A review on the role of inositol in aquaculture

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Abstract

Inositol is usually classified as an essential vitamin for most animals, and is recognised as a part of the B-complex vitamins. Among all other inositol isomer forms, *myo*-inositol possesses biological activity. It is found in the brain, skeletal, heart, and main reproductive tissues and exists as a structural component of phosphatidylinositol in biological cell membranes. *Myo*-inositol, also acts as a growth factor and affects the antioxidant capacity and oxidative status of cells. It is a major intracellular osmolyte that can be accumulated to protect cells from a variety of stresses and can also participate in transmembrane signal transfer. *Myo*-inositol is synthesised by various animal tissues and microorganisms in gut and fulfils the requirement for a few fish species. However, the supply of inositol by exogenous source (the diet) is required in most fish and shrimp for preventing deficiency signs such as inefficiency in digestion and food utilization, poor growth, fin erosion, dark skin colouration, and high accumulation of lipid in liver and muscle. The current paper aimed to provide a review on the published studies on the role of inositol in aquaculture.

Keywords: Inositol, Functions, Requirement, Deficiency, Aquaculture

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Introduction

Muscle "sugar", inositol, was discovered by Scherer in 1850 and was characterised by Maquenne (1900). Inositol is widely distributed in plants and animals, mainly as a structural component of biological membranes in the phospholipid form (Chang *et al.*, 2001). It is classified as a vitamin-like nutrient (Shiau and Su, 2005), and is an essential dietary ingredient for most aquatic animals (Michael and Koshio, 2008).

The stereo configuration of the active factor, *myo*-inositol, was proved by Posternak (1936). Keller *et al.* (1947) and McLaren *et al*. (1974) established the importance of *myo*-inositol (MI) in the diet of rainbow trout (*Oncorhynchus mykiss*). However, fish and other vertebrates, or their intestinal microbial flora may synthesise inositol (Burtle and Lovell, 1989; Aukema and Holub, 1994). In fact, MI, the biologically active isomer of inositol, is a structural component in living tissues as well as an important participant in transmembrane signal transfer in the phospholipid form, phosphatidylinositol (PI) (Aukema and Holub, 1994).

Structure of inositol

The chemical formula of inositol is $C_6 H_{12} O_6$, and the structural formula with the hydroxyl groups in position one, two, three, and five in one plane and positions four and six in the other plane (Halver, 2002). Inositol may exist in one of nine optically forms (Fig. 1). In fact, seven optically inactive and two optically active isomers of hexahydroxycyclohexane can exist and only one of these forms, *myo*-inositol, possesses biological activity (Anderson and Wallis, 1948; Weidlein, 1954).

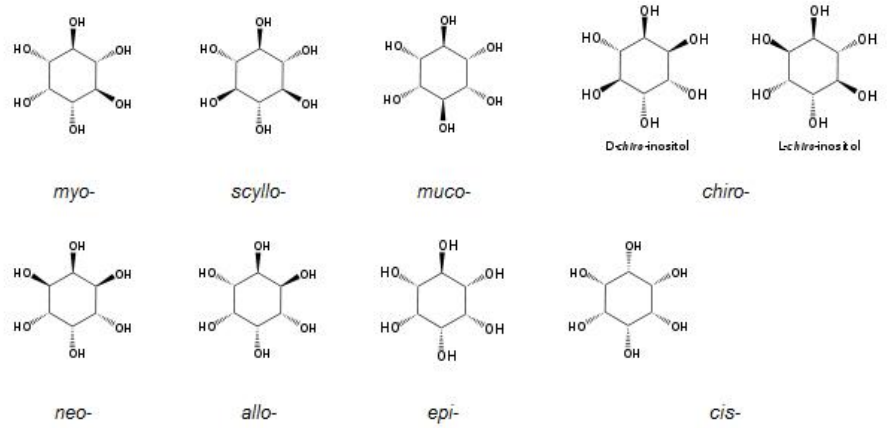

Figure 1: different optical isomers of inositol (http://www.neurosoup.com/supplements-vitamins/inositol/)

It is a biologically active cyclohexitol which occurs as a structural component in biological membranes as Phosphatidylinositol (PI) (Mathews and Van Hold, 1990; NRC, 2011).

Myo-inositol or *meso*-inositol is a white crystalline powder soluble in water and insoluble in alcohol and ether (Weidlein, 1954). The material can be synthesised, but is easily isolated from biological material in

free or combined forms (Anderson and Wallis, 1948). The mixed calcium-magnesium salt of hexaphosphate is phytin and this isomer has little biological activity, but do compete in chemical reactions (Anderson and Wallis, 1948; Weidlein, 1954). Because of the special configuration of the biologically active form and the need to fit these forms into tissue stereo biochemical structure, biologically inactive forms do not compete for critical sites in metabolism (West *et al*., 1966). However, the active cis-trans isomer does compete and introduces errors in the structural configuration of essential components. Methyl derivatives and mono-, di-, and triphosphoric acid esters occur naturally. Salts of the hexaphosphate or phytin make the bound inositol partially unavailable to the animal (Weidlein, 1954; West *et al.*, 1966; Spinelli, 1979).

Metabolic and physiological functions of inositol

Although not officially a B vitamin, inositol is recognised as part of the B-complex and is classified as a vitamin-like nutrient (Shiau and Su, 2004). MI is the most prevalent naturally occurring biologically active isomer (Aukema and Holub, 1994). It is a structural component in living tissues (West *et al.*, 1966). Inositol is found as a component in the brain, skeletal, heart, and male reproductive tissues (Shiau and Su, 2004). It acts in nerve transmission, regulation of enzyme activity, and transportation of lipids within the body (Shiau and Su, 2004). Also, it acts as a lipotropic, preventing accumulation of cholesterol in one type of fatty liver disease. WaagbØ *et al*. (1998) for *Salmo salar* and Wen *et al*. (2007) for *Ctrnopharyngodon idella* reported that plasma triglyceride (TG) was negatively correlated with dietary inositol supplementation. Peres *et al*. (2004) also showed that muscle total lipid content decreased in Nile Tilapia (*Oreochromis niloticus)* fed with a diet containing800 $mg.kg^{-1}$ of inositol.

Lee *et al*. (2009) showed the significant increase of liver unsaturated and polyunsaturated fatty acids (PUFA) and lower proportion of saturated fatty acid in olive flounder (*Paralichthys olivaceus*) fed with high dietary MI compared to the control group. They suggested that the reason for the higher liver PUFA content in MI fed fish might be due to the increased synthesis rate of phospholipids by sufficient MI. The increment long-chain PUFA of phospholipids in fish at low temperature has been attributed to their role in maintenance of proper fluidity of cell membranes (Sellner and Hazel, 1982; Farkas *et al.,* 2001;). However, Khosravi *et al*. (2015) reported that lack of significant difference in liver unsaturated fatty acids and PUFA contents in parrot fish (*Oplegnathus fasciatus*) may be due to the higher rearing temperature (18-27°C) in comparison to that in the study implemented by Lee *et al*. (2009).

Inositol deficiency has been reported to be associated with a variety of lipid metabolic disturbances resulting in accumulation of lipids in liver, decreased hepatic lipoprotein output, and intestinal lipodystrophy (Diao *et al.*, 2010). This is due to interference with chylomicron assembly and secretion, and

thus impairment of lymphatic transport of dietary fat (Hegsted *et al.,* 1973; Chu and Geyer, 1983). High acetyl-CoA carboxylase activity as well as lipolysis in the adipose tissue may also contribute to the accumulation of triacylglycerols in the liver during inositol deficiency. Inositol is metabolised to PI that is believed to prevent lipid deposition in the liver of terrestrial and aquatic animals (Lapetina *et al.,* 1981). When dietary inositol levels are re-established, synthesis and turnover of PI are increased followed by enhanced lipid clearance which leads to liver and/or intestinal recovery (Hayashi *et al.,* 1974; Chu and Geyer, 1983). PI is degraded into diacylglycerol (DAG) that is responsible for the conversion of the high-energy intermediate cytidine diphosphocholine (CDP-choline) into phosphatidylcholine (PC) (Kennedy and Weiss, 1956). According to the metabolic fate of MI, the amount of dietary inositol may affect the level of body PI, and furthermore affect the PC content of the body. PI has been shown to be involved in signal transduction in several metabolic processes (Mathewes and Van Holde, 1990; NRC, 2011). A signal transduction pathway is stimulated by certain hormones, neurotransmitters, or growth factors (Aukema and Holub, 1994). Although similar in many respects to the adenylate cyclase transduction system, the phosphoinositide system is distinctive in that the hormonal stimulus activates a reaction that generates two second messengers. Membrane bound phosphatidylinositol 5-bisphosphate is cleaved to release sn-1,2-diacylglycerol and inositol 1,4,5-triphosphate following the interaction of a hormone or agonist with the receptor on the cell membrane. Inositol 1,4, and 5-triphosphate stimulates the release of calcium from its intracellular stores in the endoplasmic reticulum, and sn-1,2-diacylglycerol activates protein kinase C to phosphorylate specific target proteins (Fig. 2). Examples of cellular processes controlled by the phosphoinositide second messenger system include amylase secretion, insulin release, smooth muscle contraction, liver glycogenolysis, platelet aggregation, histamine secretion, and DNA synthesis in fibroblasts and lymphoblasts (NRC, 2011).

Altogether, biochemical functions of PI include the mediation of cellular responses to external stimuli, nerve transmission, and the regulation of enzyme activity through specific interactions with various proteins (Chang *et al.,* 2001).

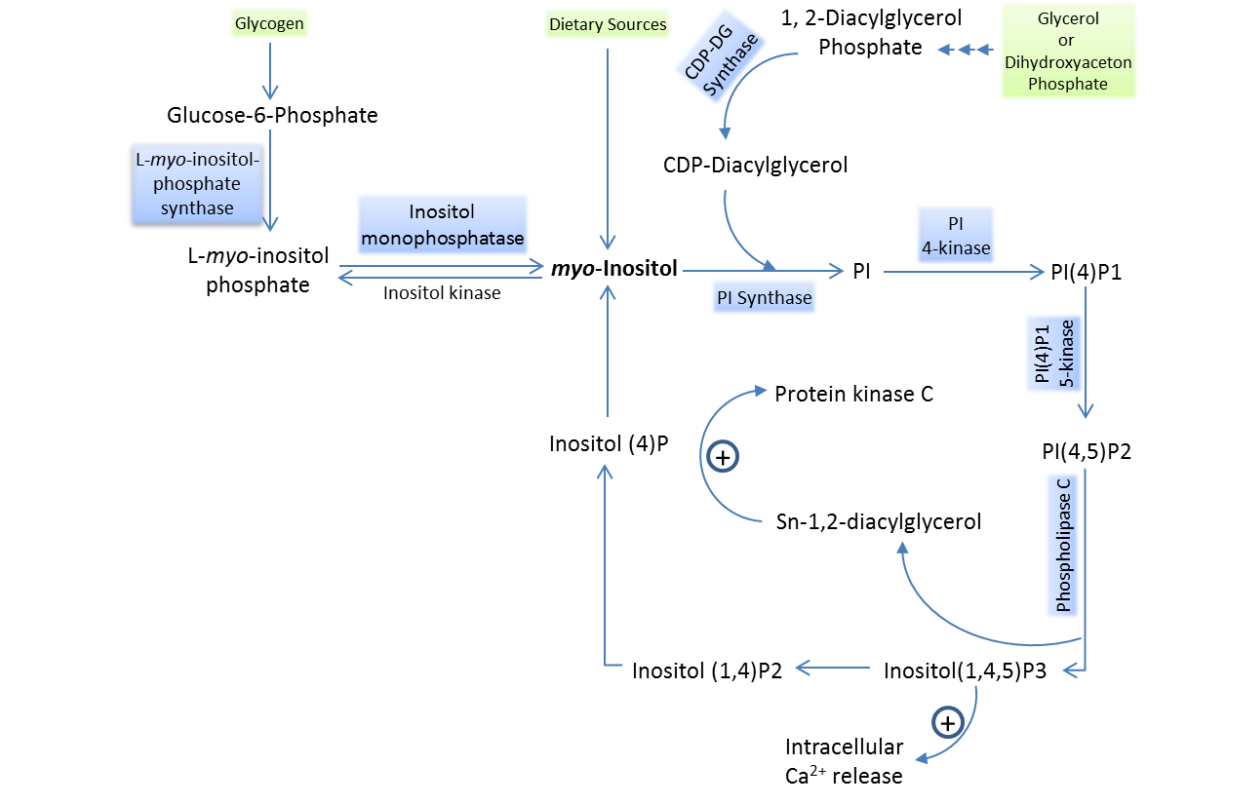

Figure 2: Biosynthesis of *myo*-inosotol (MI) and metabolism of inositol phosphate

Significant interaction has been detected between dietary choline chloride (CC) and MI as well as the choline sparing effect of inositol in shrimp. The phosphatidyl choline (PC) content of shrimp that did not receive supplemental CC or MI was significantly lower than that of the other shrimps that received either supplemental CC or MI. The addition of dietary MI significantly elevated the level of the whole body arachidonic acid in shrimp compared to those groups that did not receive dietary MI, even those that received sufficient dietary CC (Micheal and Koshio, 2008) which may be due to the initiation of PI formation that possess arachidonic acid in its structure specially at carbon 2 (Gardocki *et al.*, 2005).

In addition, it is an emergency carbohydrate source in muscle and is a major structural component of the phospholipids structures in animal tissues (Stetten and Stetten, 1946). Its primary function appears to serve as a structural element with the six hydroxyl groups available for esterification or for acid salt formation to form an integral portion of cell membranes. The stereo configuration of these cell membrane elements probably plays a major role in cell membrane permeability to various ions and molecules (West *et al.*, 1966).

Dietary inositol also affects the blood chemistry in fish (WaagbØ *et al.*, 1998; Wen *et al.*, 2007). It affects blood haemoglobin positively and plasma TG negatively, while

plasma protein and cholesterol are unaffected (WaagbØ *et al.,* 1998).

Inositol improves growth, digestive capacity, and intestinal microbial population of juvenile Jian carp and *Cyprinus carpio* var. Jian (Jiang *et al.,* 2009). Moderate effects of dietary inositol on growth have been observed in the first weeks after start of the feeding process of the Atlantic salmon (*S. salar*) (WaagbØ *et al.,* 1998). Jiang *et al*. (2013) reported that MI could improve the growth of carp enterocytes which may be partly due to the enhanced antioxidant status and depressed oxidative damage. The improvement in fish growth may be due to MI-induced intestinal structural and functional integrity. The digestion ability and the absorption function of fish have been found to be correlated with intestinal growth, development, and body weight gain (Pedersen and Sissons, 1984). MI supplementation greatly increases the proliferation of fish enterocytes. The proliferation of enterocytes may directly affect the formation of intestinal folds and so the absorption capacity is significantly improved by MI supplementation (Jiang *et al.,* 2013). High inositol levels can result in a higher midgut gland index of juvenile grass shrimp (*Penaeus monodon*) (Shiau and Su, 2004). Digestive enzyme patterns reflect the digestive capacity of fish (Smith 1980), and may be influenced by the quantity and composition of the diet (Peres *et al.,* 1998). With increasing inositol levels up to certain values, intestinal digestive enzyme activities including chymotrypsin, lipase and amylase activities are improved. Inositol increase lipase, chymotrypsin, and amylase activities in the intestine may due to the promotion of hepatopancreas development, while hepatosomatic weight, protein content, chymotrypsin, lipase ,and amylase activities are higher at high inositol levels compared with the unsupplemented diet in Jian carp (*C. carpio* var. Jian) (Jiang *et al.,* 2009). Amino acid absorption is mainly dependent on the Na1, K1-ATPase (Klein *et al.,* 1998). Na1, K1-ATPase, alkaline phosphatase (AKP), and creatine kinase (CK) serve as enterocyte differentiation markers and are considered to be involved in the absorption of nutrients (Villanueva *et al.,* 1997). Jiang *et al*. (2009) reported that with increasing inositol level, the intestine activities of AKP, Na1, K1-ATPase, gamma-glutamyl transferase (g-GT) and CK were increased. Furthermore, studies in Jiang carp (*Cyprinus carpio* var. Jian) indicated that MI improves the antioxidant ability including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in the intestine (Jiang *et al.,* 2010). SOD degrades superoxide radicals by converting them into O_2 and H_2O_2 which can be reduced to H_2O by CAT in the peroxisomes or by GPx in the cytosol (Olsvik *et al.,* 2005). Jiang *et al*. (2013) reported that the activities of CAT and GPx were increased by MI supplementation. Also MI was of benefit for scavenging superoxide and hydroxyl radicals in intestines in an in vivo experiment (Jiang *et al.,* 2010). Santoro *et al*. (2007) demonstrated that MI, as a cyclitol could donate a hydrogen atom or chelate Cu. MI pre-supplementation could

block the toxic effects of Cu on the antioxidant system, and thus protect enterocytes and gill from Cu-induced oxidative damage. MI also protects fish brain against Cu toxicity. Cu exposure decreases the GSH content in the brain which supports the decreased brain anti-hydroxyl radical (AHR) activity of the fish under Cu exposure (Jiang *et al.,* 2014). The induction of key antioxidant defences by MI pre-supplementation including SOD, CAT, GPx, glutathione-s-transferase (GST), and glutathione (GSH) may play an important role in the protection of fish against oxidative stress (Jiang *et al.,* 2011). Cell damage (medium lactate dehydrogenase activity) was induced by MI deficiency in carp, but with increasing levels of MI supplementation, the cell damage gradually disappeared. However, lipid peroxidation and protein oxidation were all gradually inhibited by higher levels of MI supplementation (Jiang *et al.,* 2013). Protein oxidation influences amino acid metabolism (Dabrowski and Guderley, 2002). In this sense, the increase in protein retention (PR) may be partly due to the decreased protein oxidation by MI. Thus, MI can reduce cell death partly by inhibiting the oxidative damage of cellular components (Jiang *et al.,* 2013).

Inositol is also a major intracellular osmolyte that can be accumulated to protect cells from a variety of stresses including increases in the osmolality of the extracellular environment (Beck *et al.,* 1992; Yamauchi *et al.,* 1994; Yancey, 2005; Alfieriand and Petronini, 2007; Burg *et al.,* 2007; Burg *et al.,* 2008; Michell, 2008;). Generally, the ability of cells to adapt to hyper osmotic stress involves an initial rapid, but transient response in which ions are moved into the cell to prevent the osmotic loss of water and cell shrinkage. This is followed by a hold up, but sustainable response characterised by either increased intracellular synthesis or active uptake of organic osmolytes such as inositol (Alfieriand and Petronini, 2007). Kalugnaia *et al*. (2010) showed that in fresh water (FW) acclimated fish, inositol levels were highest in gill followed by kidney with very low levels present in fish tissue. Salt water (SW) transfer induced significant 3, 1.8, and 10 fold increases in the inositol contents of kidney, gill, and fin respectively. The high levels of immunoreactivity within the chondrocytes of the gill and also the chondrocytes found within the fin rays of the caudal fin suggest that these cells may be particularly sensitive to changes in extracellular osmolarity (Farnum *et al.,* 2002). Since high levels of expression of the atrial natriuretic peptide clearance or type C receptor have also been reported in the chondrocytes of eel gill cartilage (Sakaguchi *et al.,* 1993), this peptide signalling system may have some regulatory role in chondrocyte inositol production and/or release. As a consequence of their high capacity for inositol production, these cells may also function as the primary source of the plasma concentrations of the osmolyte. These cells may function to produce and release inositol into the bronchial circulation allowing the epithelial cells to take up the osmolyte via active membrane transport systems such as the sodium-dependent *myo-*

inosito transporter (SMIT). This membrane transporter is responsible for the sodium-dependent accumulation of inositol and is expressed in many eel tissues including the gill. Therefore, increases in cytosolic inositol concentrations resulting from increases in the intracellular production of the osmolyte (or its accumulation from the extracellular environment *via* sodium-dependent transporters) could be responsible for the osmotic retention of water within the kidney and may also protect peripheral epithelial and epidermal cells from the dehydrating effects of the aquatic SW environment (Kalujnaia *et al.,* 2010).

Inositol monophosphatase 1 (IMPA1) has a role in the production of inositol (Alcazar-Roman and Wente., 2008). IMP1 is expressed and inositol is produced in a variety of epithelial, epidermal, and interstitial cells within the major osmoregulatory tissues of SW-acclimated eels (*Anguilla anguilla*) (Kalujnaia *et al.,* 2010). A few analyses also detected IMPA1 immunoreactivity in the fins, esophagus, and intestine. As most of these tissues are exposed to the dehydrating environment of SW, it is highly likely that the presence of IMPA1 is related to the production of inositol for the purposes of cellular osmoregulation rather than cell signalling. The large, 10-fold, increases in free inositol present within the dorsal fin of SW-acclimated fish would be in agreement with this hypothesis (Kalujnaia *et al.,* 2010). An osmoregulatory role has been reported for inositol in the Mozambique tilapia (*Sarotherodon mossambicus*) where the content of inositol increased by up to 7-fold in the brain and by up to 2-fold in the kidney, following the increases in plasma osmolality associated with the transfer of fish to SW (Fiess *et al*., 2007).

IMPA1-immunoreactive cells in kidney sections from SW-acclimated fish were considerably more abundant and of higher fluorescent intensity than the equivalent sections from FW-acclimated fish where only randomly dispersed single cells appeared to exhibit any measurable immunoreactivity. In mammals, renal inositol accumulates in medullary cells mainly by uptake from the extracellular environment via the sodium-linked transporter SMIT and not by endogenous de novo synthesis (Burg *et al.,* 2007; Ohnishi *et al.,* 2007; Burg *et al.,* 2008) suggesting that the major role for IMPA in the mammalian kidney is primarily due to the turnover of inositol phosphatase from the phospholipids pool. This restricted function is not consistent with the large increases in IMPA expression found in the SW-acclimated eel kidney. Although the toloest kidney has no comparative loop of Henle and thus is unable to concentrate urine above the osmolality of the plasma, the increased expression of IMPA within interstitial cells surrounding the tubules is responsible in some way for concentrating the tubular fluid and ultimately the urine of SW-acclimated fish. Increases in renal inositol concentrations may be required to counteract the elevated osmotic pressures of the tubular fluids found in SW-acclimated fish. The relationship between inositol production (via

IMPA), cellular uptake (via transporters such as SMIT), and the cytosolic concentrations of inositol in both tubular epithelia and peri tubular interstitial cells remain to be resolved; these results suggest that increases in renal inositol concentrations are essential to optimize tubular water retention in SW-acclimated animals (Kalujnaia *et al*., 2010).

The Impa1 gene was also expressed in the esophagus and anterior intestine organs which are important osmoregulatory tissues for the desalination of ingested SW prior to water absorption across more distal regions of the intestine (Aoki *et al.,* 2003 and Martinez *et al.,* 2005). The fact that SW transfer induces higher levels of IMPA1 mRNA expression in the esophagus (17.5-fold) rather than the anterior intestine (3.5-fold) may be related to the higher salinities of the imbibed SW in the more proximal regions of the gut (Kalujnaia *et al.,* 2010).

All intestinal segments taken from FW-acclimated fish exhibited nothing or only just detectable immunoreactivity consistent with the low levels of mRNA (Kalujnaia *et al.,* 2010). No immunoreactivity was found in the deeper tissues, suggesting that the large increases in IMPA1 mRNA expression in the whole esophagus were due to up-regulation of the gene within the epithelial tissues (Kalujnaia *et al.,* 2010). This arrangement is also similar to that found within the stratified epithelia of the skin and finswhere again the basal epithelial cell layers exhibited the highest levels of IMPA1 expression. The skin and caudal fin epidermal tissues would severely compromise osmoregulation in FW (Kalujnaia *et al.,* 2010).

Immune response and diseases resistance

A few studies have been conducted on the effect of dietary inositol on immune response and disease resistance in fish. Serum total protein (TP) levels can be used as a diagnostic tool and a valuable test for evaluating the general physiological state in fish (Pedro *et al.,* 2005). Dietary herbs (Lu *et al.,* 2009) and *Bacillus licheniformis* (Yuan *et al.,* 2009) could change some blood haematological indices of the fish. Significantly low levels of total plasma protein have been reported for some infected fish (Benli &Yildiz, 2004; Yildiz & Aydin, 2006; Rehulka & Minarik, 2007). Diago *et al*. (2010) reported that with increasing dietary inositol levels up to 507 $mg.kg^{-1}$ diet, TP increased in juvenile barramundi (*Lates calcarifer Bloch*).

MI is also involved in immune responses improving phagocytosis probably by regulation of the phosphoinositol synthesis or acting as an organic osmolyte (Kim *et al.,* 2003). It has been documented that myeloperoxidase (MPO) contributes to the bacterial activities of neutrophils and monocytes. Lincoln *et al.* (1995) reported that MPO released from neutrophils at a site of infection or inflammation can enhance phagocytosis and killing microorganisms.

The intestine micro biota is sensitive to dietary changes (Ring and Birkbeck, 1999). It has been suggested that intestine is one of the major routes of infection in fish. Hence, it is important to determine the effect of feed ingredients on the intestinal microbial

population (Birkbeck and Ring, 2005). Lactobacilli are required to maintain a healthy intestine (Aguirre and Collins, 1993); they produce bacteriocin-like substances controlling overgrowth of potentially pathogenic bacteria (Boris and Barbes, 2000). Jiang *et al*. (2009) reported that by increasing the level of dietary inositol, the intestine lactobacillus colony was increased; whereas, *Aeromonas hydrophila* and *Escherichia coli* (EC) of intestine were decreased by increasing the level of dietary inositol which may be linked to the produced compounds by *Lactobacillus casei* GR-1 that could inhibit EC growth (McGroarty and Reid, 1988). However, Peres *et al*. (2004) reported that supplementation of dietary inositol had no effect on improving the resistance of juvenile Nile tilapia (*Oreochromis niloticus*) to *Streptococcus iniae* infection.

Synthesis of inositol

Inositol is synthesised in the biologically active form by many microorganisms in the gut. Some synthesis has been reported in common carp (*C. carpio*) intestine (Aoe and Masuda, 1967) and in channel catfish (*Ictalurus punctatus*) (Burtle and Lovell, 1989).

Inositol is also synthesised by various animal tissues through a ring closure of glucose-6-phosphate catalysed by the enzyme L-*myo*-inositol-phosphate synthase (Fig. 2) (Hayashi *et al.,* 1974; Kukiss and Mookerjea, 1978; Chu and Geyer, 1982, 1983). IMPA1 plays an essential role in the generation of inositol from both the phospholipid pool (where it is important for signal transduction) and from the glucose 6-phosphate (Alcazar-Roman and Wente, 2008). Though, in rodents, the capacity for inositol biosynthesis in vivo appears to be limited since dietary inositol is needed to prevent the development of inositol deficiency (Hayashi *et al.,* 1974; Kukiss and Mookerjea, 1978; Chu and Geyer, 1982, 1983). In fish, nutritional evidence suggests that metabolic synthesis of inositol occurs to some degree in liver, kidney, brain, and other tissues (Aoe and Masuda, 1967; Burtle and Lovell, 1988; Deng *et al.,* 2002). For some fish species; however, de novo synthesis is inadequate to support their metabolic needs and thus they require exogenous source of this vitamin (Mclaren *et al.,* 1947; Halver, 1953; Aoe and Masuda, 1967; Kitamura *et al.,* 1967; Yone *et al.,* 1971; Arai *et al.,* 1972). Burtle and Lovell (1988) and Deng *et al*. (2002) demonstrated that de novo synthesis of inositol was sufficient for normal growth of channel catfish (*Ictalurus punctatus*) and sunshine bass (*Morone chrysops* × *Morone saxatilis*). Nontheless, they suggestedthat the synthesis of inositol by the microorganisms in the digestive tract was an unimportant source of this vitamin. Asian sea bass (*Lates calcarifer*) and sunshine bass do not have a requirement for exogenous source of inositol for normal growth and development. This is in contrast to rainbow trout (*O.mykiss*), chinook salmon *(O.tshawytscha),* common carp (*C. carpio*), red sea bream (*Sparus*

auratus), yellow tail (*Seriola lalandi*), and Japanese eel (*A. japonica*) which their requirement to exogenous source of dietary inositol for normal growth and for preventing clinical deficiency signs has been proved (Mclaren *et al.*, 1947; Halver, 1953; Aoe and Masuda, 1967; Kitamura *et al.*, 1967; Yone *et al.*, 1971; Arai *et al.*, 1972).

The relative rate of de novo synthesis of inositol; however, may vary among different tissues. Burtle and Lovell (1989) reported that *myo*-inositol synthetase activity and inositol concentration were higher in brain than those of in liver tissue of channel catfish. Inositol concentrations in brain tissue of sunshine bass (*Morone chrysops* × *Morone saxatilis*) were greater than their liver tissue. Inositol as PI is normally found at higher levels in neural tissue than in liver tissue of other animals (Appel and Briggs, 1980). The brain contains large amounts of PUFA which are particularly vulnerable to reactive oxygen species (ROS) and stress induction of oxidative damage (Sahin and Gümüslü, 2004). It is the center of the nervous system in all vertebrates, and its homeostasis is crucial for survival (Mustafa *et al.*, 2012). In human, MI is one of the most abundant metabolites in the brain (Haris *et al.*, 2011). The role of inositol phospholipids in signal transduction events within the central nervous system (CNS) is well established. The CNS is an atypical tissue in that it possesses relatively high concentrations of *myo*-inositol as well as the means to synthesize it (Fisher and Agranoff, 1987).

Sources of inositol

Wheat germ, dried peas, and beans are rich dietary sources of inositol, while brain, heart, and glandular tissues are very good sources of biologically active inositol. Citrus fruit pulp and dried yeast also contain inositol (Halver, 2002). Fish meal, depending on the fish species, contains about 700–800 mg inositol.kg^{-1} dry weight (700 g.kg^{-1} protein dry weight) (Boge and Brækkan, 1974).

If we consider a diet containing 10% of its dry weight as phospholipid derived from cod eggs and this being the only lipid present, we note initially that this lipid readily fulfils the published dietary requirement of fish for inositol. Such inositol is being provided solely by PI (Sargent *et al.*, 1999).

Phytic acid maybe utilised as an inositol sourceand has been found for Japanese shrimp (*P. japonicus*) after intestinal dephosphorylation (Civera and Guillaume, 1987). However, McDowell (2000) suggested that conclusive evidence for the complete dephosphorylation of phytate by phytase enzyme does not exist, so this mechanism is theoretical.

Tissue inositol concentration

The inositol concentration in the diet and tissue samples are determined using an enzymatic assay as described by Ashizawa *et al.* (2000) or according to a microbiological assay method described by Waagbø *et al*. (1998) .

Inositol is found in various tissues . Tissue inositol concentration that is related to dietary inositol has been measured in a few fish species. The liver showed

comparable concentrations of inositol to the whole body, suggesting a similar retention of inositol in liver as in other tissues for structural and metabolic purposes (Waagbø *et al.*, 1998). In common carp liver inositol concentration of fish fed by an inositol-free diet was not significantly different from that of fish fed by a 400 mg inositol .kg^{-1} diet (Aoe and Masuda, 1967). Waagbø *et al*. (1998) also found that liver inositol levels in Atlantic salmon (*S. salar*) were similar when they were fed with diets containing 300–1100 mg inositol.kg^{-1} diet. Burtle and Lovell (1989) reported that both liver and brain inositol concentrations were similar in channel catfish (*Ictaturus punctatus*) fed by an inositol-free or a diet containing 400 mg inositol.kg^{-1} diet. Also, Deng *et al*. (2002) reported that tissue inositol levels were similar in sunshine bass (*Morone chrysops* × *Morone saxatilis*) regardless of the level of dietary inositol. They detected that *myo*-inositol concentration of the brain was greater than that of liver tissue.

On the other hand, Shiau and Su (2005) reported that liver inositol concentration of tilapia was responsive to dietary MI level as a plateau was reached when the dietary MI requirement was met.

Requirements of myo-inositol

The requirement of dietary inositol in various aquatic animals like fish has been reported by Li *et al*. (2001), Shiau and Su (2005), and Wen *et al*. (2007), still it is somewhat unclear because assessment in fish has been based on a lack of deficiency signs coupled with the most efficient food conversion. The susceptibility of fish to low dietary levels of inositol appeared to vary with species, life stage, the rate of inositol biosynthesis, as well as physiological and nutritional stresses (Kukiss and Mookerjea, 1978). Dietary source and level of carbohydrate and lipid has been declared to influence the inositol requirement in some species (Kukiss and Mookerjea, 1978; Chu and Geyer, 1983). Since choline and inositol may interact with each other as both serve as regulators of lipid metabolism, their requirements might be affected in the presence of each other (Michael and Koshio, 2008).

The optimal dietary inositol requirement for the maximum growth of juvenile Jian carp (*C. carpio* var.Jian) was estimated to be 518.0 mg.kg^{-1} diet (Jiang *et al.*, 2009); juvenile tilapia *(O. niloticus* × *O.aureus*) required 400 mg.kg^{-1} diet (Shiau and Su, 2005); juvenile flounder (*Paralichthys olivaceus)* required 800-1200 mg.kg^{-1} diet (Li *et al.*, 2001); parrot fish (*Oplegnathus fasciatus*) required 100 mg.kg^{-1} (Khosravi *et al.*, 2015), and *Lateclabrax japonicus* required 500 mg.kg^{-1} diet (Zhong and Zhang, 2001); however, the suitable requirement for dietary inositol of growing grass carp (*Ctrnopharyngodon idella*) was approximately 166-214 mg.kg^{-1} (Wen *et al.*, 2007). Younger carps require a higher level of inositol than older fishes (Yone *et al.*, 1971). Although inositol is synthesized in common carp intestine (Aoe and Masuda, 1967), its amounts is not sufficient to sustain normal growth of young fish without an exogenous source of this vitamin (Yone *et*

al., 1971). Nevertheless, comparative nutritionists now consider liver and/or intestinal microbial synthesis of inositol to be adequate to meet the requirements of most animals (Deng *et al.,* 2002).

This maybe a practical diet which contains sufficient levels of inositol to meet various metabolic needs of fish because this vitamin is widely distributed in common feed ingredients (Peres *et al.,* 2004). Waagbø *et al*. (1998) reported that there is no need for inositol supplementation to fishmeal-based diets for young Atlantic salmon (*S. salar*); however, it may be advisable to supplement starter diets with 200 mg.kg^{-1} of inositol to compensate for fluctuations in the inositol concentration of the natural ingredients. They also showed that no real effect of dietary inositol supplementation has been declared on growth and mortality in Atlantic salmon feeding on practical diets. Moreover, juveniles Nile tilapia (*Oreochromis nilotica*) (Peres *et al.,* 2004), and sunshine bass (*Morone chrysops*♀ × *Morone saxatilis*♂) (Deng *et al.,* 2002) fed by puried diets did not require an exogenous source of inositol for normal growth and feed utilization. Other fish species such as channel catfish and Asian sea bass (*L. calcarifer*) do not require a dietary supplementation of inositol for normal growth and development (Burtle and Lovell, 1988; Boonyaratpalin and Wanakowat, 1993).

From a study by Burtle and Lovell (1989) on channel catfish, it was also detected that no inositol supplementation was needed because of *de novo* inositol synthesis by intestinal bacteria. Burtle and Lovell (1988) observed that altering levels of carbohydrate (dextrin) and lipid (fish and soybean oils) in diets for channel catfish affected weight gain, but did not influence the need for an exogenous source of *myo*-inositol.

The obtained requirement of inositol for juvenile grass shrimp (*P. Monodon)* 3400 mg MI .kg^{-1} diet was found to be the optimal level. It is considerably higher than that of fish (Shiau and Su 2004). For *M. japonicas,* the requirement level of dietary inositol has been reported to be either 2000 (Kanazawa *et al.,* 1976) or 4000 mg MI .kg^{-1} (Deshimaru and Kuroki, 1976).

Leaching is always a concern in a nutrition study of aquatic species especially for slow feeders such as crustacean in which particles usually remain suspended in water for extended periods before being consumed. It has been detected that the leaching rate of inositol from the experimental diets immersed 10, 30, 60 and 120 min in water were 23.5%, 43.1%, 62.1%, and 79.0%, respectively that may explain the large requirement of dietary inositol for shrimp. It may seem a possible overestimation of the inositol requirement due to leaching (Shiau and Su, 2004). The higher midgut gland lipid content of shrimp fed with inositol-deficient diets indicates lipid accumulation in the tissue (Storch *et al.,* 1984).

Deficiency signs

Good state of health for fish is the basic precondition for successful fish culture. Monitoring the physiological state of fish has become an integral part of the routine

examination of fish health (Rehulka, 2000). As an alternate, better assessment may be based on a standard muscle section or whole-carcass analysis for free or bound inositol (Halver, 2002). Signs of inositol deficiency have been reported to include poor appetite, anemia, poor growth, fin erosion, dark skin colouration, edema, slow gastric emptying, decreased cholinesterase, and certain aminotransferase activities in trout (McLaren *et al.,* 1947; Kitamura *et al.,* 1967), red sea bream (Yone *et al.,* 1971), Japanese eel, *Anguilla japonica*, (Arai *et al.,* 1972), Japanese parrotfish, *Scarus coeruleus*, (Ikeda *et al.,* 1988), and yellow tail, *Seriola lalandi*, (Hosokawa, 1989). Khosravi *et al.* (2015) concluded that fishes which lack de novo MI synthesis are more likely susceptible to anaemia by MI deficient diets. Rainbow trout fed by a diet devoid of inositol had large accumulations of neutral lipids in the liver and also increased levels of cholesterol and triglycerides, but decreased amounts of total phospholipid, phosphotidylcholine, phosphotidylethanolamine, and PI (Holub *et al.,* 1982; NRC, 2011). The higher liver lipid content of tilapia fed by inositol-deficient diets (367 $mg.kg^{-1}$ diet), indicated lipid accumulation in the tissue (Shiau and Su, 2005). In Atlantic salmon (*S. salar*), plasma triacylglycerol was negatively correlated with dietary inositol supplementation, but plasma cholesterol was unaffected (Waagbø *et al.,* 1998). Thus, the higher accumulation of lipid in liver and muscle of juvenile Nile tilapia (*Orechromis nilotica*) fed by dietary inositol at levels less than 100 and 400 $mg.kg^{-1}$, respectively could be an indication of inositol deficiency. Lee *et al*. (2009) reported that olive founder (*Paralichthys olivaceus*) fed with inositol-free diet resulted in declared abnormal lipid metabolism and decreased amount of PUFA. Khosravi *et al.* (2015) suggested that MI promoted the conversion rate of lipids into LDL in liver and caused a remarkable decrease in liver lipid content and subsequent enhancement of plasma cholesterol level. Hence, supplementation of inositol to purified diets is required to prevent lipotropic effect of inositol deficiency (Peres *et al.,* 2004). The major deficiency sign is inefficiency in digestion, food utilization, and concomitant poor growth, leading to a population of fish with distended abdomens (NRC, 2011). Anaemia has been also reported as a clinical sign of inositol deficiency in salmonids (Halver, 1982). Waagbø *et al*. (1998) observed a positive correlation between blood hemoglobin concentrations and dietary levels of inositol in Atlantic salmon (*S. salar*), but Burtle and Lovell (1988) observed that omission of inositol from the diet with or without the addition of an antibiotic did not affect haematocrit of the channel catfish. Peres *et al*. (2004) stated that supplementation of dietary inositol had no effect on mean total cell count, red cell count, haematocrit, and haemoglobin of Nile tilapia (*O. nilotica*). Therefore, Nile tilapia was probably able to synthesise sufficient quantity of inositol for normal erythropoiesis (Peres *et al.,* 2004).

Generally, it can be concluded that *Myo*-inositol is one of the water-soluble vitamins

with coenzyme functions in cellular metabolism,and possesses other functions as well. It is widely distributed in common feed ingredients especially fishmeal-based diets. Phytin that is often present in the diets, contains inositol and could be utilised as an inositol source; however, it is often unavailable to most aquatic animals. Because of the fluctuations in the inositol concentration of the ingredients and inefficient *de novo* inositol synthetises, supplementation of inositol to the practical diets is required to prevent the effects of inositol deficiency and to obtain a successful aquaculture. The essentiality of dietary MI for maximal growth has been demonstrated in several fishes. The requirement level varies from 400 to 617 $mg.kg^{-1}$ diet, but sunshine bass, Nile tilapia and Alantic salmon do not need dietary MI for growth or development (Waagbø *et al.,* 1998; Deng *et al.,* 2002; Peres *et al.,* 2004). In addition, a possible overestimation of the inositol requirement may have happened due to leaching for slow feeder such as crustacean. Inositol is usually fed in its active isomer form (MI) (Shiau and Su, 2004) and is added to aquatic feed when necessary as a dry powder in a multivitamin premix (NRC, 2011).

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