: Our interest in digestibility has been shifting from fish meal to plant ingredients. Phytase supplementation is less effective at fish's "body temperature" compared to livestock animals and birds. In vitro acid-dough incubation of plant materials (with phytase) can achieve near 100% digestion of plant P. Plant materials also contain substantial amounts of lignocelluloses. Developing technology to digest such fibrous components is becoming increasingly important to reduce the excretion of organic waste. **(5) Making low-pollution feeds** is simple and easy; as, for example, all aquarium feeds are made to be low-polluting. However, low-pollution feeds, if they are used for aquaculture, must meet various other standards, including the feed cost, fish growth, and product quality. Obviously, the goal is still far off. Sustainable development of aquaculture depends largely on problem-solving research rather than some peripheral efforts.

Introduction

Aquaculture has been one of the fastest-growing food production sectors over the past decades, now supplying half of the world seafood needs (Troell et al., 2014; FAO, 2016). Despite such great potential, aquaculture operations, especially intensive farming systems (e.g., raceway and net cage culture), result in the discharge of large amounts of phosphorus (P) into the environment (Dalsgaard and Pedersen, 2011). Since P is the rate-limiting nutrient in most lentic and lotic environments, the inflow of P into these sensitive water bodies directly stimulates algal bloom or eutrophication, leading to undesirable alterations in aquatic ecosystems as well as rendering the water unsuitable for human or animal use (Chowdhury et al., 2017).

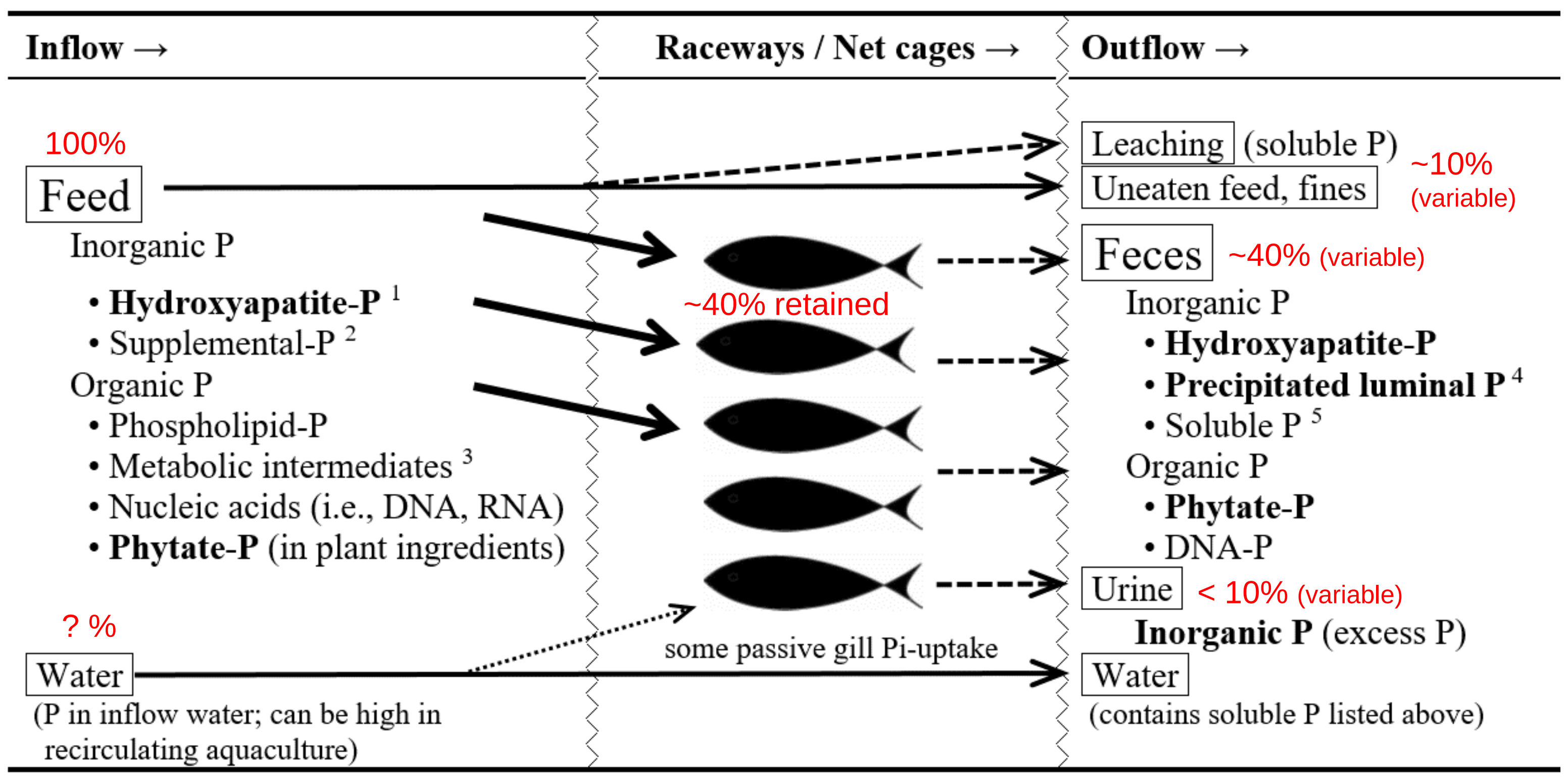
Unlike livestock and poultry production, aquaculture wastes are discharged into water and, therefore, difficult to collect. While some effluent P (i.e., fecal and particulate P) may be collected in land-based aquaculture systems, soluble-P (i.e., urinary P and soluble fecal P) is essentially non-recoverable. Where little or no regulatory mandate exists, P in aquaculture effluent is often discharged directly into the receiving waters. Thus, intensive aquaculture is more harmful to the environment than animal production systems.

The dominant source of P in aquaculture effluent is dietary P excreted by fish (see Table). While sufficient dietary P is needed to ensure fish growth, the excess dietary P and indigestible dietary P are invariably excreted, polluting the environment, often in violation of environmental regulations. Consequently, the fundamental dilemma of aquaculture today is how to sustain the continuous growth of aquacultural production, while simultaneously minimizing its environmental impact (Desai, 2002; Vandenberg and Koko, 2006).

Thus, fish P research has focused on reducing the dietary P content to the minimum level required by fish. Also, the minimum dietary P requirements have been studied for large fish that make the largest contribution to P in aquaculture effluent. In a low-P regimen, the early identification of P deficiencies is necessary to avoid clinical P deficiencies (Sugiura et al., 2007). Accordingly, much efforts have been made to identify sensitive and reliable diagnostic indicators of P. Nonetheless, there has been a veritable epidemic of bone deformities reported among some aquacultured species in response to these low-P feeds (Fjelldal et al., 2012; Ytteborg et al., 2012).

Increasing the availability of dietary P is necessary to minimize fecal P excretion by fish. Consequently, technologies have been developed to enhance the digestibility of P in various feed ingredients (e.g., Rodehutscord and Pfeffer, 1995; Vielma and Lall, 1997). In addition to low-P, fish feeds must meet various other standards to be considered suitable for sustainable aquaculture development. Therefore, P studies must be considered within the overall body of literature to best address the sustainability of fish feeds and aquaculture (Sugiura and Hardy, 2000). Within this context, the present review aims to provide solutions/suggestions to the major issues concerning P in fish feeds, including: (1) diagnostic P indicators, (2) fish growth and N:P stoichiometry, (3) the prevention of bone deformity, (4) digestibility, and (5) practical low-pollution feeds.

Flow of phosphorus (P) in intensive aquaculture systems



Each listed category with bold font indicates the major flow of P in typical aquaculture systems.

[1] P in skeletal tissues in fish meal, animal byproducts meals, etc.

[2] Calcium, sodium, or potassium phosphates, etc.

[3] ATP, G6P, phosphorylated proteins, etc.

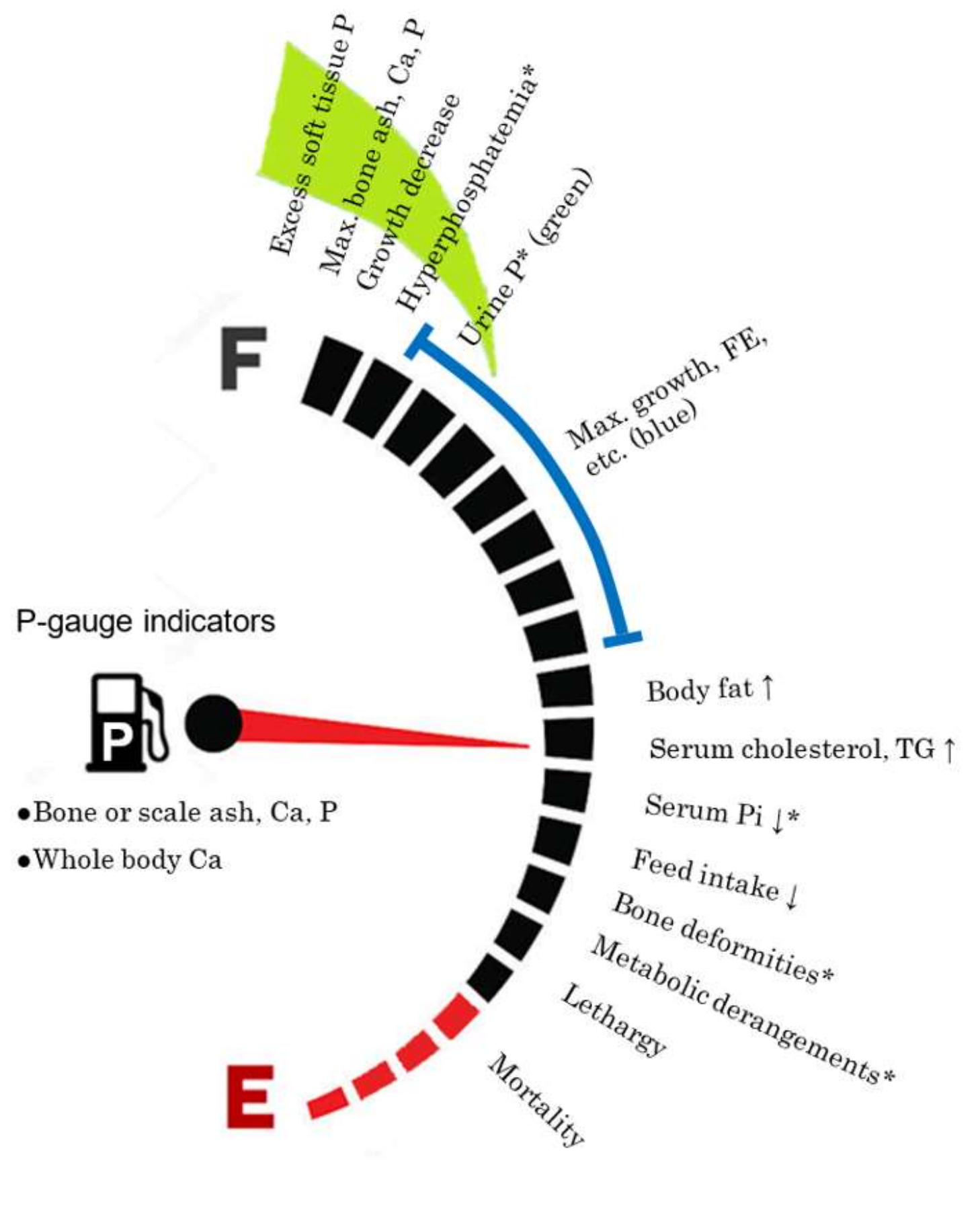
[4] Luminal P precipitation by divalent and trivalent cations, both dietary and secreted sources.

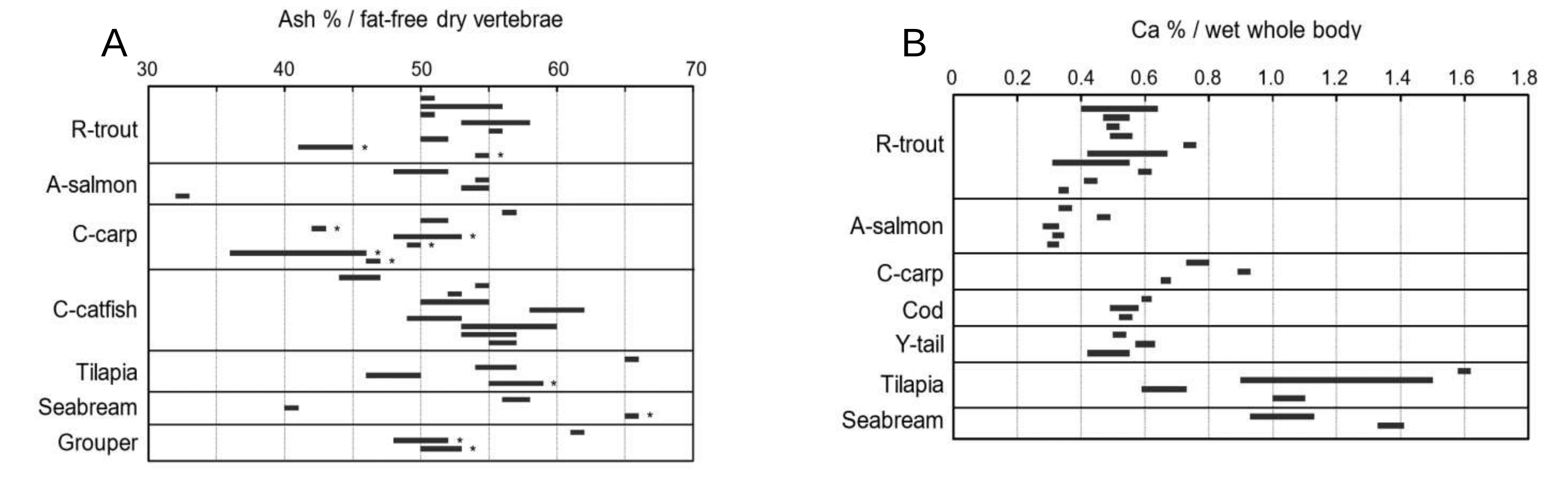
[5] Fecal soluble P originates from both bacterial degradation and endogenous secretion.

(1) Diagnostic P indicators

Among the numerous indicators of P studied in fish and animal species, certain genes have been identified that respond to early P deficiency by increasing or decreasing their expression. Such genes, however, are transient responders, and no stable or reliable genes have been found at least at the mRNA level or that will respond appropriately to practical feeds (Sugiura et al., 2007; Lake et al., 2010). For some malignant diseases in humans, molecular or biochemical markers are often used irrespective of the assay cost, as early detection and early treatment is important for diagnostic purposes. Dietary P deficiency, however, does not cause a depression in fish growth, feed efficiency, or other important performance indices until the advanced stages of deficiency (Hardy et al., 1993). Also, unlike malignant diseases, moderate P deficiency is recoverable and treatable. Therefore, the early detection of P deficiencies may not be helpful or necessary for most practical situations.

Gauge indicators are generally the most reliable and practical solution for the on-farm diagnosis of fish P status. These gauge indicators show the P-saturation level in the body, which is similar to a fuel-gauge in a motor vehicle. When the P saturation level falls below the threshold (approximately 75% of the normal P level; Hardy et al., 1993), growth depression ensues. Such P gauge indicators include the ash, Ca, and P content in the P storehouse (i.e., bones and scales) and the whole body Ca content (Figures A, B). There is considerable variability across studies (as seen in Figures A and B), which could be due to different assay protocols. Within each study or protocol, the variability should be much smaller (hence, useful as a routine diagnostic tool). Gauge indicators are more reliable and informative than point indicators, not to mention unstable molecular indicators. Gauge indicators allow for the safe reduction of P in fish feeds without risking P deficiencies in cultured fish.





Figures: Normal ranges of bone ash content (**A**) and body Ca content (**B**) of fish. Each bar represents data (as normal range) reported by an independent experiment (Data extracted from Table 2 of Sugiura, 2018). The * sign indicates that the vertebrae might not be defatted. Abbreviations: R-trout, rainbow trout; A-salmon, Atlantic salmon; C-carp, common carp; C-catfish, channel catfish; Y-tail, yellowtail.

(2) Fish growth and N:P stoichiometry

Within each species, the ratio of N and P is relatively constant, irrespective of body size or life-stage of fish; the only exception to this rule being in early ontogeny or malnutrition (e.g., Shearer, 1984; NRC, 2012). The P requirement, therefore, can be accurately expressed as per body N accretion (NRC, 2012). In practice, the dietary requirement of P can be expressed accurately by normalizing the measured P requirement based on its feed efficiency, or FE, yielding the **standard requirement** (Shearer, 1995; Sugiura et al., 2000; see Figures). The standard requirements are constant, within the same species, irrespective of fish size (life-stage) and feed composition (e.g., energy density, protein content, fiber content, etc.). Also, the available P content of a diet should be normalized based on the FE of each diet in order to assess, rationally and accurately, the adequacy of dietary P levels. Without such normalization, the dietary requirements or available P content will be highly inaccurate, resulting in P deficient cultured fish or the excretion of excess P to the environment.

Normalization (i.e., standardization) is becoming increasingly important as more plant ingredients are included in modern fish feeds. Unlike highly digestible animal ingredients, plant feed ingredients often contain large percentages of indigestible fibers. It has been shown that when a diet is diluted with various proportions of cellulose, animals can adjust their voluntary food intake, regardless of the bulk, so that their intake of digestible energy per day remains fairly constant (Kleiber, 1975). This has also been verified in fish (Rozin and Mayer, 1961; Bromley and Adkins, 1984). As most nutrients, including P, are required per unit of growth, the expression of nutrient requirements as per unit of bulk feed is changeable.

Figure: Nutrient requirements can not be expressed accurately as per weight of feed or percent of feed due to different feed efficiency from one feed to another.

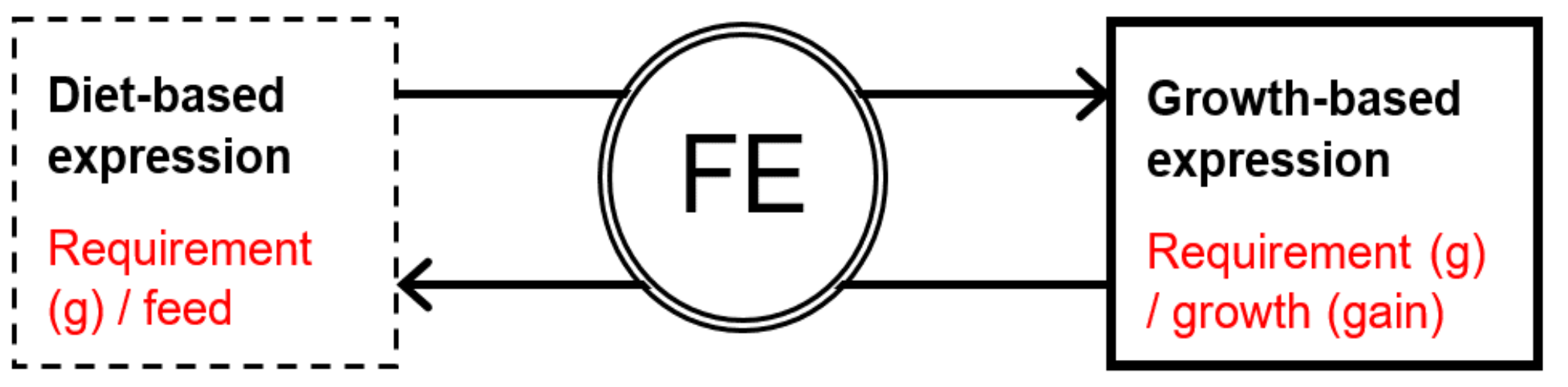


Figure: Phosphorus is required per unit of growth (gain); not per unit of feed. Feed is merely a vehicle of nutrients required for growth (and other metabolic needs). Feed efficiency (FE: gain/feed) of each feed is the conversion factor between growth-based and feed-based requirements.

(3) Prevention of bone deformity

Bone deformities occur due to insufficient calcification of collagen. **Larval-early juvenile fish are particularly vulnerable to bone deformities because their collagen matrix is only partially calcified and stabilized** (Kronick and Cooke, 1996). Collagens have species-specific low melting temperatures (Leikina et al., 2002). Therefore, only a slight temperature increase is needed to cause bone malformation, especially in under-mineralized collagen (i.e., high temperature, low-P, or low-Ca causes bone deformity). Vitamin C, which is necessary for collagen formation, is also thought to be involved in bone malformation during embryogenesis and larval development, especially when vitamin C in eggs is depleted by maternal malnutrition, frequent egg disinfection, etc. (Wedemeyer, 1971; Soliman et al., 1986). Since small fish contribute little to overall effluent P, reducing dietary P during this vulnerable period should be avoided.

In larval-early juvenile fish, the entire skeleton, including vertebrae and mandible, may become deformed, with this deformity becoming more pronounced as the fish grows larger. For post-juvenile fish, however, the P-deficient deformity develops at the growing end, especially at the tip of thin bones, such as the pleural ribs, where the collagen is not well-calcified. Therefore, for post-juvenile fish, the ribs might be the sensitive indicator for P deficiency (Shearer and Hardy, 1987).

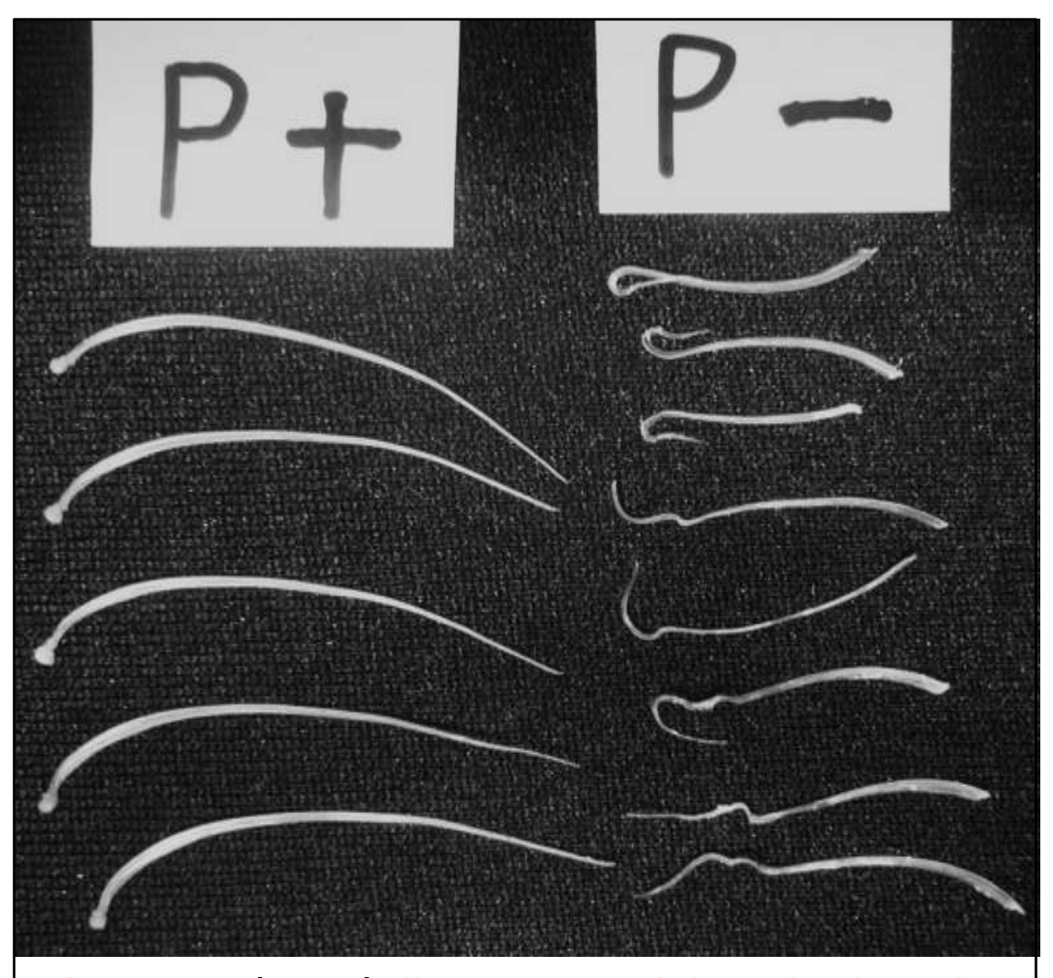


Figure: Pleural ribs of P-sufficient (P+) and P-deficient (P-) rainbow trout.

(4) Digestibility and absorption

Many species of fish absorb dietary P through pyloric caeca. Rainbow trout have ~50 pyloric caeca, which account for ~70% of the total surface area of the GI tract (Buddington and Diamond, 1987). An active component of Pi (inorganic P) transport in the caeca was estimated with Km = 0.52 mM and Vmax = 64.5 nmol/g tissue/min (Sugiura and Ferraris, 2004). At physiological luminal Pi concentrations (20 mM), however, Pi in the caeca is transported predominantly by the passive, non-carrier mediated (i.e., NaPi-independent) process, indicating that P absorption is largely unregulated, as also confirmed by in vivo fractional Pi absorption (Sugiura et al., 2003). Therefore, dietary P absorption depends almost exclusively on the chemical forms of P in the diet as well as luminal interactions between P and other solutes (see Figure).

As plant ingredients are increasingly used in fish feeds, thus replacing traditional fish meal, our research interest in digestibility has shifted from fish meal to plant ingredients (Gatlin et al., 2007; Olsen and Hasan, 2012; FAO, 2016). Most plant ingredients are lower in P compared with fish meal and animal byproduct meals. Thus, modern plant-based fish feeds contain less P than traditional fish meal-based feeds, resulting in less P being excreted by aquacultured fish (Jahan et al., 2003; Bureau and Hua, 2006).

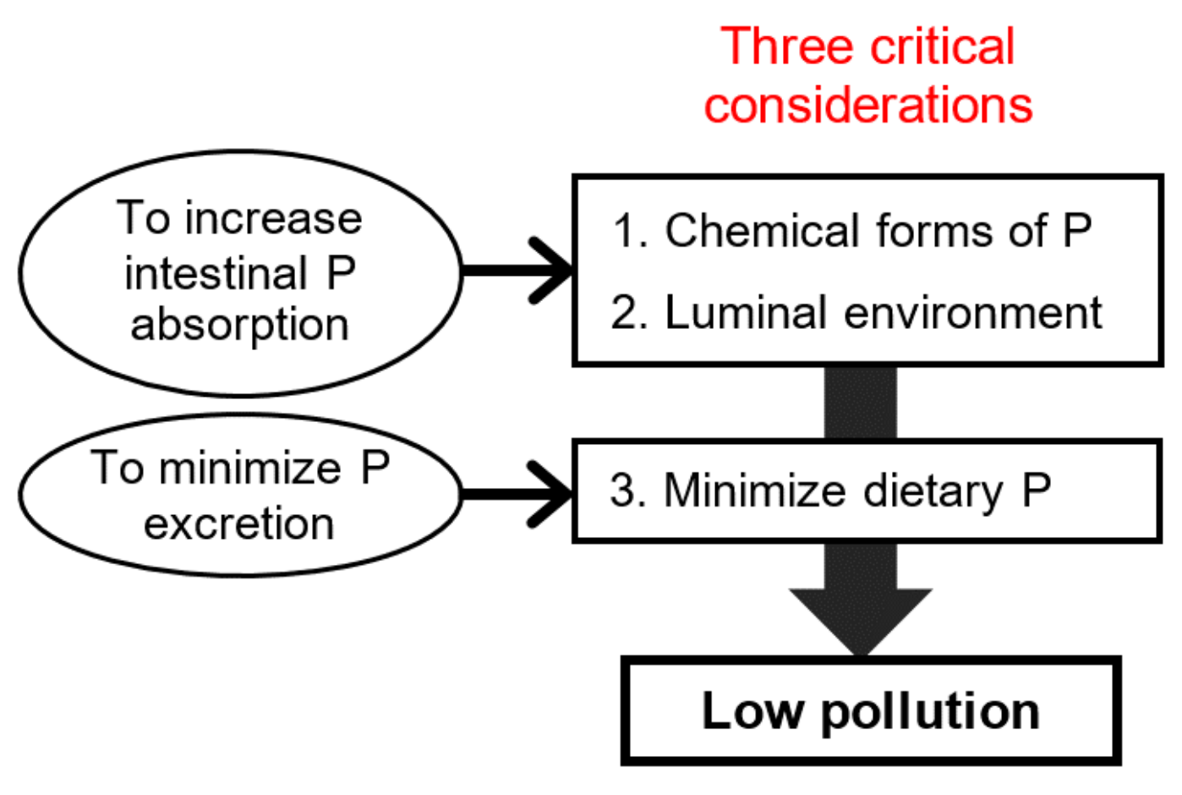
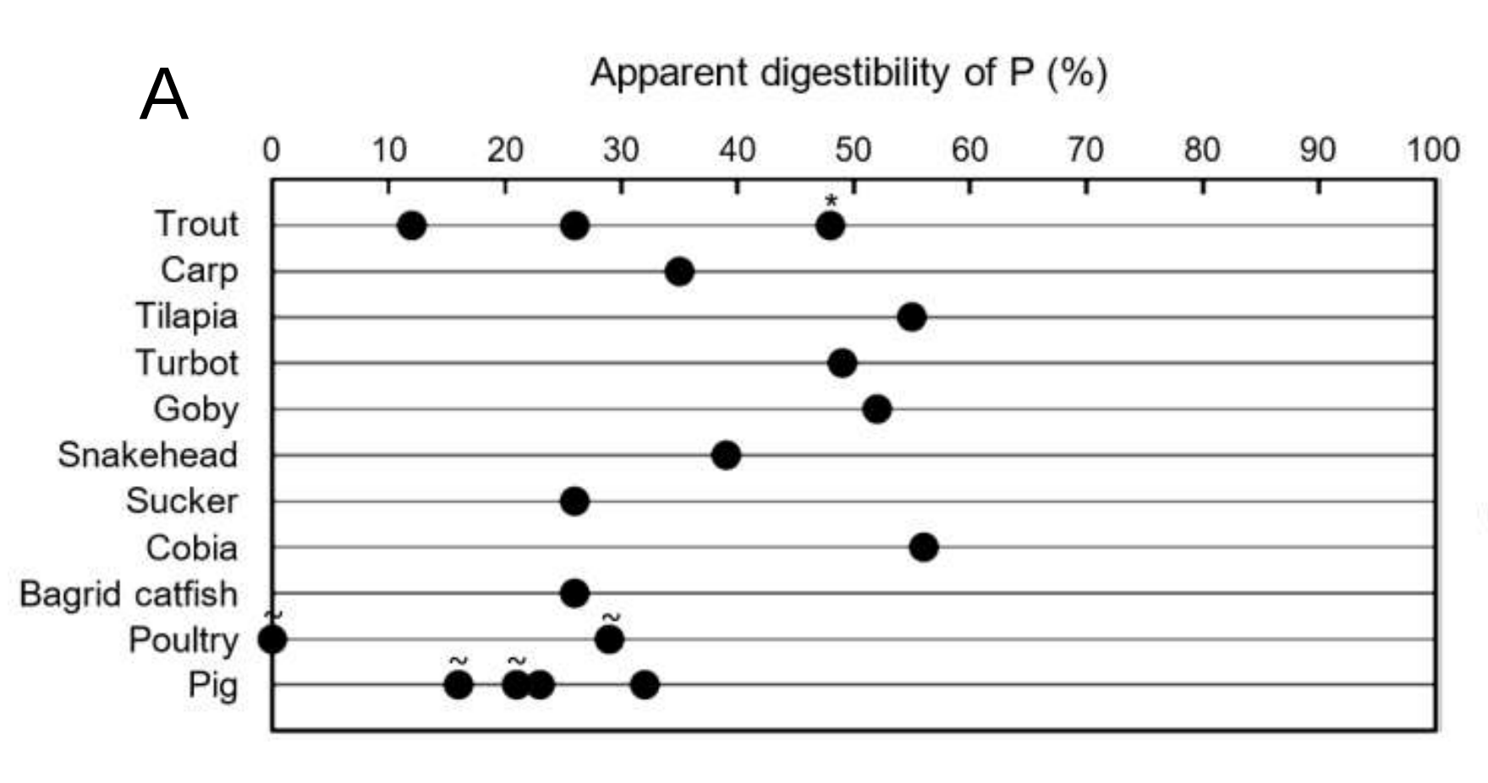
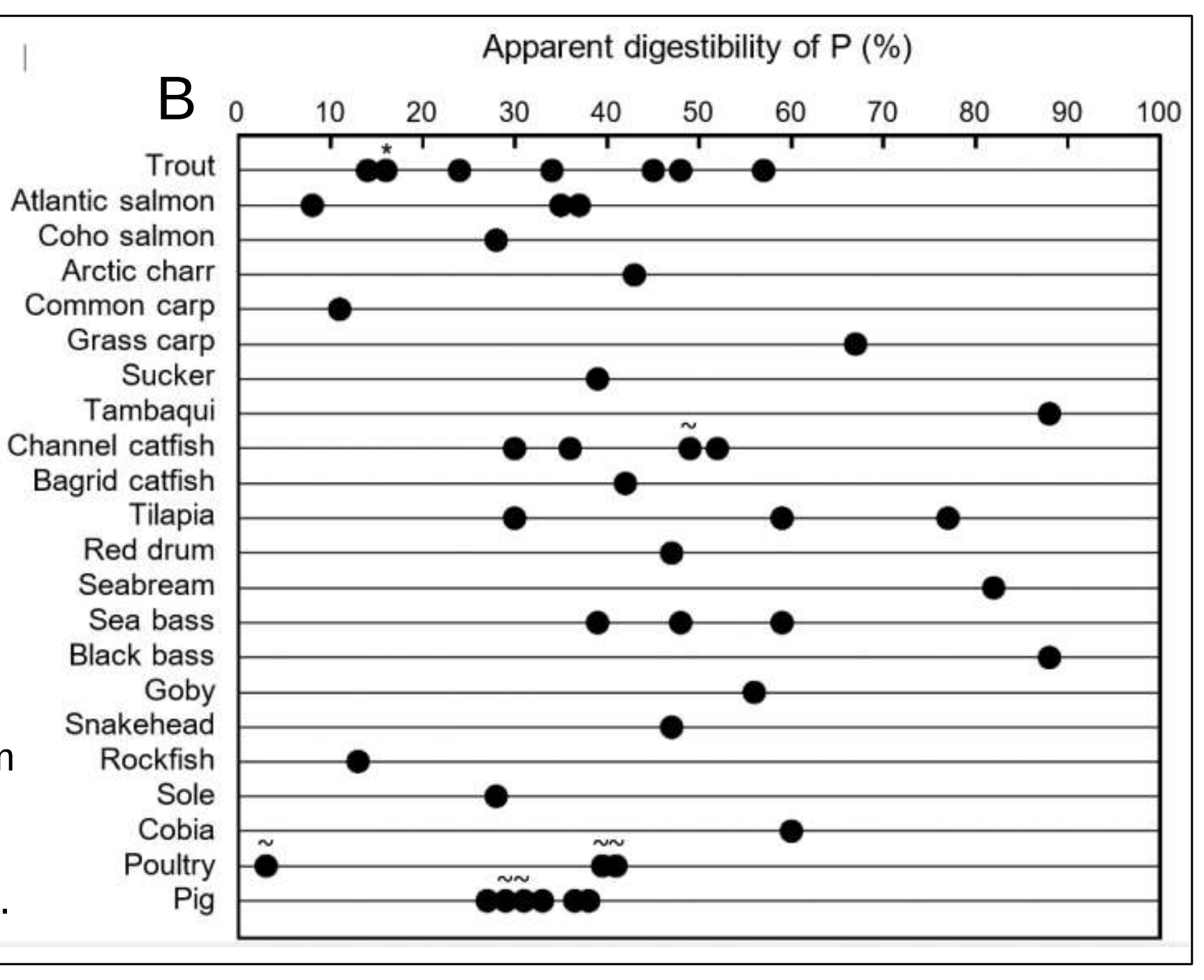


Figure: Three critical points to minimize P excretion by fish. In fish, Pi transporters do not functionally limit the intestinal Pi absorption. Hence, chemical rather than physiological approaches are important to minimize P excretion by fish.





Figures: Digestibility of P in rapeseed meal or canola meal (**A**) and soybean meal (**B**) by selected species. Each point indicates data from an independent experiment (c.f. Table 3 of Sugiura, 2018). The * sign indicates true digestibility. The ~ sign indicates relative bioavailability based on bone ash, bone breaking strength, or growth of the animals.

Approximately two-thirds of total P in plant ingredients are phytate-P which are not well-digested by fish. Thus, the use of phytase enzyme is critical for increasing the P digestibility of plant sources. Various types of commercial phytase are available as feed supplements (Lei et al., 2013). Dietary phytase, however, is considerably less active at the "body temperature" of fish compared to that of livestock animals or birds. Alternatively, the **in vitro incubation of sour dough**, consisting of plant materials with phytase and acidulant, can achieve near **100% digestion of plant P** (Sugiura et al., 2001; Vielma et al., 2002). Modern fish feeds, containing a high percentage of plant ingredients, should include such a pre-incubation step in the manufacturing process.

Also, it is clear from the above figures that the digestibility of P is highly variable, even within the same species/ ingredients. Thus, unlike macro-nutrients, the digestibility of P for each feed ingredient should not be considered as a fixed value. Digestibility of P varies depending on many factors, including luminal pH, interactions with soluble Ca, Fe (III), Al, bicarbonate, chelating agents, dietary fats, and various other natural and synthetic compounds (Sugiura, 2018).

Also important is the fact that most plant materials contain substantial amounts of lignocellulose. Developing the technology to facilitate the digestion of such fibrous components is becoming increasingly important (Castillo and Gatlin, 2015; Tanemura et al., 2016; see Figure).

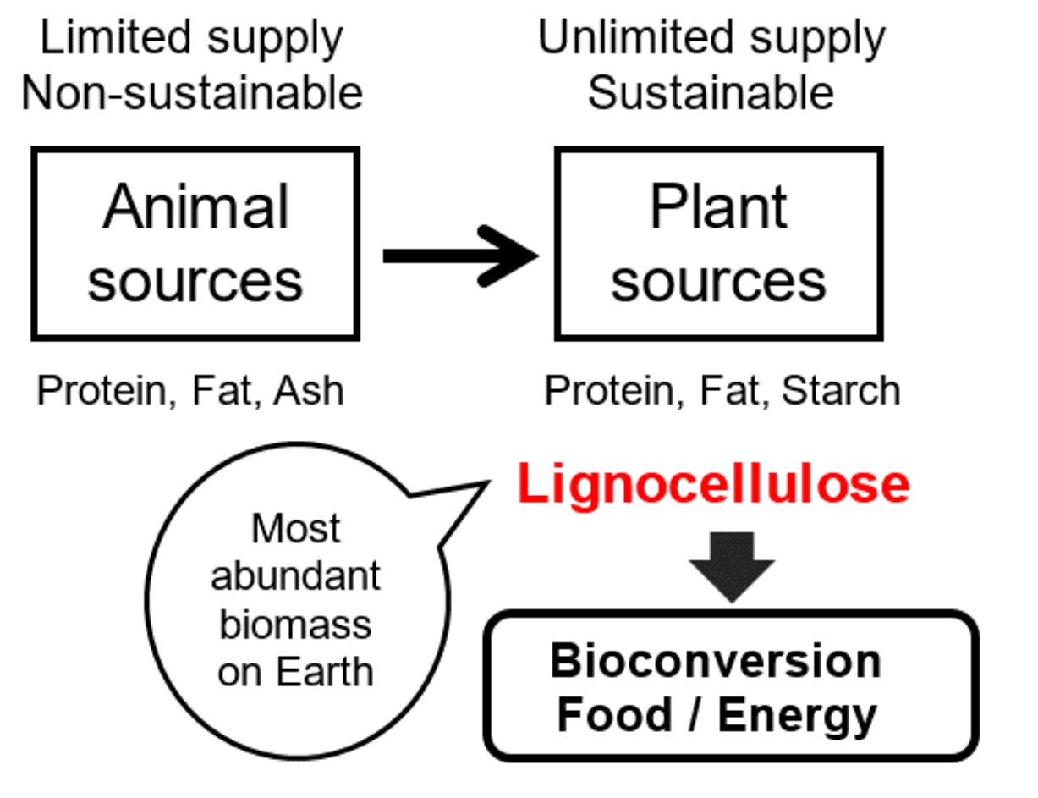


Figure: Global transition to sustainability. Plant ingredients contain lignocellulose that can cause organic matter pollution. In vitro pre-digestion of lignocellulose is necessary to use high-fiber ingredients in low-pollution feeds.

(5) Making low-pollution feeds

Making low-pollution feeds is fairly simple and easy; for example, all aquarium feeds are made to be low-polluting by the use of highly digestible ingredients often considered edible for humans. However, if used for commercial aquaculture, low-pollution feeds must also address issues of feed cost, fish growth, and product quality (Sugiura and Hardy, 2000). Indeed, low-polluting commercial feeds are more expensive than regular feeds (Sugiura et al., 2006). As fish feed is the single largest cost in intensive aquaculture (representing 30-90% of the total expense, depending on the country and the culture system; Hasan, 2007), the high cost of low-pollution feeds inevitably limits their widespread use in commercial aquaculture. Consequently, reducing the cost is an important hurdle to be overcome for the development of practical low-pollution feeds and for reducing the environmental burden of commercial aquaculture. In other words, the use of materials otherwise considered waste (rather than edible ingredients) for the manufacture of fish feed will become increasingly important for the future of seafood production by aquaculture (Troell et al., 2014).

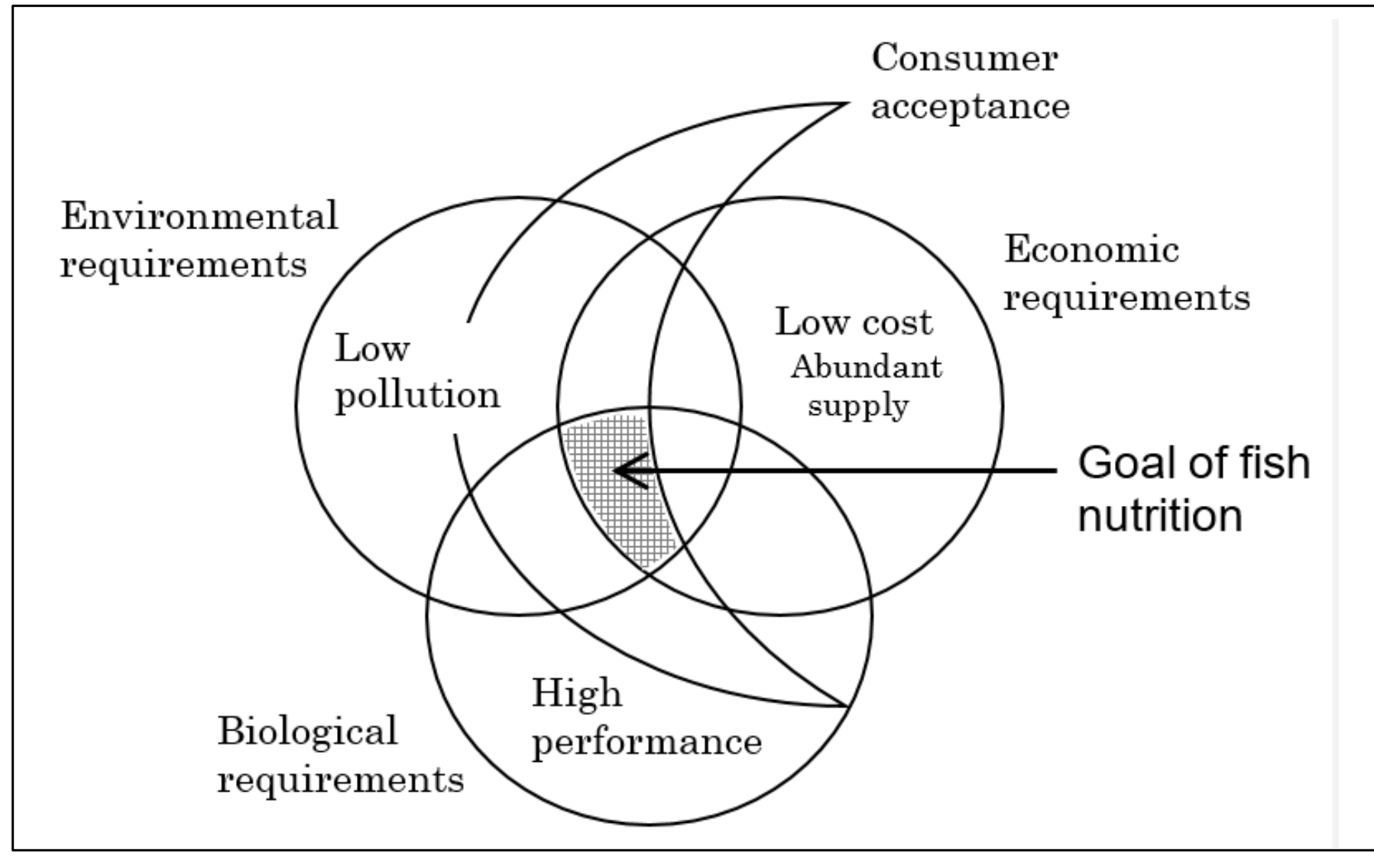


Figure: The goal of fish nutrition in aquaculture.
Four basic requirements of fish feeds are given below. Note that each of these requirements has no end (i.e., the higher/lower, the better). The figure does not apply to areas unrelated or only partly related to aquaculture production (e.g., larval nutrition, etc.).

1. Biological requirements: nutritionally adequate (support optimum fish growth, FE, health, disease resistance, etc.)
2. Economic requirements: low cost (abundant supply)
3. Environmental requirements: low-polluting (low excretion of P, N, organic matter, minimum feed loss)
4. Consumer acceptance: high product quality (flavor, health benefits, safety, etc.), traceability and transparency (of feeds and ingredients), public image (positive images for feed and feeding)

Conclusion

Phosphorus is a key nutrient in fish feed. Several important progresses have been made in this field over the past few decades that have contributed to the continuous increase in aquaculture production while reducing its environmental impact. The goal of sustainable aquaculture, however, is still far distant with fish retaining only ~40% of P in modern fish feeds. This review highlighted five major issues concerning P and explored practical approaches toward their resolution. The sustainable development of aquaculture is largely contingent upon the outcomes of **problem-solving research rather than efforts to generate knowledge or to simply collecting data**.