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Supplementation of materials in fish feed for efficient growth performance and body indices: An extensive review on the role of functional feed additives in tilapia nutrition

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Abstract

Aquaculture feeds are formulated with a vast pool of ingredient to meet nutritional requirements of fish for normal physiological functions, including maintaining a highly effective natural immune system, growth, and reproduction. To ensure the dietary nutrients are ingested, digested, absorbed, and transported to the cells, an increasing diversity of non-nutritive feed additives are being used in aquatic feeds. Feed additives are supplemented in small amounts to tilapia for a specific purpose in aquaculture. Feed containing functional feed additives promote the growth and health of tilapia, improve their immune systems, and induce physiological benefits beyond traditional feeds. Probiotics, prebiotics, phytogenic substances, immune-stimulants, enzymes, hormones, mycotoxin binders, organic acids etc., are best functional feed additives to manage and regulate tilapia performance and improve aquaculture profit.

Keywords: functional feed additives, immune-stimulants, mycotoxin binders, organic acids, phytogenic substances, prebiotics

Introduction

Products that improve feed efficiency are particularly important since feed costs are a major expense in aquaculture production. Nonnutritive feed additives are being used in aquatic feeds to ensure ingestion, digestion, and absorption of dietary nutrients. Feed additives may be both nutritive and non-nutritive ingredients and work by either direct or indirect methods on the animal's system [1-3]. According to Bai *et al*. [2], feed additives are supplemented in small amounts (alone or in combination) for a specific purpose, such as to improve the quality of fish as a final product, to preserve the physical and chemical quality of the diet or to maintain the quality of the aquatic environment. The range of feed additives used in aquatic feeds is very diverse. Additives are used in fish feed to preserve the nutritional characteristics of a diet or feed ingredients prior to feeding (e.g. antioxidant and mold inhibitors) [2], enhance ingredient dispersion or feed pelleting (e.g. emulsifiers, stabilizers and binders) [1, 2, 4], facilitate feed ingestion and consumer acceptance of the product (e.g. feed stimulants or attractants) [5-8] and promote growth (e.g. growth promoters, including antibiotics, probiotics and hormones) [2, 9-14].

Enzymes also used to improve the availability of certain nutrients (e.g. proteases, amylases) or to eliminate the presence of certain antinutrients (e.g. phytase) [15, 16].

Methods

Types of feed additives in tilapia nutrition

Nowadays, there are more sustainable ways to modulate the health and performance of tilapia by supplementing feeds with nutraceuticals or functional foods. Functional feed (feed containing functional feed additives) promote the growth and health of cultivated organisms, improve their immune systems, and induce physiological benefits beyond traditional feeds. According to Barrows *et al*. [1] feed additives can be categorized into: (1) additives that affect fish performance and health (functional feed additives) and (2) additives that affect feed quality and feed up take. There are several options available to manage and regulate fish performance and health such as the fish gut environment which includes probiotics, prebiotics, immune stimulants, phytogenic substances, enzymes, hormones, mycotoxin binders and organic acids. There are also different feed additives such as pellet binders, attractants, antioxidants, color/pigmentation agents and antimicrobial compounds used to maximize feed up take and maintain feed quality in tilapia culture.

Selecting fish foods

There are three types of food used in fish ponds:

- Natural food.
- Supplementary feeds.
- Complete feeds.

Natural food is found naturally in the pond. It may include detritus*, bacteria*, plankton*, worms, insects, snails, aquatic plants and fish. Their abundance greatly depends on water quality. Liming and fertilization in particular organic fertilization, can help you to provide a good supply of natural food to your fish.

Supplementary feeds are feeds regularly distributed to the fish in the pond. They usually consist of cheap materials locally available such as terrestrial plants, kitchen wastes or agricultural by-products.

Complete feeds may also be regularly distributed. They are made from a mixture of carefully selected ingredients to provide all the nutrients necessary for the fish to grow well. They must be made in a form which the fish find easy to eat and digest. These feeds are quite difficult to make on the farm and are usually quite expensive to buy.
The system of production can be defined according to the type of food given to the fish.

- **Extensive:** fish production depends entirely on natural food;
- **Semi-intensive:** fish production depends on both natural food and supplementary feed; more fish may be reared in the pond;
- **Intensive:** fish production depends entirely on complete feed, and the stocking rate no longer depends on food availability but on other factors such as water quality.

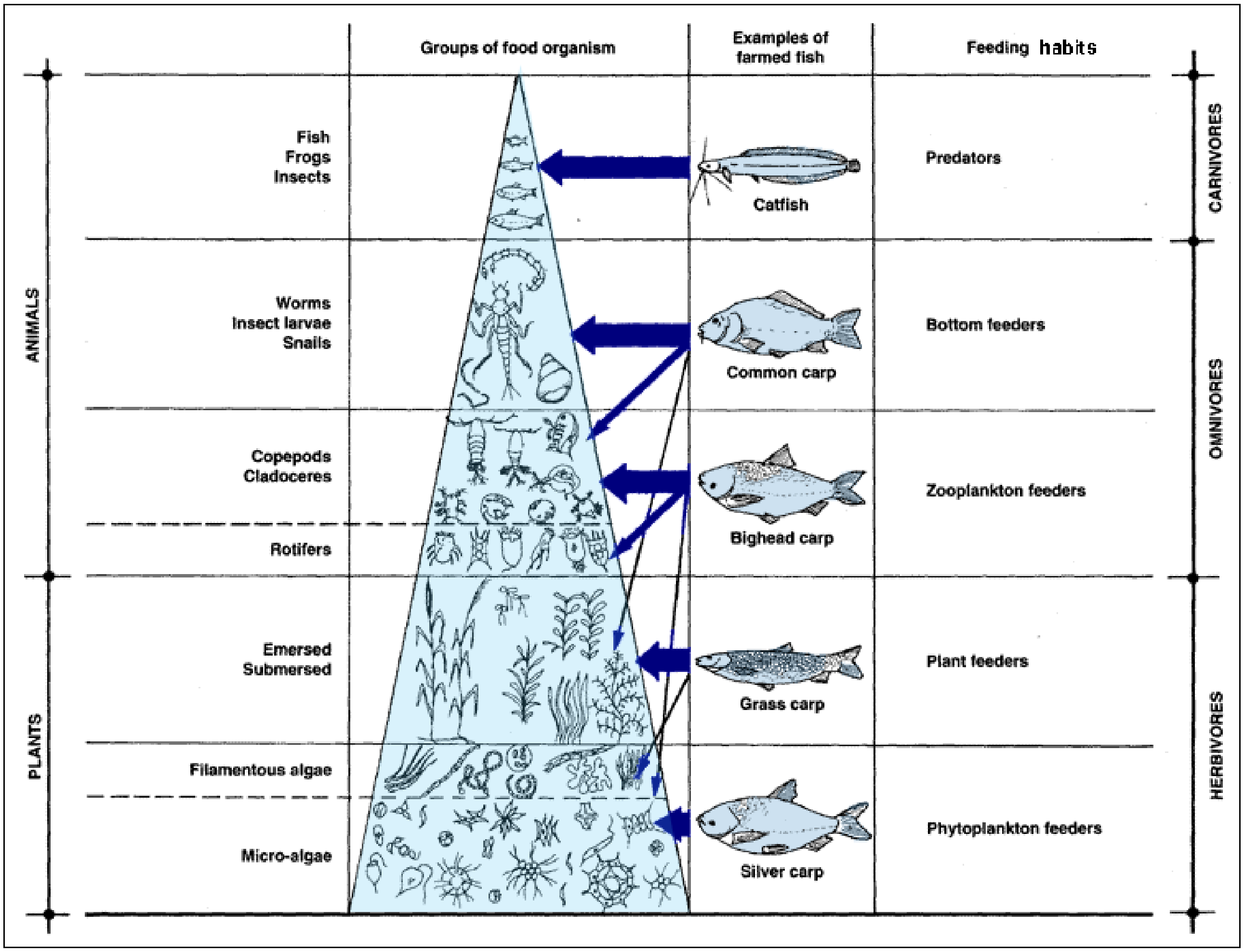

Fig 1: Natural foods for fish

There are several kinds of natural foods

1. Natural fish foods present in a fish pond are very diverse and usually consist of a complex mixture of plants and animals.
   a) They range from microscopic to relatively large size.
   b) They may be alive or dead (detritus) and available from bacterial decomposition.
   c) They are generally present in various parts of the pond:
      - Near the shore such as rooted high plants;
      - Floating in the water such as plankton;
      - On the surface of or within the bottom (benthic material or benthos*) such as worms, insect larvae and snails;
      - Covering the surface of submerged objects (biological cover or autwuchs*);
      - Swimming around such as aquatic insects, frogs and fish (nekton*).

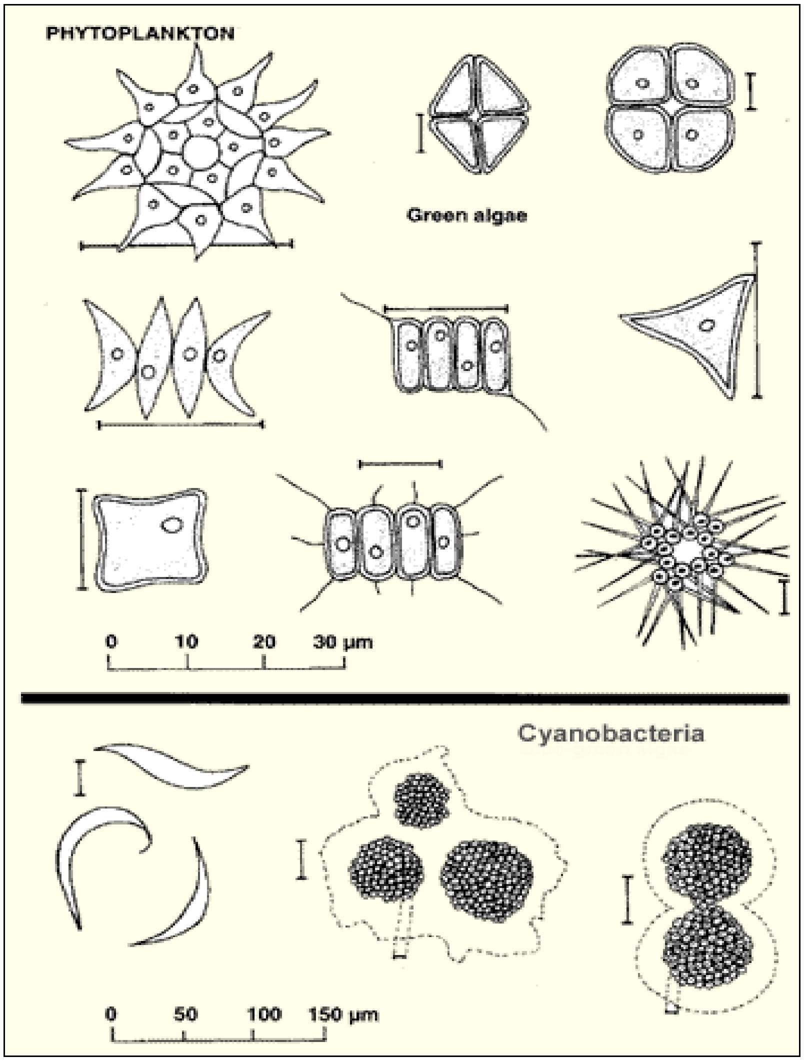

Fig 2: Phytoplankton

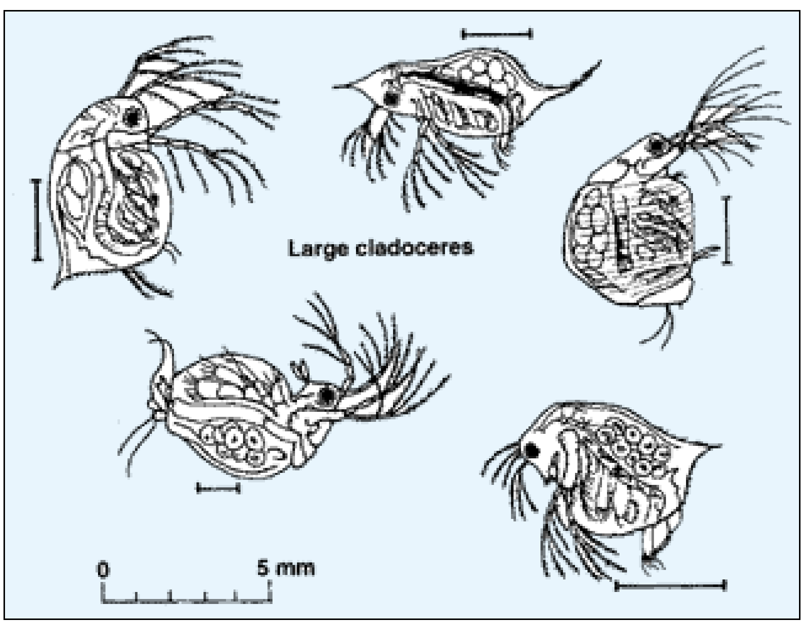

Fig 3: Zooplankton

Table 1: Natural feeding habits of adult fish

Fish species	*Natural food items*[1]										
	Bottom detritus	Bottom fauna	Biological covers	Filamentous algae	Phytoplankton	Zooplankton	Macrophytes[2]	Fruits/seeds	Insects	Molluscs	Fish
HERBIVORES											
Tilapia rendalli			+				+ +				
Silver carp	+				+ +	+	dec				
Grass carp							+ +				
Puntius javanicus				+			+ +				
OMNIVORES											
Nile tilapia	+	+	+		+ +	+ +	+		+		
Mozambique tilapia	+	+	+ +		+ +	+	+		+		
Common carp	+ +	+ +	+		+	+	dec				
Bighead carp					+	+ +					
Colossoma spp.				+		+ +	+	+ +	+	+	
Catla	+			+	+	+ +	dec		+	+	
Mrigal	+ +			+ +	+		dec				
Rohu	+ +		+ +		+	+	dec		+		
CARNIVORES											
Clarias spp.	+	+ +				+		+	+ +	+	+ +
Pangasius spp.									+ +		+ +
Rainbow trout									+ +	+	+

[1] From relatively less important (+) to more important (+ +)
[2] dec.: decayed material from macrophytes (higher plants)

Roles of functional feed additives in tilapia nutrition

Phytogenic substances

Phytogenics are plant-derived products which are added to the feed in order to improve palatability of feeds or animal performance [17, 18]. These plant active ingredients can exert multiple effects on the organisms, including improvement of feed efficiency and digestion, reduction of nitrogen excretion and improvement of gut flora and health status. Phytogenic feed additives are an extremely heterogeneous group of feed additives originating from leaves (e.g. extract of Moringa oleifera) [19], roots, tubers (e.g. Garlic, Allium sativum [20]; Ginger, Zingiber officinale) [14] or fruits of herbs, spices or other plants. They are either available in a solid, dried or ground form or as extracts or essential oils [17, 21].

Gbadamosi *et al*. [19] tested hepatoprotective and stress-reducing effects of dietary M. oleifera extract against *Aeromonas hydrophila* infections and transportation-induced stress in *O. niloticus* fingerlings. They reported that a dose of 0.10 g per 100 g dietary moringa leaf supplementation was sufficient as a hepatoprotective and stress reducing agent in Nile tilapia. Pachanawan *et al*. [22] also evaluated effect of dry leaf powder of *Psidium guajava* and ethanol extract of *P. guajava* leaf as feed additive to control *A. hydrophila* infection in tilapia culture. Fish diets containing either dry leaf powder of *P. guajava* or dried ethanol extract of *P. guajava* leaf reduced mortality of *A. hydrophila* infected tilapia compared with commercial tilapia diet supplemented with oxytetracycline. According to Zilberg *et al*. [23], Nile tilapia fed with dried rosemary (Rosmarinus officinalis) leaves significantly reduced mortality following infection with *Streptococcus iniae*. They reported that, 44% mortality in the group fed 8% rosemary, similar to oxytetracycline treatment (43% mortality), and significantly lower than the control (65%). Goda [24] reported that Nile tilapia fingerlings fed diets containing at least 200 mg/kg ginseng herb for 17 weeks enhanced growth performance, diet utilization efficiency, and hematological indices. The fingerlings also had significantly higher protein efficiency ratio (PER) compared to fish fed the control diet. This is also the case in Dada [25] report in which Nile tilapia supplied with 5.0 g kg-1 of commercial herbal growth promoter feed additive powder (Superliv). Chinese herb, Astragalus radix can modulate the innate immune system of tilapia (*Oriochromis niloticus*). They reported that feeding tilapia with 0.1 and 0.5% Astragalus radix for one week enhanced lysozyme activity and for three weeks stimulated phagocytosis by phagocytic blood cells [26].

Probiotics are live microorganisms when supplied in adequate amount to cultured organisms confer a health benefit of the host. Probiotics are live non-pathogenic and nontoxic microorganisms without undesirable side-effects when administered to aquatic organisms. Probiotics are live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance [27-30]. The range of probiotics examined for use in aquaculture has included both Gram-negative and Gram-positive bacteria, yeasts [31-33], bacteriophages, and unicellular algae [31, 33].

Probiotics have several uses in the host animals in aquaculture. They have antiviral activities against infections; they produce inhibitory chemicals [30, 34] they improve water quality by participating in turnover of organic nutrients and toxic NH3 and NH2 in aquaculture system [27, 29, 30, 35-38] they enhance immune response by increasing the phagocytic activity of leucocytes [30, 35] they compete for nutrients otherwise consumed by pathogenic microbes; they also compete for adhesion site and food with the pathogens in the gut epithelial surface and prevent colonization [32] and probiotics are sources of nutrients and secrete a variety of enzymes to increase feed degradation assimilation enhancing its nutritional values [29, 30, 32, 39, 40].

Zhou *et al*. [41] reported that adding Bacillus coagulans and *Rhodopseudomonas palustris* at concentration of 1× 9107 CFU ml-1 every two days had significantly higher final weight, daily weight gain, and specific growth rate compared with without probiotics (control) in Nile tilapia. Wang *et al*. [42] analyzed the effect of a probiotic bacterium, E. faecium on growth performances and immune responses of tilapia (*O. niloticus*). Tilapia was treated with E. faecium at concentration of 1×107 CFU ml-1 in aquaria water every four days. Tilapia supplemented with the probiotic showed significantly better final weight and daily weight gain (DWG) than those fed the basal diet (Control). In addition, myeloperoxidase activity and the respiratory burst activity of blood phagocytes were higher ($P<0.05$) in E. faecium treated tilapias than the controls. This was also the case where Nile tilapia fingerlings supplemented with a diet containing probiotics Bacillus amyloliquefaciens at level of 1 × 9106 CFU ml-1 [43]. Pigott *et al*. [4] evaluated the effects of three types of probiotics, two bacteria (bacterial mixture containing *Streptococcus faecium* and *Lactobacillus acidophilus*) and yeast (Saccharomyces cerevisiae) on growth performance in Nile tilapia. Fry fed diets with a probiotics supplement exhibited greater growth than those fed with the control diet without supplements. Of the probiotic treatments, the 40% protein diet supplemented with yeast produced the best growth performance and feed efficiency. Aly *et al*. [44] also studied on *Bacillus subtilis* and *L. acidophilus*, as potential probiotics, on the immune response and resistance of *O. niloticus* to pathogenic bacterial infections. Both *B. subtilis* and *L. acidophilus* inhibited the growth of *A. hydrophila*. In addition, *B. subtilis* inhibited development of *P. fluorescens* while *L. acidophilus* inhibited the growth of *S. iniae*

Prebiotics: A prebiotic was defined by Gibson *et al*. [45] as: 'a non-digestive food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'. Prebiotics are food for bacterial species, which are considered beneficial for health and well-being and important dietary additions for modulating the growth and activity of specific bacterial species in the colon [46]. In order for a food ingredient to be classified as a prebiotic, it should (1) be neither hydrolysed nor absorbed in the upper part of gastrointestinal tract; (2) be fermentable by intestinal microbiota; (3) be a selective substrate for one or a limited number of beneficial bacteria to the colon, which are stimulated to grow and/or are metabolically activated; and (4) consequently, be able to alter the colonic flora in favour of a healthier composition [28, 45, 47, 48].

Prebiotics bring about a specific modulation of the gut microbiota, particularly increased numbers of bifidobacteria and/or lactobacilli cell counts or a decrease in potential harmful bacteria is a sufficient criterion for health promotion [46]. The most common prebiotics used in fish are carbohydrates like inulin, fructooligosaccharides, short chain fructooligosaccharides, oligofructose, mannan oligosaccharides, trans-galactooligosaccharides, which are nondigestible but can be fermented by the intestinal flora [49, 50].

Tiengtam *et al*. [51] evaluated inulin as prebiotic ingredients in the diet of juvenile Nile tilapia (*O. niloticus*). Fish fed the inulin diets exhibited better growth performance compared with control groups. Dietary inulin (5 g kg-1) increased red blood cell number, goblet cell number, magnesium, calcium, iron content, increased the height of intestinal villi and lysozyme activity. Supplementation of 0.4% prebiotic (mannan oligosaccharides) increased intestinal fold height and intestine muscular layer thickness in Nile tilapia [52]. Abu-Elala *et al*. [53] tested S. cerevisiae as a whole yeast cell (probiotic), its extract (mannan-oligosaccharide-Prebiotic) and Pre-Probiotic mixture (synbiotic) as growth promoters and immunostimulants in cultured *O. niloticus*. Synbiotic feed additive has showed significant enhancement of fish innate resistance against selected fish pathogens (*A. hydrophila*, *P. fluorescens* and *F. columnare*) as well as positively increased the growth performance of challenged fish. Hassaan *et al*. [54] also showed that increasing dietary B. licheniformis and yeast extract levels significantly improved growth performance and nutrient utilization in *O. niloticus*. Contrarily to this, Shelby *et al*. [55] reported that incorporation of yeast and yeast subcomponents consisting mainly of β-glucan or oligosaccharide feed additives to juvenile Nile tilapia have no effect on growth, antibody responses or survival following *S. iniae* or *Edwardsiella tarda* infection.

Organic acids

Organic acids are organic carboxylic compounds of general structural formula R-COOH whose acidity is associated with their carboxyl group (-COOH). They are weak acids because they partially dissociate in water to form a hydrogen ion (H^+) and a carboxylate ion ($-COO^-$) (e.g. acetic CH2COOH) [56]. Dietary acidification by the addition of organic acids has been widely used in animal nutrition and organic acids have

become a promising feed additive to improve gut health and performance [57]. There are two different mode of action of organic acids in the intestinal tract of fish: the pH-decreasing action of organic acids in stomach and small intestine contributes to an improved activity of digestive enzymes and some organic acids can penetrate the cell wall of certain types of bacteria, disrupt the normal physiology and inhibits the growth of pathogens bacteria [3, 56]. Organic acids also used in fish feeds to reduce the potential threat of microbial contamination including pathogenic bacteria and molds or fungi (due to Aspergillus, Penicillium, and Fusarium) that may grow during feed storage [56]. The most commonly used organic acids as feed additive includes: (1) individual or combinations of organic acids such as propionic, sorbic, benzoic, butyric acids, malic acid, lactic acids, and acetic acids, and (2) salts of organic acids such as calcium propionate, potassium sorbate, and sodium benzoate [2, 3, 56].
According to Lim *et al*. [56] during periods of high feed intake, such as when the animals are young or when the feeds are high in protein, hydrochloric acid concentrations in the stomach are reduced. This reduction negatively impacts pepsin activation and pancreatic enzyme secretion and impairs digestion. Abu-Elala *et al*. [58] reported that *O. niloticus* fed on 0.2% and 0.3% organic acid, potassium diformate (KDF) exhibited significant improvements in their feed intake, live weight gain, specific growth rate, feed conversion ratio (FCR) and protein efficiency ratio compared with control. The reduction of the stomach and the upper gut pH in KDF supplemented fishes may be the primary reason for improving the growth performance and protein digestibility. The lower gastric pH associated with a higher pepsin activity contributes to improve the protein digestibility and nitrogen retention. According to them, the second reason for improving the growth performance may be KDF supplemented di*et al*so markedly decreased the total bacterial counts in faeces. Because the low molecular weight organic acids can diffuse across the cell membrane of gram-negative bacteria, acidification of their metabolism can lead to bacterial cell death.
Koh *et al*. [59] evaluated the effects of oxytetracycline or organic acids (consists of five organic acids, formic acid, lactic acid, malic acid, tartaric acid and citric acid) supplementation on growth, nutrient utilization and faecal/gut bacterial counts of red hybrid tilapia. Tilapia fed 0.5% oxytetracycline or 0.5% organic acids blend diet had significantly higher resistance to S. agalactiae than those fed the control diet (no additives). They reported that dietary organic acids
can potentially replace oxytetracycline as a growth promoter and antimicrobial in tilapia feeds. This also the case in which Nile tilapia supplemented with organic acids, formic and propionic acid/salt mixture in 1 g/kg and 2 g/kg respectively [60]. The best protection against challenged A. sobria was also observed in fish supplemented with formic acid and propionic acid compared with oxytetracycline. Khaled [61] evaluated the effect of sodium diformate as commercial feed additives on growth performance and feed utilization of hybrid tilapia (*O. niloticus* × O. aureus) fingerlings. Supplementation of 3 g/ kg-1 sodium diformate showed significant improvement in FCR and PER compared with other groups of fish at various supplementation levels of organic acids salts and better than control. Protein and lipid digestibility among fish groups fed the experimental diets supplemented by sodium diformate also improved significantly compared to the control group.

Enzymes

Nowadays, a number of exogenous enzymes (e.g. phytase, carbohydrase, protease and lipase) are used in aquaculture feeds to overcome the negative effects of anti-nutritional factors, and to improve the digestion of dietary components and enhance growth of fish [15, 16]. For instance, phytase, an enzyme specific to hydrolyze indigestible phytate, has been increasingly used in fish feed during the past two decades [62]. According to Bai *et al*. [2] up to 80% of phosphorus (P) in plant seeds are in the form of phytate. The digestibility and availability of phytate phosphorus for fish is very low and consequently lost to the environment as waste. Thus, use of phytase enhances bioavailability phytate phosphorous [62-64]. However, effect of temperature on the stability of enzymes applied in the feed processing, leaching loss of the enzyme in water and the effectiveness of some enzymes (e.g. microbial enzymes) that have 37°C optimum temperature, when applied in cold water aquatic animals that have low body temperatures are constrains regarding the effectiveness of enzyme applications in aqua feeds.
Liebert *et al*. [65] conducted a study on nutrient utilization of *O. niloticus* fed plant based low phosphorus diets supplemented with microbial (yeast) phytase. Significant improvements due to phytase addition were found for growth, feed conversion ratio, protein efficiency ratio, and specific growth rate compared with control. Phytase supplementation also increased protein and phosphorus utilization significantly. In addition, the mineral composition of scale and vertebra was significantly affected. Similar result was reported by Nwanna *et al*. [16, 66] in which Nile tilapia supplemented with phytase compared with control. Goda *et al*. [24] also conducted a research to evaluate the effect of baker yeast, S. cerevisiae and exogenous digestive enzymes (pepsin, papain and amylase) dietary supplementation on growth performance, feed utilization and hematological indices of *O. niloticus* fingerlings. They reported that, growth performance and feed utilization efficiency were significantly higher in all treatments receiving S. cerevisiae and enzymes supplemented diets than the control diet (without additives).

Hormones

Various hormones have been used for different reason in aquaculture (e.g. growth hormone, steroid hormones etc.). For instance, growth hormone [11] and thyroid hormone (thyroxin) [13] plays an essential role in the stimulation of somatic growth and survival of tilapia. Monosex fish production is common practice in aquaculture and possible in 47 species using steroid hormone (e.g. methyl testosterone) [9, 10]. According to Beardmore *et al*. [10], the potential advantages sought from monosex production in different fish species may include one or more of the following features: achievement of higher average growth rate, elimination of reproduction, reduction of sexual/territorial behaviour, reduction of variation in harvest size, and reduction of risk of environmental impact resulting from escapes of exotic species. Early sexual maturity in tilapia culture is well recognized problem which results in inbreeding in overstocked fish ponds, reduced production, and farmed stocks of generally low quality. To overcome these problems, mono-sex (all male) production is a solution and they (male of tilapia) grow faster than females. Sex reversal by oral administration of feed incorporated with methyl testosterone is the most effective and practical method for production of all male tilapia [10, 12, 14, 67]. However, currently there is an argument on use of methyl testosterone hormone due to some research reports that concluding its effect on consumer, fish feed producer and the environment [67, 68].

Mycotoxin binders

Mycotoxins are toxic metabolites produced by a diverse group of fungi (e.g. Aspergillus) that contaminate agricultural crops prior to harvest or during storage post-harvest [69]. Mycotoxins represent a serious problem in fish production worldwide. Its effects includes reduction of weight gain and feed efficiency, causing liver and kidney damage, worsening the overall health of the fish and which can result in serious economic implications to farmers [70,71]. According to Selim *et al*. [72] 0.5% of hydrated sodium calcium aluminosilicates (HSCAS) effectively reduced aflatoxin B1 (AFB1) toxicity in *O. niloticus*. HSCAS binds aflatoxin in the gastrointestinal tract, thereby reducing overall bioavailability to the bloodstream. Muanglai *et al*. [73, 74] reported that 1% bentonite clay reduced growth inhibitory effect, bioaccumulation of AFB1 in muscle of Nile tilapia as well as tissue lesions due to AFB1.

Immunostimulating agents

An immunostimulant is a naturally occurring compound that modulates the immune system by increasing the host's resistance against diseases that in most circumstances are caused by pathogens [75]. *O. niloticus* supplied with diet containing plant additives 0.25% E. purpurea, 3% garlic (A. sativum) or 3% Nigella sativa showed higher survival in response to challenge infection than fed on control (without additives) [76]. According to Aly *et al*. [77] survival rate was significantly high (>85%) after challenge infection using pathogenic *A. hydrophila* in *O. niloticus* supplemented with feed containing E. purpurea and garlic (3%). Shalaby *et al*. [78, 79] also reported that adding 3% garlic to fish diet can promote growth, reduce total bacteria and improve fish health. According to Acar *et al*. [80] 0.1%, 0.3% or 0.5% oil extracted from sweet orange peel (Citrus sinensis) enhanced growth rate of tilapia (O. mossambicus) and disease resistance against *S. iniae*. Hassanin *et al*. [33, 81] also reported that ginger (Z. officinale) supplementation of *O. niloticus* protected against pathogenic strain of *A. hydrophila* in aquaculture.

Conclusion

Nutrition is one of the most important factors influencing the ability of cultured tilapia to exhibit its genetic potential for growth and it is greatly influenced by factors such as behavior of fish, stocking density, quality of feed, daily ration size, feed frequency and others. In addition to the above factors, use of functional feed additives in tilapia nutrition improve feed conversion ratio, improve the digestion of dietary components, boosts immune system, binds toxic substances in the gastrointestinal tract, thereby reducing overall bioavailability to the bloodstream and functional feed additive like prebiotics modulate the gut microbiota.

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