Review Article

Prospects of Using Citric Acid as Fish Feed Supplement

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Abstract

Fishes usually have low levels of acid secretion in the gut as compared to mammals. Inclusion of organic acids in their diet reduces the pH in the gut. This lowering of pH increases the phytate hydrolysis, kills the pathogens, decreases the rate of gastric emptying and improves mineralization and nutrient absorption. Among the organic acids, citric acid (CA) has been used extensively for diet acidification due to its unique flavor and high buffering capacity. It has great potential to replace fish meal (up to 70%) with plant based protein sources. Being a strong chelator of calcium and phosphorus, CA enhances the phytate hydrolysis. It improves the bioavailability of minerals by solubilizing the bones and competing with other chelators. It also increases the endogenous as well as exogenous phytases efficiency by providing an optimum pH in gut. Besides, it acts as antimicrobial agent and stimulates feeding in fish. Purpose of this review is to appraise the applications of citric acid supplementations in fish feed and to highlight its role in improving the growth performance, nutrient digestibility, minerals availability and phytase efficacy. © 2015 Friends Science Publishers

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Introduction

Use of the antibiotic growth promoters in fish feed improves their growth, feed conversion and survival rate. However, these antibiotics produce resistance in micro-biota of fish that may lead to cross–resistance among human. These public concerns led to a worldwide ban on the use of these antibiotic growth promoters in fish feed. Consequently, researchers focused on alternative additives such as organic acids, probiotics, herbs, enzymes and essential oils. Among them, short-chain organic acids are of special interest due to their beneficial effects in preservation of feed (Luckstadt, 2006; Atapattu and Senevirathne, 2013; Sing *et al.*, 2014).

Many studies have been conducted on broilers (Brenes *et al.*, 2003; Ali *et al.*, 2013; Ahmad *et al.*, 2013), pigs (Li *et al.*, 1998) and rabbits (Debi *et al.*, 2010) to investigate the effects of organic acids in diet (. However, a little information is available concerning the fish nutrition. Few available studies showed improved production of Rainbow trout (*Onchorynchus mykiss*) (Sugiura *et al.*, 1998), Red sea bream (*Pagrus major*) (Sarker *et al.*, 2005) and Rohu (*Labeo rohita*) (Baruah *et al.*, 2005) in response to organic acids.

Sugiura *et al.* (2006) reported two specialized types of cells in mammalian stomach (parietal and peptic) which secrete HCl and pepsinogen, for the acidification of lumen and digestion of protein. In contrast, stomach of other non-mammalian vertebrates including fish has only one type of

cells called oxynticopeptic cells which are responsible for the secretion of both HCl and pepsinogen. However, the acid secretion of these cells is not as efficient as in mammalian parietal cells (Koelz, 1992). Therefore, rainbow trout has high postprandial gastric pH i.e. ~4.0 (Sugiura and Ferraris, 2004) as compared to pH 2 or less in mammalian stomach (Berne and Levy, 1990). This problem can be tackled by adding organic acids in fish feed (Eidelsburger, 1997). Inclusion of these organic acids in diet lowers the pH of feed and intestinal digesta (Baruah *et al.*, 2005). This lowering in pH increases the nutrient absorption (Boling-Frankenbach *et al.*, 2001) and phytate solubility (Jongbloed, 1987). Besides this, rate of gastric emptying is also being reduced by acidification of diet (Mayer, 1994). In addition, due to their antimicrobial effect, they also improve the gut health of the animal (Ravindran and Kornegay, 1993; Partanen and Mroz, 1999).

Bioavailability of dietary minerals is also being greatly influenced by acidification through organic acids in several ways. Firstly, they modify the mineral transport mechanism by altering the gastric acidity. Secondly, chelating and complex forming ability of elements (Cross *et al.*, 1990; Ravindran and Kornegay, 1993) is also affected by inclusion of these organic acids in diet. Chelation of these acids with calcium (Ca) ions reduces the antagonistic interactions, including precipitation and co-precipitation between Ca and phosphates or trace elements at the

intestinal brush border. Thus, ensure the increased absorption of phosphorus (P) and other trace elements (Sugiura *et al.,* 1998). Thirdly, these organic acids stimulate the proliferation of the epithelial cells in gastrointestinal mucosa (Sakata *et al.,* 1995) thereby increasing the absorption area for minerals (Baruah *et al.,* 2007a). Further, these organic acids have high gross energy values (Freitag and Lückstädt, 2007) and therefore are used in various metabolic processes for energy generation such as production of ATP in citric acid cycle (Diebold and Eidelsburger, 2006), and also used as substrates in intermediary metabolism (Kirchgessner and Roth, 1988).

Among these organic acids, citric acid (CA), due to its unique flavor and high buffering capacity, has been extensively used for diet acidification (Hossain *et al.*, 2007;). Chemically, it is known as 2-Hydroxy-1,2,3-Propanetricarboxylic acid and its chemical formula is $COOHCH_2C(OH)(COOH)CH_2COOH$ (Dibner and Buttin, 2002). Physically, it is solid having molecular mass and density of 192.14 g/mol and 1.67 g/mL respectively. It has good water solubility and its gross energy is 2460 kcal/kg (Freitag, 2007). Like other tri- and dicarboxylic acids, its mode of absorption is Na^+-dependent transport mechanism across the intestinal brush border membrane (Wolffram *et al*., 1990, 1992).

In history, CA was first used in diet by Shohl (1937) at Harvard University. Rats were fed with rachitogenic diet deficient in Ca or P or both with supplementations of CA/sodium citrate (1:1) mixture. Results showed no rickets in rats which indicated improved phytate-P utilization. Later on, Pileggi *et al.* (1956) inveterated these results and further reported that response to CA addition was showed by phytate-containing diets only and no effect on non-phytate or available P was observed.

Citric acid has the great potential to replace fish meal up to 70% in a low P containing plant protein diets (Sarker *et al.,* 2012b). It has been reported that CA intensifies the phytate dephosphorylation *in vitro* (Zyla *et al.,* 1995; Baruah *et al.,* 2007b). It may also activates the proteolytic enzymes, stimulates feed consumption, depresses the microbial metabolites including ammonia and decreases the chances of subclinical infections (Chowdhury *et al.,* 2009; Ou *et al.*, 2013).

In this article, the authors review the applications of CA supplementation in fish feed in relation to the growth performance and nutrient utilization. The main objective of this review is to contribute in the current understanding of CA use in fish feed, and to locate gaps for potential future research work.

Implications of Using CA in Fish Feed

Effect on Growth and Feed Performance

Growth rate is considered as an important index for the determination of economic efficiency of commercial fish culture and is affected by number of factors. Studies conducted to investigate the growth and feed performance responses to CA acidified diet have shown the positive results (Sarker *et al.*, 2005; Pandey and Satoh, 2008) (Table 1).

Citric acid (3%) has been found to perk up the weight gain and specific growth rate while it has decreased the feed conversion rate in Beluga (Khajepour and Hosseini, 2012), Rohu (Baruah *et al.,* 2007b) and Common carp (Khajepour *et al.,* 2012). In the same concentration, CA improved feed performance in Red sea bream (Sarker *et al.,* 2005) and protein efficiency ratio in Beluga (Khajepour and Hosseini, 2012) and Rohu (Baruah *et al.,* 2007b). In other studies, 1% CA improved weight and feed conversion ratio in Red sea bream (Hossain *et al.,* 2007; Sarker *et al.,* 2007) while a similar increase in weight was also observed in Yellowtail (Sarker *et al.,* 2012b).

In another trial, a blend of organic acids (acetic acid, lactic acid and CA) was used to evaluate the growth performance in Rainbow trout. Higher feed conversion ratio, total length and feed intake were found in organic acid treated group (Tabrizi *et al.,* 2012). However, Sarker *et al.* (2012b) observed no effect of CA (0.5%) on specific growth rate and feed conversion ratio in Yellowtail.

Effect of CA on Mineralization

Effect on Phosphorus (P) Availability

Phosphorus is essential for fish like other animals, as it is the part of skeleton, nucleic acids, ATP and phospholipid. Its absorption, transport and metabolism are not well studied in fish (Gatlin III, 2000) and from the limited available studies it is suggested that 5 to 8 g/kg P is required for optimum growth, feed utilization and bone mineralization in rainbow trout and other fish species (Ogino and Takeda, 1978; Watanabe *et al.,* 1980; Sugiura and Hardy, 2000). Fish can take up P and other minerals from water across the gill membrane (Lall, 1989). However, natural water bodies have very low (usually 5-50μg/L) level of available inorganic P (Nose and Arai, 1976), therefore, fish still require a dietary source of P to fulfill its metabolic requirements.

In fishmeal, P is present in the form of hydroxyapatite and tricalcium phosphate and in phytate form in plant proteins. As tricalcium phosphate and phytate are structurally complex compounds, availability of P from them is less in some fish species (Takamatsu *et al.,* 1975; Shitanda *et al.,* 1979; Watanabe *et al.,* 1980). This unavailable organic P remains in water and is decomposed by microorganisms causing the pollution especially when the temperature of water increases (Sarker *et al.,* 2005).

It is evident from several studies that acidification of diet helps in the conversion of unavailable form of P to available form, therefore the diets containing CA supplementations do not require additional P (Table 1).

Table 1: Impact of citric acid supplemented feed on growth performance and mineral metabolism in various fish species

Reference	CA[a] (%)	Feeding trial	Species	Initial weight (g)	Diet No.	Fish Meal	Plant Protein sources Meal 1	Meal 2	Observation
Baruah *et al*., 2005	3	60 d[b]	Rohu (*L. rohita*)	12.61-13.72	1	FM[c] (5)	SBM[d] (20)	RP[e] (28.55)	Improved P retention and muscle ash contents
					2	FM (5)	SBM (40.85)	RP (8)	
Baruah *et al*., 2007a	3	60 d	Rohu (*L. rohita*)	13.16	1	FM (5)	SBM (20)	RP (28.55)	Increased Ca contents of muscle and serum but no effect on Ca content of scute; enhanced absorption of minerals (Na, P, K, Mn, Mg, Fe, Cu, Ca and N) and their contents in plasma and whole body
					2	FM (5)	SBM (40.85)	RP (8)	
Baruah *et al*., 2007b	3	60 d	Rohu (*L. rohita*)	12.61-13.72	1	FM (5)	SBM (20)	RP (28.55)	Improved weight gain, specific growth rate and protein efficiency ratio while decreased feed conversion rate, improved P digestibility
					2	FM (5)	SBM (40.85)	RP (8)	
Hossain *et al*., 2007	1	75 d	Red sea bream (*P. major*)	5.56±0.01	1	JMM[f] (35)	D-SBM[g] (20)	CGM[h] (10)	Improved weight and FCR; increased absorption and retention while reduced excretion of P
Khajepour and Hosseini, 2010	2 and 3	8 w[i]	Beluga (*H. huso*)	25.1±1.9	1	KFM[j] (30)	SBM (41.1)	WM[k] (6)	Improved P contents in muscle, serum and scute; increased Ca contents of muscle and serum but no effect on scute
Khajepour and Hosseini, 2011	3	8 w	Beluga (*H. huso*)	25.1±1.9	1	KFM (45)	SBM (20)	WM (8)	Improved P contents in muscle, serum and scute; increased Ca contents of muscle and serum but no effect on scute
					2	KFM (37)	SBM (30)	WM (8)	
					3	KFM (30)	SBM (41.5)	WM (6)	
Khajpour and Hosseini, 2012	2 and 3	8 w	Beluga (*H. huso*)	25.1±1.9	1	KFM (30)	SBM (41.1)	WM (6)	Improved weight gain, specific growth rate and protein efficiency ratio while decreased feed conversion rate; improved P contents in muscle, serum and scute; increased Ca contents of muscle and serum but no effect on scute
Khajepour *et al*., 2011	3	8 w	Common carp (*C. carpio*)	10.6±1.5	1	KFM (11)	SBM (24)	CM[l] (22)	Improved weight gain and specific growth rate while decreased feed conversion rate
Pandey and Satoch, 2008	1	12 w	Rainbow trout (*O. mykiss*)	9.71±0.35	1	JMM[m] (15)	D-SBM (26)	CGM (17)	Positive growth; increased P retention; increased P and Zn contents in carcass
Sarker *et al*., 2005	3	12 w	Red sea bream (*P. major*)	7.14±0.01	1	JMM (50)	D-SBM (5)	CGM (5)	Improved growth and feed performance; improved absorption and retention while reduced excretion of P; improved N retention; increased P, Ca, and K contents in carcass
Sarker *et al*., 2007	1 and 2	12 w	Red sea bream (*P. major*)	12.6±0.36	1	JMM (35)	D-SBM (20)	CGM (10)	Improved weight and FCR; increased P absorption and retention; improved N retention
Sarker *et al*., 2012a	0.5	12 w	Yellowtail (*S. quiqueradiata*)	129.7±6.34	1	AM[n] (31)	D-SBM (11)	CGM (11)	No effect on specific growth rate and feed conversion ratio; improved retention and lowered excretion of P; increased whole body ash content; increased retention of Ca, Mg, Na, K, Zn and Mn
					2	AM (23)	D-SBM (15)	CGM (15)	
Sarker *et al*., 2012b	1	16 w	Yellowtail (*S. quiqueradiata*)	135.8	1	AM (35)	SBM (10.5)	CGM (10.5)	Improved weight; increased retention of P, Ca, Mg, Na, K, Zn and Mn and lowered excretion of P
Tabrizi *et al*., 2012	1	7 w	Rainbow trout (*O. mykiss*)	150±05	1	-	-	-	Higher FCR, total length and feed intake

[a] Citric acid, [b] Days, [c] Fish meal, [d] Soybean meal, [e] Rice Polish, [f] Jack mackerel meal, [d] Deffated soybean meal, [h] Corn gluten meal, [i] Week, [j] Kilka fish meal, [K] WM, [l] Corn meal, [m] Anchovy meal. Only concerning diets are mentioned from the experiments

CA might liberate the P from tricalcium phosphate and phytate, in adequate quantity for the better fish growth. It makes the phytate soluble with subsequent chelation of the released P (Sarker *et al.,* 2005, 2007).

Retention efficiencies of nutrients such as P and nitrogen (N) are important for evaluation of feed quality (Cho *et al.,* 1994). Elevated absorption and retention with reduced excretion of P has been observed in Red sea bream when supplemented with 1% (Hossain *et al.*, 2007) and 3% CA in diet (Sarker *et al.,* 2005). In Yellowtail, improved retention and lowered excretion of P has been pragmatic in response to 0.5% (Sarker *et al.,* 2012a) and 1% CA supplementation (Sarker *et al.,* 2012b). Also in Rohu 3% CA supplementation improved P retention (Baruah *et al.,* 2005) and its absorption (Baruah *et al.,* 2007a). This reduced excretion, increased absorption and retention of P in red sea bream would in turn reduces its loading to the environment (Sarker *et al.*, 2007). A similar increased retention of P was also observed in Rainbow trout by Pandey and Satoh (2008) in 1% CA supplemented groups.

Improved P digestibility was observed with the use of 3% CA in Rohu (Baruah *et al.,* 2007b) as well as in Beluga (Khajepour and Hosseini, 2012).

Citric acid acidification also increased the P content in serum, muscles, scutes (Khajepour and Hosseini, 2012), bones (Pandey and Satoh, 2008) and in whole body (Baruah *et al.,* 2007a) of fish. Feeding of Beluga with 2% and 3% CA supplemented feed also enhanced the P contents in muscles, serum and scutes of fish (Khajepour and Hosseini, 2010; 2011; 2012). Also in carcass studies, increased P contents have been observed by means of 1% CA supplementation in Rainbow trout (Pandey and Satoh, 2008) and by 3% CA in Red sea bream (Sarker *et al.,* 2005).

Effect on Bioavailability of Other Minerals

Ash content is a well-documented parameter to estimate mineralization of bones and muscles. The administration of 3% CA increased the ash contents of muscle (Baruah *et al.,* 2005) and reduced the fecal ash content up till 28.6% in Rohu (Baruah *et al.*, 2007a). Reduction in fecal ash content could be a result of increased bioavailability of minerals from diet (Brenes *et al.,* 2003; Baruah *et al.*, 2005). Increase in muscle ash content of Beluga was also reported by Khajepour and Hosseini (2012). Sarker *et al.* (2012b) reported an increase in whole body ash contents in Yellowtail when fed with 0.5% CA supplemented diet.

Calcium (Ca) is an important component of skeleton, involved in blood coagulation, contraction of muscles, transmission of nerve and osmoregulation (Khajepour and Hosseini, 2011). Calcium contents of muscles and serum increased significantly in response to 2% and 3% CA in Beluga, however, it has no significant effect on Ca contents of scutes (Khajepour and Hosseini, 2010; 2011; 2012).

Citric acid supplementation also improves the retention of minerals in body of fish. The diets supplemented with 0.5% and 1% CA, increased the retention of Ca, Mg, Na, K, Zn, and Mn in Yellowtail (Sarker *et al.,* 2012a; b). It also caused enhanced nitrogen retention in Red sea bream with its different supply levels i.e., 1, 2 and 3% in the diet (Sarker *et al.,* 2005, 2007).

Baruah *et al.* (2007a) found that 3% CA improved the absorption of minerals (Na, P, K, Mn, Mg, Fe, Cu, Ca and N) and their concentration in plasma and whole body of Rohu. Carcass studies showed increased level of Zn in 1% CA supplemented group of Rainbow trout (Pandey and Satoh, 2008), while, Ca and K contents in carcass were improved by 3% CA in Red sea bream (Sarker *et al.,* 2005). This increment in carcass mineral deposition suggests that the organic acids and other supplements enhanced the mineral utilization of dietary fish meal and plant protein meal (Hossain *et al.,* 2007).

Citric acid increases the bioavailability of minerals in several ways. It solubilizes the bones present in fishmeal and releases the bounded minerals (Sarker *et al.,* 2005).

Also, being a strong chelator of Ca and P, it removes these minerals from the phytate; making it less stable and more susceptible to endogenous phytases (Khajepour and Hosseini, 2010). Moreover, CA increases the bioavailability of minerals by competing with the dietary mineral inhibitors (Ashmead, 1993).

Effect of CA on other Nutritional Attributes

Phytate not only complexes with minerals but also with proteins (Gifford and Clydesdale, 1990). It inhibits the activity of protein digesting enzymes like trypsin and pepsin (Caldwell, 1992). Citric acid physically affects the chemical bonds of phytic acid with amino acids, proteins and fibers which might make them more accessible to endogenous enzymes (Atapattu and Nelligaswatta, 2005). Furthermore, it increases pepsin activity by lowering gastric pH, stimulates pancreatic secretions, influences the mucosal morphology and decreases the intestinal microbial activity, which otherwise may utilize nutrients and reduce the availability to host animal (De Wet, 2005; Hossain *et al.,* 2007). However, responses of nutrients other than minerals to CA are somewhat inconsistent.

Sarker *et al.* (2007) reported improved nutrient retention in Red sea bream supplemented with 1% CA in the diet. In other studies, 3% CA showed no effect on moisture and muscle proteins while it caused reduction in the lipid contents in Common carp (Khajepour *et al.,* 2012) and Beluga (Khajepour and Hosseini, 2012). Dry matter and crude protein contents also remained unaffected in response to 1% and 3% CA in Red sea bream (Hossain *et al.,* 2007) and juveniles of Rohu (Baruah *et al.,* 2007b) respectively. These contradictory results may be due to the difference in feed composition, feed processing methodology, ecological variables and different species and therefore needs further exploration.

Citric acid and Feeding Behavior

Some studies have shown stimulatory effects of organic acids on fish feeding behavior (Adams *et al.*, 1988; Hidaka *et al.,* 1992). A citric acid content of 10^{-2} to 10^{-6} M stimulated feeding in *Tilapia nilotica* (Xie *et al.,* 2003) while in *Tilapia zilli,* 10^{-2} M concentration was found effective stimulant however, 10^{-3} M did not stimulate feeding (Adams *et al.,* 1988). Negative effect of CA supplementation (11.6%) on feed intake was also reported by Fauconneau (1988) in Rainbow trout, *Onchorhynchus mykiss.* The possible phenomenon of these variations might be the different concentrations of CA which may have created different pH levels, affecting differently on feeding behavior of fish along with species specificity (Yoshii *et al.,* 1979). It is needed to confirm stimulatory effects of organic acids, which may contribute in the improvement of fish feed quality with respect to both storage and feeding stimulation.

Table 2: Synergistic effect of citric acid and phytase on growth performance and mineral metabolism in feed of various fish species

Reference	CA[a] (%)	PHY[b] level	Feeding trial	Species	Initial weight (g)	Diet No.	Fish Meal	Plant Protein sources Meal 1	Meal 2	Observation
Amanat, 2011	2	750 FTU/kg	60 d[c]	Rohu (*L. rohita*)	13.98	1	FM[d] (5)	SFM[e] (67)	RP[F] (8)	Improved weight gain and digestibility of crude protein, crude fat and gross energy
Arshad, 2012	3	500 FTU/kg	60 d	Rohu (*L. rohita*)	7.06	1	FM (12)	CM[g] (56)	RP (12)	Improved weight gain, total length, FCR and digestibility of dry matter, crude protein, crude fat and gross energy
Baruah *et al.,* 2005	3	500 U/kg	60 d	Rohu (*L. rohita*)	12.61-13.72	1 / 2	FM (5) / FM (5)	SBM[h] (20) / SBM (40.85)	RP (28.55) / RP (8)	Improved bone ash content and P retention
Baruah *et al.,* 2007a	3	500 U/kg	60 d	Rohu (*L. rohita*)	13.16	1 / 2	FM (5) / FM (5)	SBM (20) / SBM (40.85)	RP (28.55) / RP (8)	Observed significant interaction for mineral (Zn, Na, P, K, Mn, Mg, Fe, Cu, Ca, and N) absorption, their content in plasma (except Mg and Fe) and whole body
Baruah *et al.,* 2007b	3	500 U/kg	60 d	Rohu (*L. rohita*)	12.61-13.72	1 / 2	FM (5) / FM (5)	SBM (20) / SBM (40.85)	RP (28.55) / RP (8)	Improved weight gain %, specific growth rate and protein efficiency ratio
Baruah *et al.,* 2009	3	500 U/kg	60 d	Rohu (*L. rohita*)	13.16	1 / 2	FM (5) / FM (5)	SBM (20) / SBM (40.85)	RP (28.55) / RP (8)	Increase in Hb, haematocrit, total serum proteins, albumin and globulin values while no effect on WBC and RBC counts
Farooq, 2012	5	750 FTU/kg	60 d	Rohu (*L. rohita*)	-	1	FM (5)	SFM (67)	WF[i] (15)	Improved apparent crude protein, crude fat and gross energy digestibility
Iqbal, 2012	3	1000 FTU/kg	60 d	Rohu (*L. rohita*)	7.50	1	FM (12)	CM (56)	-	Improved weight gain, total length, FCR and digestibility of dry matter, crude protein, crude fat and gross energy
Khajepour *et al.,* 2012	3	500 IU/kg	60 d	Common carp (*C. carpio*)	10.6±1.5	1	KFM[j] (11)	SBM (24)	WM (34)	No effect on growth performance
Phromkunthong *et al.,* 2010	0.22	750 FYT/kg	60 d	Common carp (*C. carpio*)	-	1	FM (17)	SBM (55)	-	Improved growth, feed efficiency, P availability, bone mineralization, bone ash, bone P, P digestibility and ash of whole body and decreased fecal P while no effect on serum P
Saeed, 2012	5	750 FTU/kg	70 d	Rohu (*L rohita*)	-	1	FM (5)	SFM (67)	RP (12)	Improved weight gain and digestibility of crude protein and crude fat and higher apparent digestibility of minerals (Zn, Na, P, K, Mn, Mg, Fe, Cu, Ca, and N)

[a] Citric acid, [b] Phytase, [c] Days, [d] Fish meal, [e] Sunflower meal, [f] Rice polish, [g] Corn meal, [h] Soybean meal, [i] Wheat flour, [j] Kilka fish meal. Only concerning diets are mentioned from the experiments

Citric Acid Effects on Hematological Parameters

Hematological parameters including red blood cells (RBCs) count, hemoglobin (Hb) and white blood cells (WBCs) count are considered valuable indices to assess fish health (Roberts and Rodger, 1978; Khajepour *et al.,* 2011). In Beluga, 3% CA showed no effect on RBCs and WBCs count which reflects that diet acidification does not cause any stress (dietary imbalance) that usually cause an increase in WBCs count. Similarly, mean cell volume (MCV), mean cell hemoglobin (MCH), serum glucose and total proteins were not affected by CA inclusion which indicates that acidification does not cause any metabolic stress. Furthermore, Beluga also showed increased Hb against diet acidification (Khajepour *et al.,* 2011). Increased heamatocrit values by 1% and 2% CA in Red sea bream (Sarker *et al.,* 2007) and by 3% CA in Common carp has also been observed (Khajepour *et al.,* 2012). On the other hand, 3% CA showed no effect on haematocrit values in Red sea bream (Sarker *et al.,* 2005). CA addition might cause maximum liberation of Fe, Cu, Ca and P from phytic acid complex which result in increased Hb and heamatocrit values (Khajepour *et al.,* 2011).

Synergistic Action of Citric Acid and Phytase

Due to uncertain and limited supply of fish meal, efforts are being made to replace fish meal with plant protein sources (Khajepour *et al.,* 2012). However, these plant proteins contain phytate as anti-nutritional factor (Khajepour and Hosseini, 2011). Phytate reduces the availability of protein by binding with trypsin (Singh and Krikorian, 1982; Spinelli *et al.,* 1983) and also interferes with the digestibility of starch and lipids (Cosgrove, 1966). 60-70% of P is found in bounded form with this phytate which decreases its availability (Storebakken *et al.,* 1998) and availability of other minerals including Zn, Mg and Ca to the fish (Denstadli *et al.,* 2006; Fredlund *et al.*, 2006).

Phytase is a group of specific type of enzymes that hydrolyze the phytate. Fish also has intestinal phytase, however, its hydrolyzing activity is poor in most of the teleosts (Ellestad *et al.,* 2003). Addition of phytase in diet

increases the phytate containing P availability up till 60% (Rodehutscord and Pfeffer, 1995; Vielma *et al.,* 1998). Phytase also has protein-sparing effect by releasing protein from phytate (Baruah *et al.,* 2007b). Its activity vary along the gut (Yi and Kornegay, 1996) and it performs optimum activity at pH 2.5 and 5.0-5.5 (Simons *et al.,* 1990). Therefore, performance of phytase can be enhanced, at least theoretically, by adding them in feed combination with organic acids. Plentiful literature is available on synergistic effect of CA and phytase in poultry and pig. However, few studies have also been conducted in fish (Table 2).

Phromkunthong *et al.* (2010) reported a synergistic effect of CA (0.22%) and phytase (750 FTU/kg) in the improvement of growth and feed efficiency in Common carp. Baruah *et al.* (2007b) reported significant interaction between CA and phytase on phytate hydrolysis *in vitro* and found that, at sub-optimal protein level; their combination (Phytase, 500U/kg and CA, 3%) improves weight gain %, specific growth rate and protein efficiency ratio in Rohu juveniles. Similarly, improved weight gain, total length and better feed conversion ratio were observed in response to 3% CA with 500 (Arshad, 2012) and 1000 FTU/kg phytase (Iqbal, 2012) in a canola meal based diet in *Labeo rohita* fingerlings. Furthermore, an increase in weight was observed in sunflower meal based diet supplemented with 750 FTU/kg phytase in combination with 2% (Amanat, 2011) and 5% CA (Saeed, 2012) respectively in *Labeo rohita* fingerlings. However, Khajepour *et al.* (2012) observed no significant effect of 3% CA and 500 FTU/kg phytase on growth performance of Common carp.

In various studies conducted on *Labeo rohita* fingerlings to investigate the nutrient digestibility; significant interactions have been found between CA and phytase. Dry matter, crude protein, crude fat and gross energy digestibility was higher in group fed on 3% CA with 500 FTU/kg (Arshad, 2012) and 1000 FTU/kg phytase (Iqbal, 2012) in a canola meal based diet. On the other hand, in sunflower meal based diet, 2% (Amanat, 2012) and 5% CA (Farooq, 2012) with 750 FTU/kg phytase also improved apparent crude protein, crude fat and gross energy digestibilities. On the contrary, Baruah *et al* (2007b) did not observe any effect on crude protein and dry matter digestibility in response to their interaction.

Citric acid and phytase also showed significant positive interaction for mineral (Zn, Na, P, K, Mn, Mg, Fe, Cu, Ca, and N) absorption, their contents in plasma (except Mg and Fe), whole body (Baruah *et al.,* 2007a) and their apparent digestibility (Saeed, 2012) in Rohu fish fingerlings. Among the minerals, their synergistic effect has special influence on P availability, digestibility (Phromkunthong *et al.,* 2010) and retention (Baruah *et al.,* 2005) in fish. Combination of both the additives affects the haemato-biochemical parameters as well. Baruah *et al.* (2009) reported a significant increase in Hb, haematocrit, total serum proteins, albumin and globulin values while no effect

was observed on WBCs and RBCs counts in groups fed on 3% CA and 500 U/kg phytase.

In conclusion, despite the limited literature on the use of CA in fish feed, the results from the available studies indicate that CA has promising potential to improve growth and feed performance and mineralization in fish. Its acidification in fish feed can efficiently be used to formulate cost effective and environment friendly feed.

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