World Journal of Fish and Marine Sciences 4 (1): 87-101, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.01.56264

A Perspective on the Ingestion and Nutritional Effects of Feed Additives in Farmed Fish Species

O.O. Ajiboye, A.F. Yakubu and T.E. Adams

Nigerian Institute for Oceanography and Marine Research, Sapele, Delta State, Nigeria

Abstract: The use of feed additives as natural growth promoters has received considerable attention in the aquaculture industry. Besides, the utilization of antibiotics, antioxidants, feed stimulants, feed colourants as well as the use of hormones has been well established by several authors. However, the absorption or ingestion rate of the widely used feed additives by cultured fish species has not been thoroughly quantified and researched in fish nutrition while on the other hand; further investigation needs to be focused on the nutritional effects of the commonly used feed additives in the aquaculture industry. In spite of the growing interest and success obtained using feed additives as supplements in farmed fish diets, several physical anomalies or alterations in the normal development of farmed fish species have been reported by several authors. The present review therefore emphasizes on new areas of further research to improve growth, feed utilization, absorption or ingestion rates and disease resistance in cultured fish species fed feed additives. Special emphasis is focused on the elucidation of the biochemical contents and dietary supplementation levels of feed additives by cultured fish species which has received relatively little attention in the aquaculture industry. The development of a cost-effective new natural source of antioxidant; the development of algal meal-based diets as feed additives; adoption of a reliable and cost-effective method to quantify the absorption or ingestion rate in cultured fish species as well as the utilization and adoption of acidifiers consisting of organic acids and their salts as a potential replacement for antibiotic growth promoters are recommended as real and achievable research goals for future.

Key words: Feed Additives % Ingestion % Cultured Fish % Fish Growth % Nutrition % Immunity

INTRODUCTION

The active search for a wide variety of feed additives as natural growth promoters is starting to extend into fish farming where continued consumer pressure may bring about a ban on antibiotics during production in most countries [1-3]. On the other hand, the use of feed additives in the aquaculture industry has received considerable attention in recent years. The rationale behind their dietary use has been to preserve the nutritional characteristics of a diet or feed ingredient prior to feeding (i.e. antioxidants and mould inhibitors) and at the same time by enhancing ingredient dispersion or feed pelleting (i.e. emulsifiers, stabilizers and binders), facilitating feed ingestion and consumer acceptance of the product (i.e. feed stimulants and food colourants) thereby promoting growth (i.e. growth promoters, including antibiotics and hormones) and at the same time supplying essential nutrients in purified form (i.e. vitamins, minerals, amino acids, cholesterol and phospholipids).

The fishes need aliments to lead their lives, keep their health and grow [4]. Similar to the other animals, basic aliments such as proteins, fats, carbohydrates as well as vitamins for sufficient and balanced nutrition are indispensable in the diets of farmed fish species. The use of feed additives has proven to be successful in aquaculture and its nutritional effects on fish culture (as will be discussed in this review) is of great concern to fish nutritionists. However, with the growth of fish culturing, it is necessary to search for additives that can stimulate effective growth, but without affecting the environment, as occurs with antibiotics [2, 5 and 6]. Besides, feed additives that could enhance the immune systems of farmed fish species, promotes growth, facilitates ingestion

Corresponding Author: Ajiboye, Olufemi Oluseun, Nigerian Institute for Oceanography and Marine Research, P.M.B. 4015, Sapele, Delta State, Nigeria.
Tel: +234 8036993337.

rates, attains the desired flesh and skin pigmentation, as well as improving the organoleptic properties of the farmed product without having negative effects to both the farmed fish species and the farming environment is of paramount importance in fish culture.

In an attempt to gain further insight into the ingestion and nutritional effects of feed additives in farmed fish species, this present review emphasizes on low feed attractability and palatability; the nutritional components of aquafeeds; dietary factors such as dietary phospholipids (PL) or lecithin, which are the major factors responsible for the poor ingestion rates and physical anomalies reported in some farmed fish species (Table 1) and need to be further investigated from a nutritional point of view. Special emphasis is also made on the biochemical contents and dietary supplementation levels of feed additives as well as the interactions between nutrition and immunity of cultured fish species on feed additives as will be discussed in this review.

Ingestion of Feed Additives in Cultured Fish Species: The poor growth performance in some fish species could be attributed to the low palatability of the diets. For example, high inclusion of low-cost vegetable protein sources, such as soybean meal, in shrimp diets is often linked with poor performance and this has been attributed to decreased feed consumption led by low feed attractability and palatability [7]. In spite of the fact that meat and bone meal (MBM) has been successfully used to partially or totally replace fish meal (FM) in the diets for some fish [8,9] low palatability is also linked with poor performance in some fish species fed diets containing MBM [10,11]. From a nutritional point of view, low feed attractability and palatability could be responsible for the poor ingestion rates in some fish species. Furthermore, some off-flavoured feed ingredients or additives reduced the feed intake of salmonids, as reported with drugs [12, 13] or oxidized oil [14]. Early on, researchers realized that the poor performance of micro-diet (MD) is related to the variable acceptance and attraction of the inert particle for the larvae compounded by inadequate ingestion, digestion and assimilation [15]. Besides the palatability of diets and feeding rate, the nutritional components of aquafeeds are also of paramount importance to the growth performance of the farmed fish species. Thus, formulation of a diet that meets up with the nutrient requirements of the cultured fish species is imperative for the rapid growth rate and survival of farmed fish species.

It is worthy to note that dietary factors could also be responsible for the low palatability of fish diets and this has relatively contributed to the poor ingestion rates in some farmed fish species. In view of this, the incorporation of feedstuffs that could be well ingested and assimilated by cultured fish species has a pivotal role to play in the optimum performance of farmed fish species. Besides, the beneficial effect of dietary phospholipids (PL), such as lecithin, on growth has been demonstrated in the larvae and juveniles of a number of marine species [16-18]. For example, the effect of dietary phosphatidylcholine (PC) and its constituent medium-chain fatty acids on micro-diet ingestion (µg diet larva^{-1} h^{-1}) and the absorption rate of the free fatty acid

Table 1: Anomaliies in cultured fish species fed feed additives

Anomalies	Species	Reference
• Skeletal deformity	*Hippoglossus hippoglossus*	[38]
• Exudative diathesis	*Ictalurus punctatus*	[39]
	Onchorhynchus mykiss	[40]
• Growth depression,	*Onchorhynchus mykiss*	[81]
• Microcytic anaemia		
• Liver lipoid degeneration		
• Low red blood cell numbers,	*Onchorhynchus mykiss*	[82]
• Haemoglobin content		
• Haematocrit		
• Increased haemolyis		
• Polychromatocyte development		
• Splenic haemosiderosis		
• Hepatic ceroidosis		
• Scoliosis (spanning the cephalic and prehemal regions and the anterior hemal region of the vertebral column)	*Hippoglossus hippoglossus*	[38]
• Histological lesion	*Ictalurus punctatus*	[39]
	Onchorhynchus mykiss	[40]

Table 2: Dietary feed attractants for cultured fish species

Feed attractants	Species	Reference
(Glycine betaine, trimethylglycine)	Red sea bream (*Chrysophyrys major*)	[20]
	Dover sole (*Solea solea*)	[21]
	European eel (*Anguilla anguilla*)	[22]
(Free amino acids + glycine betaine)	Rainbow trout (*Oncorhynchus mykiss*)	[24] Beklevik and Polat (unpublished data)
	European eel (*Anguilla anguilla*)	[22]
	Japanese eel (*Anguilla japonicus*)	[25]
	Sea bass (*Dicentrarchus labrax*)	[26]
	Red sea bream (*Chrysophyrys major*)	[20]
	Dover sole (*Solea solea*)	[21]
	Puffer (*Fugu pardalis*)	[27]

[^{14}C] 16:0 (pmole larva^{-1} h^{-1}) was tested on 15, 20, 21, 25, 26, 30 and 31-day-old gilthead sea bream, *Sparus aurata* L. larvae by [18]. The research findings [18] showed that larvae from one or both of the PC micro-diets demonstrated significantly ($P < 0.05$) higher ingestion rates (µg diet larva^{-1} h^{-1}) than the non-PL micro-diet control in 15, 21, 22, 25 and 26-day-old larvae and the *Artemia* PL micro-diet in 15, 22 and 26-day-old larvae. This suggests that PC stimulates feeding by acting as a feed attractant. Therefore, dietary supplementation of phospholipids that could act as feed attractants in the stimulation of feeding in cultured fish species need to be given more attention in aquaculture and need to be further investigated. Also, the absorption or ingestion rate of dietary phospholipids in cultured fish species is of paramount importance in fish nutrition. Generally, the absorption or ingestion rate of aquafeeds by cultured fish species has not been thoroughly quantified and researched and thus need to be well established in fish nutrition. On the other hand, adoption of a reliable and cost-effective method to quantify the ingestion rate in cultured fish species need to be given more attention in fish nutrition.

Supplementation of a trace amount of feed stimulants or attractants that could promote feed intake, supply additional nutrients for protein and energy metabolism with the aim of enhancing the growth performance and ingestion rates of farmed fish species is indispensable in aquaculture. Betaine (glycine betaine, trimethylglycine) and free amino acids (L-alanine, L-glutamic acid, L-arginine, glycine, inosine) are widely used as dietary feeding attractants for many fish species (Table 2). Some researchers have suggested that dietary free amino acids may be responsible for stimulating feeding behaviour or affecting hormones that control the release of digestive enzymes into the lumen of the gut [19]. Betaine, a highly water soluble and diffusible compound which has the ability to stimulate the olfactory bulb of fish is now widely used as dietary feed attractants in fish culture. Betaine has been shown to act as a dietary feeding attractant in some fish species such as red sea bream, *Chrysophyrys major* [20], dover sole, *Solea solea* [21] and European eel, *Anguilla anguilla* [22]. In a study conducted by Beklevik and Polat (unpublished data) to investigate the effects of betaine and alanine as dietary feeding attractants (at 1.5% of the total diet) on the growth and carcass composition of rainbow trout (*Onchorhynchus mykiss*), a significant improvement in ingestion rate and increased growth in rainbow trout fed diet supplemented with traces amount of betaine as a feeding attractant and growth promoter was reported. Similarly, L-alanine, L-glutamic acid, L-arginine and glycine are also highly water soluble and diffusible compound, like betaine and have been reported to have dietary attractant properties. According to [23], protein-rich ingredients of marine origin, such as fishmeal and squid meal contain these substances which are feeding effectors for shrimp. In a study conducted by [7], the use of fishmeal-Brazilian origin (FMBO) and fishmeal-Peruvian origin (FMPO) confirm the superior nutritional ability of marine fishmeal to stimulate white shrimp, *Litopenaeus vannamei* chemoreceptors. On the other hand, various studies conducted using mixtures of dietary free amino acids (L-alanine, L-glutamic acid, L-arginine and glycine) with glycine betaine or inosine, as feeding stimulants (Table 2) in rainbow trout, *O. mykiss* [24], European eel, *A. anguilla* [22], Japanese eel, *A. japonicus* [25], sea bass, *Dicentrarchus labrax* [26], red sea bream, *C. major* [20], Dover sole, *S. solea* [21] and puffer, *Fugu pardalis* [27] showed that they are more effective than individually free amino acids. Evidences that mixtures can be more stimulatory than individual or single compounds by either additive or synergistic interactions, has accumulated in both behavioural and electrophysiological studies [28]. However, the bioactive compounds in these dietary free amino acids could be responsible for the differences in their stimulatory actions in cultured fish species. In view of these reasons, future research effort should focus more on the bioactive compounds in the dietary feed stimulants commonly used as feed additives in fish diet. Concordantly, elucidation of the attractive properties of the free amino acids commonly used as feed attractants in fish culture is also of paramount importance in fish nutrition.

Furthermore, researchers must be aware of the fact that the ingestion of aquafeeds supplemented with traces amount of feed stimulants or feed attractants as feed additives would depend on whether the fish species in question is mainly a visual feeder or a chemosensory feeder. For example, cultured marine fish species generally rely on sight to locate their food. Also, they rely on chemoreceptors located in the mouth or externally on appendages (lips, barbells, fins) to 'sense' their food before ingestion. Thus, more emphasis should therefore be placed on this area. According to [29], the practical importance of feed attractants and diet palatability is particularly critical during the weaning of cultured marine fish larvae from a live to a non-living diet. This is due to the fact that the feeding of most cultured marine fish species still relies on live feeds such as *Artemia* and rotifers during the early life stages and thus need to be given more attention in marine larviculture. Besides, it must be well noted that nutrition at first feeding is a critical factor affecting the growth performance of most cultured marine fish species. In view of this, further research work need to be conducted on this area if we seriously aim at harvesting marine fish species at marketable size.

Fish Growth and Yield of Cultured Fish Species on Feed Additives: Feed additives make the bulk of chemicals used in animal production, thus representing a major issue for safety of foods of animal origin [2]. Besides, compounds intended for deliberate addition/use in animal feed should have a proven efficacy, should be safe for animals and consumers at the intended dose levels [2]. Routine use of antibiotics as growth promoters is a matter of debate in the animal farming industry. The use of low levels of these antibiotics in animal feeds possesses the possibility to transfer bacterial immunity to species pathogenic in animals and humans [5]. However, [3] reported that the worldwide search for alternatives to antibiotic growth promoters (AGP) has resulted in a variety of different strategies.

The use of feed additives as growth promoters is gaining more attention in the field of aquaculture. However, further research need to be conducted to investigate the nutritional effect of these feed additives in farmed fish species. A recent study conducted [4] to determine the effects of commercial diet supplemented with a trace amounts of Mannanoligosaccharide (MOS) and Vitamin B_{12} on the growth and body composition of the carp (*Cyprinus carpio*) showed that the best live weight was recorded in the group fed MOS and vitamin B_{12} combination in group C (4.51±0.13g) while the best growth in terms of length was recorded in the group fed MOS in group A (6.68±0.08cm). These reported findings are in agreement with [30] for the effects of the nutritional MOS on immune function and growth of the salmon and carp fries. Concordantly, [31] reported MOS supplemented with feeds leads to substantial development in weight and feed assessment rates as well as decrease in the mortality rates of carp fries. Also, a study conducted by [32] to determine the effects of MOS on the growth performance and feed assessment for juvenile sea bass (*D. labrax*) indicated that growth and specific growth is about 100% higher when compared with the control group. On the other hand, a recent study conducted [33] on cultured shrimp *L. vannamei* juveniles showed that the assayed doses of microencapsulated bovine insulin administered to shrimp as a feed additive affects mainly the metabolism of carbohydrates and proteins, rather than acting as a growth hormone. Besides, it is worth noting from a nutritional point of view that one of the main classic anabolic roles of the hormone insulin is to increase protein synthesis. Also, insulin and insulin-like peptides (ILPs) in crustaceans have been found to increase glucose uptake and glycogen synthesis in different tissues such as hepatopancreas and gills [34-36]. Despite all the significant progress in the use of insulin or ILPs either *in vitro* or injected into animals, their nutritional effect as growth hormone has not been elucidated.

Biochemical Contents and Dietary Supplementation Levels of Feed Additives: In spite of the fact that increasing attention has been focused on the use of feed additives as growth promoters in farmed fish species, relatively little attention has been focused on the nutritional effect of the biochemical contents of the commonly used feed additives fed farmed fish species. From a nutritional point of view, the nutritional effects of the biochemical contents of feed additives used as growth promoters and their dietary supplementation levels in cultured fish species need to be further investigated or explored. For example, when a lipid-oxidized diet was fed to fish, various detrimental symptoms of fish, such as reducing growth performance [37], skeletal deformity [38], exudative diathesis [39] and histological lesion [39,40] were reported (Table 1). According to [41], a high lipid diet is commonly supplied to fish at fish farms to achieve effective fish production rather than a high protein or carbohydrate diet in terms of saving feed and fish production costs and lowering the source of water pollution. Besides, it is worth noting that aquafeeds with high lipid content gets oxidized during storage. Concordantly, fish fed aquafeed with high lipid content are prone to deterioration in terms of quality due to lipid oxidation during storage. In view of these reasons, future research efforts should explore the potentials of antioxidant as a supplement to feed additives to prevent the lipid oxidation of feed.

Synthetic antioxidants have been widely used to retard lipid oxidation in fish and fish diet and their importance has been reported [42, 43]. On the other hand, animal fed diet containing high concentration of synthetic antioxidants displayed suppressed growth performance, histopathology and hepatomegaly [44, 45]. Based on these reasons, the development of a cost-effective natural source of antioxidant that could retard lipid oxidation in fish and which does not deteriorate the quality of the fish is of paramount importance in aquaculture. Interestingly, the potentials of new natural source of antioxidants such as green tea [46-48], fig (*Ficus carica*) containing polyphenols [49-51] and Haeroc product [41] has attracted international attention due to its antioxidative properties (Table 3). However, despite the high potentials of these natural sources of antioxidant for aquaculture, the elucidation of the active ingredients in the antioxidants is not thoroughly investigated. For example, deterioration of weight gain and specific growth rate of fish (olive flounder) fed the antitox (AT) diet containing a commercially available antioxidant, antitox (as shown in Table 3), in a recent study conducted by [41] conflicted with the study showing that dietary inclusion of 200ppm butylated hydroxyanisole (BHA) in the moist pellet did not affect the growth of olive flounder [52]. This might indicate that the commonly used antioxidants contain some organic acids or bioactive compounds which thus need further investigation. For example, fig was known to contain some organic acids such as citric, succinic and tartaric acids [53, 54] and dietary supplementation of some organic acids, such as citric, metacetonic and acetic acids improved the storage time and feed utilization [55-56]. However, the effects of these organic acids and other bioactive compounds on the growth performance of cultured fish species fed feed additives need to be thoroughly explored and moreover, their concentrations in aquafeeds must be adjusted accordingly. According to [57], dietary inclusion of citric acid at a concentration of 10^{-6}-10^{-2} had stimulatory effect on feeding behavior of *Tilapia nilotica*. Besides the antioxidative activity of feed additives or antioxidants used widely as growth promoters in aquaculture, their biochemical composition which has received relatively little attention in aquaculture need to be elucidated. Based on research findings reported by [58] on the evaluation of the antioxidative activity of propolis (a plant extract made by bees) samples collected in four regions of Iran, differences in terms of composition and capacity were observed. [6] also reported that the composition of propolis varies due to several factors and so does its biological activity.

Table 3: Survival (%), weight gain (g fish^{-1}) and specific growth rate (SGR) of olive flounder fed the experimental diets containing various sources of green tea for 8 weeks

Sources	Initial weight (g fish^{-1})	Final weight (g fish^{-1})	Survival (%)	Weight gain(g fish^{-1})	SGR*
CT	12.7 ± 0.16	40.9 ± 0.45	100 ± 0.00^{a}	28.1 ± 0.57^{ab}	2.4 ± 0.05^{a}
AT	12.9 ± 0.02	38.8 ± 0.24	99.2 ± 0.83^{a}	25.9 ± 0.21^{ab}	2.3 ± 0.01^{b}
GE	13.0 ± 0.02	42.8 ± 0.55	100 ± 0.00^{a}	29.8 ± 0.56^{a}	2.5 ± 0.03^{a}
FE	12.8 ± 0.11	43.0 ± 0.89	99.2 ± 0.83^{a}	30.2 ± 0.86^{a}	2.5 ± 0.04^{a}
HR	12.9 ± 0.05	37.2 ± 1.23	98.3 ± 0.83^{a}	24.3 ± 1.18^{b}	2.2 ± 0.06^{b}
BG	12.9 ± 0.08	37.5 ± 0.53	100 ± 0.00^{a}	24.6 ± 0.58^{b}	2.2 ± 0.04^{b}

Values (mean ± SE) in the same column sharing the same superscript letter are not significantly different (P<0.05).

*SGR = (In final weight of fish - In initial weight of fish) x 100/days of feeding trial.

CT, control; AT, antitox; GE, green tea extract; FE, fig extract; HR, Haeroc product; BG, by-product of green tea. (c) [41]

According to [6], the large number of propolis bioactive components is responsible for the antiviral, antifungal, antimicrobial, antiprotozoal properties, as well as for immune modulatory and anti-inflammatory activities and could therefore be attributed to the improved performance of Nile tilapia fingerlings. More research work is therefore needed on this area and most especially on the effectiveness of the feed additives in the several growing phases of the Nile tilapia culture under several sanitary and handling challenges.

It is worth noting that the optimum dietary supplementation levels of feed additives and its effects on growth performance may be different among fish species and thus need to be further investigated. For example, in a study conducted by [59] on Korean rockfish (*Sebastes schlegeli*), the growth performance of the species tended to decrease as dietary supplementation of *Chlorella* powder increased to 1.5% or higher. On the other hand, research findings reported by [60] indicated that dietary supplementation of 5% *Chlorella* meal increased weight gain and feed efficiency ratio in nibbler. Further, a study conducted by [59] indicated that high level of *chlorella* powder gave negative effects on growth and feed utilization of rockfish (*S. schlegeli*). Besides, parameters such as the mean final total length, standard length, head length, carcass yield with and without heads of Nile tilapia fingerlings feed with increasing levels of BPE did not vary significantly with an increase of BPE levels added to the feed ($P > 0.05$) as reported by [6]. Based on these research findings, it might indicate that the optimum dietary supplementation levels for the positive effects on growth performance may be different among fish species. Similarly, data for the optimum dietary supplementation levels of feed additives for entirely different species should not be extrapolated for other species since the optimum dietary supplementation levels may be different among fish species. Future research should also focus more on the effects of the dietary supplementation levels on the physiological condition of the farmed fish species.

Macro and micro-algae, such as *Ascophyllum*, *Laminaria*, *Undaria*, *Porphyra*, *Ulva*, *Spirulina* and *Chlorella*, as feed additives have been reported to improve growth performance of farmed fish species [60-62]. However, the biochemical contents of these algae and their nutritional effect on cultured fish species need to be thoroughly researched or explored in aquaculture. Among these algae, *Chlorella* sp. is one of the most common microalgae used widely in aquaculture and it contains high levels of protein, pigments, vitamins, minerals and unknown *Chlorella* growth factors [63]. Dietary supplementation of *Ascophyllum* and *Spirulina* as feed additive to red sea bream, *Pagrus major*, produced remarkable effects on growth, feed utilization and protein deposition [64]. However, supplementation of fish feed with *Spirulina* at a high level might produce physiologically negative effects [62]. In a study conducted by [59], dietary supplementation of 0.5% *Chlorella* powder improved the growth performance of juvenile Korean rockfish (*S. schlegeli*) (Table 4). Despite the fact that it is generally believed that *Chlorella* is an excellent protein source, there is no evidence to prove the efficacy of *Chlorella* powder as the protein source in fish culture. This might indicate that the biochemical contents of some of the widely used feed additives in aquaculture need to be thoroughly researched and thus need to be examined if we seriously aim at utilizing feed additives as growth promoters in aquaculture.

Interactions Between Nutrition and Immunity of Cultured Fish Species on Feed Additives: Researchers need to be aware of the fact that an understanding of the immune system of farmed fish species is indispensable in fish nutrition most especially in the use of feed additives as growth promoters in fish diet. Besides, the use of antibiotics to treat illness, prevent infections or promote growth in animal production is not a new phenomenon. However, concerns on the part of the scientific community and general public about development of antibiotic-resistant bacterial strains are strongly challenging the use of antibiotics in agriculture [1]. According to [65], these concerns have been heightened by the occasional cases of human death from antibiotic-resistant bacteria reported in recent years. Nevertheless, the development of alternatives to antibiotics that may keep fish healthy such as probiotics, prebiotics and immunomodulators is receiving considerable attention in fish nutrition as well as aquafeed research [66, 67].

The use of feed additive for growth and health management of cultured fish species is widely used in aquaculture. However, the interactions between nutrition and immunity of cultured fish species on feed additives are not thoroughly investigated. For example, a recent research findings [6] on the effects of the increasing supplementation of feed with brown propolis extract (BPE) as a growth promoter in Nile tilapia (*Oreochromis niloticus*) fingerling feeds showed that the level of best performance parameters was 2.22g KgG^{1}, between the levels of 1.83 and 2.74g BPE KgG^{1} feed inclusion (Table 5). The research finding agrees with the results presented by [68], who observed a significant effect of

Table 4: Weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), hepatosomatic index (HSI), visceral index (VI), condition factor (CF) and survival of juvenile Korean rockfish fed six experimental diets supplemented with *Chlorella* powder (CHP) at 0, 0.5, 1.0, 1.5, 2.0 and 4.0% for 12 weeks[1]

	Diets[2]						
	CHP_0	$CHP_{0.5}$	$CHP_{1.0}$	$CHP_{1.5}$	$CHP_{2.0}$	$CHP_{4.0}$	Pooled SEM[3]
WG (%)[4]	1054^b	1094^a	$1062^{a,b}$	1034^b	1039^b	1037^b	6.14
SGR (%)[5]	2.91^b	2.95^b	$2.92^{a,b}$	2.89^b	2.90^b	2.89^b	0.01
FCR[6]	97.9^c	102.3^a	100.0^b	$98.2^{b,c}$	$98.1^{b,c}$	$99.0^{b,c}$	0.44
PER[7]	1.96^b	2.05^a	2.00^b	1.96^b	1.98^b	1.98^b	0.01
HSI (%)[8]	2.88	2.89	2.81	2.83	3.1^2	3.05	0.07
VI (%)[9]	14.7	14.1	14.3	14.8	13.8	13.7	0.30
CF[10]	1.83	1.87	1.83	1.90	1.90	1.87	0.01
Survival (%)	100	100	100	100	0.00	100	100

[1]Means of triplicate groups; values in the same row with different superscripts are significantly different ($P < 0.05$).

[2]*Chlorella* powder.

[3]Pooled standard error of mean: SD/v*n*.

[4]Weight gain :[(final weight - initial weight)/initial weight] x 100.

[5]Feed conversion ratio: (dry feed intake/wet weight gain) x 100.

[6]Specific growth rate: [(In_e final weight - In_e initial weight)/feeding days] x 100.

[7]Protein efficiency ratio: wet weight gain/protein intake.

[8]Hepatosomatic index: (liver weight/body weight) x 100.

[9]Visceral index: (visceral weight/body weight) x 100.

[10]Condition factor: [wet weight (g)/body length $(cm)^3$] x 100.© [59]

Table 5: Mean final length and carcass yield of Nile tilapia fingerlings fed with increasing levels of brown propolis extract into feed (BPE)

Treatment*	Total length (cm)†	Standard length (cm)†	Head length (cm) †	Carcass yield with head (%)†	Carcass yield without head (%)†
0.00	10.02	8.06	2.62	90.09	64.43
0.91	10.35	8.30	2.65	89.63	63.11
1.83	10.23	8.17	2.58	89.70	62.48
2.74	10.22	8.21	2.62	89.13	61.67
3.65	10.25	8.25	2.61	89.13	62.11
VC	2.97	2.74	4.09	1.94	2.92

*BPE g kgG[1] feed.

†Not significant.

(c) [6]

Table 6: Performance of hybrid striped bass fed diets containing various levels of lavamisole for 3 weeks in feeding trial 1[a]

Lavamisole (mg/kg)	Growth (% increase)[b]	Feed efficiency (g gain/g feed)	Survival (%)
0	50.2^y	0.84^{yz}	97.6
100	55.2^x	0.93^x	97.6
250	53.4^{xy}	0.90^x	100
500	52.4^{xy}	0.87^{xy}	97.6
1000	46.6^z	0.78^{yz}	97.6
P[c]	0.014	0.004	0.903
Pooled S.E	1.412	0.020	2.128

[a] Values represent means of five replicate groups. Values in a column that do not have the same superscript are significantly different at P = 0.05 based on Duncan's multiple range test.

[b] Fish initially weighed 40.6±0.6 (mean±0.6) g each.

[c] Significance probability associated with the *F* statistic.

(C) [78].

propolis extract on quails. In contrast, [69] and [70] reported no significant difference between broilers fed diets supplemented with propolis extract and control diets. In view of these research findings, it is interesting to note that the immune response in animals could be responsible for their growth performances. Similarly, it needs to be well emphasized that the immune response in fish fed growth promoters [71,72] differs from that of mammals [71] and thus need to be further investigated. Besides, [72] reported that propolis may stimulate the immune response of carps (*Carassius auratus gibelio*) against *Aeromonas hydrophila*.

Although the research findings reported by [6] showed a beneficial effect when BPE was added to feed as a growth promoter (Table 5); however, an understanding of immune system of cultured fish species is imperative in the use of feed additives as growth promoters and thus need to be given more attention in aquaculture. In addition, the significant reports on its use (BPE) in other animal species [68, 71] suggest that it may have a significant impact on fish culture, mainly in the initial phases of Nile tilapia culture [6]. Based on these reasons, a comprehensive research work is therefore needed in this area to elucidate the use of propolis and other growth promoters that could be of good potential as feed additive as well as health promoters in the several growing phases of the Nile tilapia culture and other farmed fish species. Lavamisole (a potent immunostimulant) is also widely used in aquaculture. It was the first drug reported to increase the functions of cellular immunity of healthy laboratory animals [73]. This phenomenon was confirmed with rainbow trout in the late 1980s [74]. Besides the immunostimulating influence and potential use in fish health management, lavamisole was also recognized to enhance growth of gilthead sea bream [75], carp [76] and several terrestrial animals [77]. In a study conducted by [78] to evaluate the effects of dietary lavamisole on growth performance, innate and adaptive immunity and resistance of hybrid striped bass to *Streptococcus iniae* and *A. hydrophila* infection, juvenile hybrid striped bass fed low doses of lavamisole (100-250 mg/kg) significantly enhanced feed efficiency and growth by approximately 10% after 3 weeks of feeding (Table 6). On the other hand, previous research work conducted on hybrid striped bass [79] indicated that excessive dietary lavamisole suppressed their growth performance. In view of these reasons, fish farmers as well as researchers need to be aware of the fact that the use of feed additives that is safe for fish species at the intended dose levels and at the same time ensures that possible residues in animal products would not pose any appreciable risk to consumers is of paramount importance in fish culture. In spite of the fact that lavamisole is one of the most widely used immunostimulants for aquaculture which also has been shown to enhance growth of carp [76] and gilthead seabream [75], further research is also needed to explore the nutritional effects of other immunostimulants on growth performance of cultured fish species.

Researchable Issues to Enhance Positive Effects of Feed Additives in Cultured Fish Species: In the final analysis, it is very important to stress the following real and achievable research goals for future if we seriously aim at promoting positive effects of feed additives in aquaculture:

The Development of a Cost-Effective New Natural Source of Antioxidant: Because most marine fish require food organisms which have relatively high concentrations of the long chain, n-3 polyunsaturated or essential fatty acids (EFA) such as 20:5n-3, Eicosapentaenoic Acid (EPA) and 22:6n-3, Docosahexaenoic Acid (DHA), fish oil or squid liver oil containing high content of n-3 highly unsaturated fatty acids (HUFA) is commonly included in fish diet. According to estimates, the global aquafeed industry currently uses approximately 87% of the global supply of fish oil as a lipid source [80]. Besides the development of a nutritionally balanced formulated diet and its pivotal role to the success of any aquaculture activity, it is also very important to note that the dietary requirement for lipid varies widely among species and life stages and thus need to be well examined. However, fish fed on high lipid diet are likely to produce high body lipid content, because the dietary surplus lipid is stored in body lipid after utilization [41] and moreover, the fish is prone to deterioration in terms of quality due to the fact that lipid oxidation takes place during storage. The high content of n-3 highly unsaturated fatty acids (HUFA) in fish diet is readily susceptible to oxidation due to the high number of unsaturated carbon-carbon bonds in the fatty acids. [81] reported growth depression, microcytic anaemia and liver lipoid degeneration in 13g rainbow trout fed a rancid diet. Low red blood cell numbers, haemoglobin content and haematocrit and increased haemolysis, polychromatocyte development, splenic haemosiderosis and hepatic ceroidosis have also been observed in rainbow trout fed highly or extremely oxidized oils [82] (as shown in Table 1). Scoliosis, spanning the cephalic/prehemal regions and the anterior hemal region of the vertebral column were recorded in juvenile Atlantic halibut which increased in frequency as the level of dietary oxidized lipid increased [38].

To retard lipid oxidation in fish and fish diet, synthetic antioxidants have been widely used and their importance has been reported [42, 43]. However, despite the inclusion of synthetic antioxidants to prevent lipid oxidation in fish and fish diet, alterations in the normal growth performance of fish species fed diet containing high concentration of synthetic antioxidants has been reported [44,45]. In view of this, the search for a new natural source of antioxidant that does not deteriorate the growth performance of farmed fish species is of paramount importance in fish culture. It is also very important to note that the preparation of feedstuff rations rich in polyunsaturated fatty acids (PUFA) without the inclusion of these natural source of antioxidant are highly prone to oxidative decomposition (oxidative rancidity) which in turn may cause a reduction in the nutritive value of the constituent lipids, protein and vitamins.

Antitox, "-tocopherol, butylated hydroxyanisole (BHA), green tea and its extract, Haeroc product, fig (*F. carica*) containing polyphenols and fig extract have gained more attention as natural source of antioxidants in the aquaculture industry due to its antioxidative properties [41,50]. On the other hand, little attention has been focused on the investigation of the bioactive compounds in these natural antioxidants and thus need to be explored. It is worth noting that these antioxidants contain bioactive compounds (as already discussed in this review) and moreover, it is very important to note that the bioactive compounds in the natural source of antioxidants used in the aquaculture industry have a significant role to play in reducing lipid oxidation in fish diet. For example, crude tea containing catechins has been reported to be more effective in reducing lipid oxidation than "-tocopherol or butylated hydroxyanisole [83]. On the other hand, a possibility of the application of mixtures of these natural antioxidants and its effect on farmed fish species need to be investigated from a nutritional point of view. For example, in a recent study conducted by [41], deterioration of weight gain of fish fed Haeroc product diet containing a mixture of fig and green tea extracts and green tea powder compared with that of fish fed green tea extract and fig extract diets in the study might indicate that green tea extract or fig extract was solely effective to improve the growth of fish, but their mixture with green tea powder was not (Table 3). Besides, fig (*F. carica*) containing polyphenols [49] is reported to have antioxidant effect [50, 51]. Green tea extract is also useful as dietary additives and it has been reported to improve the growth and body composition of juvenile olive flounder *Paralichthys olivaceus* [41] (as shown in Table 3). However, the potentials of the mixtures of the commonly used antioxidants and its nutritional effects on cultured fish species are not thoroughly investigated and need to be well examined. Future research efforts should focus more on this area and emphasis should be placed on the practicality, reliability and cost-effectiveness of using natural antioxidants as feed additives. Moreover, the use of natural source of antioxidants in commercial settings is rare. Thus, conscious efforts need to be made to upscale the production of natural antioxidants to commercial levels. This would ensure production of a reliable, continuous supply of antioxidants on a large scale. Also, the appropriate technology needed in the production of cost-effective natural antioxidants is still in the research stage. Intensive research is therefore needed in the development of the most appropriate production techniques that will promote the practicality and cost-effectiveness of using natural antioxidants in the aquaculture industry.

The Development of Algal Meal-Based Diets as Feed Additives: The efficacy of algae as a feed additive has been confirmed in fish [59, 61, 64]. It has also been confirmed that small amounts of algae added to fish feed exert pronounced effects on growth [61], lipid metabolism [84, 85] and disease resistance [86]. However, research on algal meal-based diets as feed additives is fragmentary and is still in the research stage. In spite of the fact that the application of an inexpensive and locally available algal meal-based diet as feed additives is well established in aquaculture, however, its use in commercial settings is rare. Thus, more attention should be focused on the production of a reliable, continuous supply of cost-effective algal meal-based diets on a large scale.

If we seriously aim at developing algal-meal based diets that would promote positive effects on growth, feed utilization and body composition of cultured fish species; nutrient digestibility by farmed fish species should be given more attention in aquaculture and thus need to be well examined. It has been observed that most nutritionists or fish farmers based the nutritional value of a feed or feedstuff solely on its chemical composition instead of the amount of the nutrients or energy the fish can absorb and use [87]. Thus, further studies should be conducted to ascertain the digestibility of the major feed ingredients widely used in aquafeeds. For instance, the effect of dietary inclusion of various sources of additives such as antitiox (AT), green tea extract (GE), fig extract (FE), Haeroc product (HR) and by product of green tea (BG) as well as the control (CT) without antioxidant, on growth and body composition of juvenile olive flounder *P. olivaceus* [46] showed that the weight gain of fish fed the GE and FE diets was significantly ($P < 0.05$) higher than that of fish fed the HR and BG diets, but not significantly ($P > 0.05$) different from that of fish fed the CT and AT diets (as shown in Table 3). This therefore shows that a thorough study of the digestibility coefficients of the local raw materials used in feed formulation as feed additives for farmed fish species needs to be investigated. Additionally, it must be well noted that digestibility is one of the major factors that affects the efficiency of feed utilization.

Besides the digestibility coefficients of the local raw materials used in the aquaculture industry, the nutritional requirements of the cultured fish species need to be elucidated. Moreover, the possession of enzyme in given species is a critical factor that can enhance digestibility of a number of nutrients in aquafeeds [87]. It is evident that many of the differences observed between species, with respect to the digestibility of a given ingredient or nutrient, are a function of whether a given species possess an endogenous source of a digestive enzyme. For example, the result of the experiment confirmed from the gut analysis of *Tilapia guineensis* shows that the stomach pH values are extremely low; 1.0-3.7 with 75% of the observations being less than 2.0. Besides, a pH value of less than 2 will considerably help in the digestion of algae and bacteria [88]. In view of this, there is a need for further research in biological engineering for production of enzymes on a commercial scale. This will enhance the digestibility of a number of nutrients in the widely used feed additives of cultured fish species.

Utilization and Adoption of Acidifiers as a Potential Replacement for Antibiotic Growth Promoters: Acidifiers consisting of organic acids and their salts present a promising alternative and they have received much attention as a potential replacement for antibiotic growth promoters (AGP) in order to improve growth, feed utilization and disease resistance in fish. In aquaculture, the use of acidifiers has a long history [3]. Besides, several studies have been conducted with different tropical species including herbivorous filter feeders (tilapia) and omnivorous fish (sea bream, carp, catfish). In animal nutrition, acidifiers exert their effects on performance via three different ways [89]: (a) in the feed; (b) in the gastro-intestinal tract of the animal; and (c) due to effects on the animal's metabolism. Acid preservation of fish and fish viscera to produce fish silage is common practice and is widely used in fish feeds with reported beneficial effects [90, 91] and thus attracted attention from the scientific community.

It is also very important to note that the concentrations of the commonly used organic acids as acidifiers in aquafeeds need to be adjusted accordingly. For example, it was evident that fish tended to avoid acetic acid at10^{-3} M. It has also been confirmed that dietary inclusion of citric acid at a concentration of 10^{-2} to 10^{-6} M and lactic acid at10^{-2} to 10^{-5} M stimulated feeding, in a study conducted in Tilapia (*O. niloticus*) to determine the effects of several organic acids on their feeding behaviour (as already stated in this review). This is also an indication that the understanding of the feeding behaviour of farmed fish species has a significant role to play in the utilization of acidifiers (consisting of organic acids and their salts) as a potential replacement for antibiotic growth promoters in order to improve growth, feed utilization and disease resistance in fish.

The use of acidifiers can be an efficient tool to achieve sustainable, economical and safe fish production. In view of this, conscious efforts must be directed towards the development of acidifiers that could promote growth, feed efficiency, mineral absorption and disease prevention in cultured fish species. However, aquafeed manufacturers should be compelled to consider using acidifiers in fish diets.

CONCLUSION

It is evident from the above review that the use of feed additives as natural growth promoters is of great interest to aquaculturists. However, few nutritional studies conducted shows that several physical anomalies or alterations occurred in the normal development of farmed fish species. The scope of this review is to show that the development of a cost-effective new natural source of antioxidant that does not deteriorate the growth performance of farmed fish species; the production of a reliable, continuous supply of cost-effective algal meal-based diets on a large scale; adoption of a reliable and cost-effective method to quantify the absorption or ingestion rate in cultured fish species as well as the utilization and adoption of acidifiers as a potential replacement for antibiotic growth promoters may be an example for a group of additives which can play an important role in future aquaculture diets.

In addition, further researches need to be directed towards the elucidation of the biochemical contents and optimum dietary supplementation levels of feed additives by cultured fish species. In view of this, improvement in the growth, feed utilization, absorption or ingestion rates and disease resistance in cultured fish species fed feed additives may promote growth and feed conversion as well as improve survival rates.

REFERENCES

1. FAO, 2002. Antibiotics residue in aquaculture products. The state of world fisheries and aquaculture 2002, Rome, Italy, pp: 74-82.
2. Mantovani, A., F. Maranghi, I. Purificato and A. Macri, 2006. Assessment of feed additives and contaminants: an essential component of food safety. Ann 1st Super Sanità, 42(4): 427-432.
3. Lückstãdt, C., 2008. Utilization of acidifiers in nutrition and feeding of tropical fish - a mini-review. Bulletin of Fish Biol., 10: 105-109.
4. Atar, H.H. and M. Ates, 2009. The effects of commercial diet supplemented with Mannanoligosaccharide (MOS) and vitamin B_{12} on the growth and body composition of the carp (*Cyprinus carpio* L. 1758). Journal of Animal and Veterinary Advances, 8(11): 2251-2255.
5. Liem, D.T., 2004. *E. coli* resistant to most antibiotics in Vietnam. Asian Pork, 8(9): 22-24.
6. Meurer, F., M.M. Costa, D.A.D. Barros, S.T.L. Oliveira and P.S. Paixào, 2009. Brown propolis extract in feed as a growth promoter of Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758) fingerlings. Aquaculture Res., 40: 603-608.
7. Nunes, A.J.P., M.V.C. Sá, F.P. Andriola-Neto and D. Lemos, 2006. Behavioural response to selected feed attractants and stimulus in Pacific white shrimp, *Litopenaeus vennamei*. Aquaculture, 260(1-4): 244-254.
8. Robinson, E.H. and M.H. Li, 1993. Protein quantity and quality of catfish feeds. Technical Bulletin, 189: 1-10.
9. Allan, G.L., S.J. Rowland, C. Mifsud, D. Glendenning, D.A.J. Stone and A. Ford, 2000. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: V. Least cost formulation of practical diets. Aquaculture, 186: 327-340.
10. Rodriguez, S.M., N.M. Olvera and O.C. Carmona, 1996. Nutritional value of animal by-product meal in practical diets for Nile tilapia *Oreochromis niloticus* (L.) fry. Aquaculture Res., 27: 67-73.
11. Robaina, L., F.J. Moyano, M.S. Izquierdo, J. Socorro, J.M. Vergara and D. Montero, 1997. Corn gluten and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus autata*): nutritional and histological implications. Aquaculture, 157: 347-359.
12. Schreck, J.A. and C.M. Moffitt, 1987. Palatability of feed containing different concentrations of erythromycin thiocyanate to chinook salmon. Progressive Fish-Culturist, 49: 241-247.
13. Hustvedt, S.O., T. Storebakken and R. Salte, 1991. Does oral administration of oxolinic acid or oxytetracycline affect feed intake of rainbow trout? Aquaculture, 92: 109-113.
14. Ren, Z., 1999. The nutritional values of oxidized fish oil and its effects on carp (*Cyprinus carpio*). Doctoral dissertation of ChineseAcademy of Agricultural Sci., pp: 29.
15. Koven, W., S. Kolkovski, E. Hadas, K. Gamsiz and A. Tandler, 2001. Advances in the development of microdiets for gilthead seabream, *Sparus aurata*: a review. Aquaculture, 194: 107-121.
16. Kanazawa, A., S. Teshima, S. Inamori, T. Iwashita and A. Nagao, 1981. Effects of phospholipids on growth, survival rate and incidence of malformation in the larval ayu. Mem. Fac. Fish. Kagoshima University, 30: 301-309.
17. Kanazawa, A., 1993. Nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish. J. World Aquac. Soc., 24: 162-166.
18. Koven, W.M., G. Parra, S. Kolkovski and A. Tandler, 1998. The effect of dietary phosphatidylcholine and its constituent fatty acids on microdiet ingestion and fatty acid absorption rate in gilthead seabream, *Sparus aurata*, larvae. Aquac. Nutr., 4: 39-45.
19. Langdon, C., 2003. Microparticle types for delivering nutrients to marine fish larvae. Aquaculture, 227: 259-275.
20. Goh, Y. and T. Tamura, 1980. Olfactory and gustatory responses to amino acids in two marine teleosts-red sea bream and mullet. Comp. Biochem. Physiol., 66c: 217-224.
21. Mackie, A.M. and A.I. Mitchell, 1982. Further studies on the chemical control of the feeding behaviour in the Dover sole, *Solea solea*. Comp. Biochem. Physiol., 73(1): 89-93.

22. Mackie, A.M. and A.I. Mitchell, 1983. Studies on the chemical nature of feeding stimulants for the juvenile European eel, *Anguilla anguilla* (L.). J. Fish. Biol., 22: 425 - 430.
23. Smith, D.M., S.J. Tabrett, M.C. Barclay and S.J. Irvin, 2005. The efficacy of ingredients included in shrimp feeds to stimulate intake. Aquaculture Nutrition, 11: 263-271.
24. Adron, J.W. and A.M. Mackie, 1978. Studies on the chemical nature of feeding stimulants for rainbow trout, *Salmo gairdneri* Richarson. J. Fish Bio., 12(4): 303-310.
25. Takeda, M., K. Takii and K. Matsui, 1983. Identification of feeding stimulants for juvenile eel. Bull. Jap. Soc. Scient. Fish., 59: 645-651.
26. Mackie, A.M., 1982. Identification of the gustatory feeding stimulants In: Chemoreception in Fishes, Hara, T.J. (ed.). Elsevier Scientific Publication Co. Amsterdam, pp: 275-291.
27. Oshugi, T., I. Hidaka and M. Ikeda, 1978. Taste receptor stimulation and feeding behaviour in the puffer, *Fugu pardalis*. 2. Effects produced by mixtures of constituents of clam extracts. Chemical Senses and Flavour, 3(4): 355-368.
28. Lee, P.G. and S.P. Meyers, 1997. Chemoattraction and feeding stimulation. In: L.R. D'Abramo, D.E. Conklin and D.M. Akiyama, Editors, *Crustacean Nutrition*, *Advances in World Aquaculture* vol. 6, The World Aquaculture Society, Baton Rouge, USA, 6: 292-352.
29. Tacon, A.G.J., 1987. The nutrition and feeding of farmed fish and shrimp: a training manual. A report prepared for the FAO Trust Fund GCP/RLA/075/ITA Project Support to the Regional Aquaculture Activities for Latin America and the Caribbean. FAO of the United Nations, Brasilia, Brazil, pp: 117.
30. Staykov, Y., P. Spring and S. Denev, 2005. Influence of dietary bio-mass on growth, survival and immune status of rainbow trout (*Salmo gairdnei irideus* G.) and common carp (*Cyprinus carpio* L.). Proceedings of the 21st Alltech Annual Symposium: Nutritional Biotechnology in the Feed and Food Industries, (AASNBFFI005), Nottingham University Press, Nottingham, UK. pp: 333-343.
31. Cagiltay, F., 2007. Icsu Baliklari Yetistiriciligi, 1. Baski, Nobel Yayinlari, Ankara, Turkey, pp: 45.
32. Torrecillas, A., M.J. Caballero, J. Sweetman, A. Makol and M.S. Izguierdo, 2006. Effect of bio-mos on European sea bass (*Dicentrarchus labrax*) juveniles: Growth performance and feed utilization. Proceedings of 22nd Alltech Annual Symposium, pp: 24-26. Lexington, KY, USA.
33. Gonzalez, J.P., O.C. Farnés, G. Gaxiola, C. Pascual and C. Rosas, 2010. The effects of microencapsulated bovine insulin given to *Litopenaeus vannamei* juveniles as a feed additive on growth, metabolism and digestive enzyme activities. Aquaculture, 306: 252-258.
34. Sanders, B., 1983. Insulin-like peptide in the lobster *Homarus americanus*. II. Insulinlike biological actsivity. Gen. Comp. Endocrinol., 50: 374-377.
35. Kucharski, L.C., V. Schein, E. Capp and R.S.M.D. Silva, 2002. *In vitro* insulin stimulatory effect on glucose uptake and glycogen synthesis in the gills of the estuarine crab *Chasmagnathus granulata*. Gen. Comp. Endocrinol., 125: 256-263.
36. Gutiérrez, A., J. Nieto, F. Pozo, S. Stern and L. Schoofs, 2007. Effect of insulin/IGF-I like peptides on glucose metabolism in the white shrimp *Penaeus vannamei*. Gen. Comp. Endocrinol., 153: 170-175.
37. Kim, K.D., Y.J. Kang, H.Y. Lee, K.W. Kim and S.M. Lee, 2006. Effect of dietary oxidized squid liver oil and DL-a-tocopherol level on growth and body composition of juvenile flounder (*Paralichthys olivaceus*). J. Aquaculture, 19: 140-146.
38. Lewis-McCrea, L.M. and S.P. Lall, 2007. Effects of moderately oxidized dietary lipid and the role of vitamin E on the development of skeletal abnormalities in juvenile Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture, 262: 142-155.
39. Murai, T. and J.W. Andrews, 1974. Interactions of dietary a-tocopherol, oxidized menhaden oil and ethoxyquin on channel catfish (*Intalurus punctatus*). J. Nutrition, 104: 1416-1431.
40. Cowey, C.B., E. Degener, A.G. Tacon, A. Youngson and J.G. Bell, 1984. The effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*) grown at natural, varying water temperatures. British J. Nutrition, 51: 443-451.
41. Cho, S.H. and C.I. Kim, 2009. Effect of dietary inclusion of various sources of additives on growth and body composition of juvenile olive flounder *Paralichthys olivaceus*. Aquaculture Res., 40: 625-629.

42. Gonzalez, M.J., J.I. Gray, R.A. Schemmel, L. Dugan and C.W. Welsch, 1992. Lipid peroxidation products are elevated in fish oil diets even in the presence of added antioxidants. J. Nutrition, 122: 2190-2195.
43. Kaitaranta, J.K., 1992. Control of lipid oxidation in fish oil with various antioxidative compounds. J. the American Oil Chemists Society, 69: 810-813.
44. Branen, A.L., 1975. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. J. the American Oil Chemists Society, 55: 85-89.
45. Surak, J.G., A.L. Branen and E. Shrago, 1976. Effect of butylated hydroxyanisole on *Tetrahymena pyriformis*. Food and Cosmetics Toxicol., 52: 85-89.
46. Rhi, J.W. and H.S. Shin, 1993. Antioxidant effect of aqueous extract obtained from green tea. Korean J. Food Science and Technol., 25: 758-763.
47. Park, B.H., H.K. Choi and H.S. Cho, 2001. Antioxidant effect of aqueous green tea on soybean oil. J. The Korean Society of Food Science and Nutrition, 30: 552-556.
48. Paul, R. and J.H. Michael, 2007. The kinetics and mechanisms of the complex formation and antioxidant behaviour of the polyphenols EGCg and ECG with iron (III). J. Inorganic Biochemistry, 101: 585-593.
49. Vinson, J.A., 1999. The functional food properties of figs. Cereal Foods World, 44: 82-87.
50. Jeong, M.R., B.S. Kim and Y.E. Lee, 2002. Physicochemical characteristics and antioxidative effects of Korean figs (*Ficus carica* L.). Journal of the East Asian Society of Dietary Life, 12: 566-573.
51. Lim, K.T., S.J. Lee, J.H. Ko and P.S. Oh, 2005. Hypolipidemic effects of glycoprotein isolated from *Ficus Carica* Linnoeus in mice. Korean J. Food Science and Technol., 37: 624-630.
52. Kim, S.R. and S.H. Kim, 2002. The safety of food treated with gamma radiation and butylated hydroxyanisole in the feed of flounder (*Paralichthys olivaceus*) and mouse. J. Veterinary Clinics, 19: 1-6.
53. Kim, S.S., C.H. Lee, S.L. Oh and D.H. Chung, 1992. Chemical components in the two cultivars of Korean figs (*Ficus carical* L.). J., 35: 51-54.
54. Kang, S.K., D.O. Chung and H.J. Chung, 1994. Purification and identification of antimicrobial substances in phenolic fraction of fig leaves. Agriculture Chemistry and Biotechnol., 38: 293-296.
55. Kumar, D., H. Kaller, N. Bhaskar, M.H. Bhandary, M.J. Antony, C.V. Raju and V.M. Biradar, 1997. Lipid oxidation and subsequent browning in salted-dried mackerel (*Rastrelliger kanagurta* Cuvier). Indian J. Fisheries, 44: 377-385.
56. Sugiua, S.H., F.M. Dong and R.W. Hardy, 1998. Effects of dietary supplements on the availability of minerals in fish meal; preliminary observation. Aquaculture, 160: 283-303
57. Xie, S., L. Zhang and D. Wang, 2003. Effects of several organic acids on the feeding behavior of *Tilapia nilotica*. J. Applied Ichthyol., 19: 255-257.
58. Mohammadzdeh, S., M. Sharriatpnahi, M. Hamedi, Y. Amanzadeh, S.E.S. Ebraimi and S.N. Ostad, 2007. Antioxidant power of Iranian propolis extract. Food Chemistry, 103: 729-733.
59. Bai, S.C., J.W. Koo, K.W. Kim and S.K. Kim, 2001. Effects of *Chlorella* powder as a feed additive on growth performance in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquaculture Res., 32: 92-98.
60. Nakazoe, J., S. Kimura, M. Yokoyama and H. Iida, 1986. Effect of the supplementation of alga or lipids to the diets on the growth and body composition of nibbler, *Girella punctata* Grey. Bulletin of Tokai Regional Fisheries Research Laboratory, 120: 43-51 [in Japanese, with änglish summary].
61. Yone, Y., M. Furuichi and K. Urano, 1986. Effects of dietary wakame *Undaria pinnatifida* and *Ascophyllum nodosum* supplements on growth, feed efficiency and proximate composition of liver and muscle of red sea bream. Nippon Suisan Gakkaishi, 52: 1465-1468.
62. Watanabe, Y., W.L. Liao, T. Takeuchi and H. Yamamoto, 1990. Effect of dietary *Spirulina* supplementation on growth performance and flesh lipids of cultured striped jack. Journal of the Tokyo University of Fisheries, 77: 231-239. [in Japanese, with English summary].
63. Shubert, L.E., B.D. Larsen and P.E. Johnson, 1985. Nutritional value of *Spirulina* and *Chlorella* for human consumption. Journal of Phycology, 21. Abstracts of Papers Scheduled for the Annual Meeting of The Phycological Society of America at the University of Florida, pp: 11.
64. Mustafa, M.G., T. Takeda, T. Umino, S. Wakamatsu and H. Nakagawa, 1994. Effects of *Ascophyllum* and *Spirulina* meal as feed additives on growth performance and feed utilization of Red sea bream, *Pagrus major*. J. Fac. Appl. Biol. Sci., 33: 125-132.

65. Ferber, D., 2000. Superbugs on the hoof ? Sci., 288: 792-794.
66. Salamatdoustnobar, R., A. Ghorbani, S.S.G. Maghami and V. Motallebi, 2011. Prebiotic could affect fingerlings rainbow trout meat protein? (*Oncorhynchus mykiss*). World J. Fish and Marine Sci., 3(4): 332-334.
67. Vahedi, G. and S. Ghodratizadeh, 2011. Effect of chitin supplemented diet on innate immune response of rainbow trout. World J. Fish and Marine Sci., 3(6): 509-513.
68. Denli, M., S. Cankaya, S. Silici, F. Okan and A.N. Uluocak, 2005. Effect of dietary addition of Turkish propolis on the growth performance, carcass characteristics and serum variables of quail (*Coturnix japonica*). Asian Australian J. Animal Sci., 18: 848-854.
69. Acikgoz, Z., B. Yucel and O. Altan, 2005. The effects of propolis supplementation on broiler performance and feed digestibility. Achiv fur Ge£ugelkunde, 69: 117-122.
70. Franco, S.S., A.P. Rosa, S. Lengler, R. Uttpatel, I. Zanella, C. Gressler and H.M. Souza, 2007. I£ndices produtivos e rendimento de carcac_a de frangos de corte alimentados com dietas contendo ni veis de extrato etano. ico de propolis ou promotores de crescimento. CieOE ncia Rural, 37: 1765-1771.
71. Cuesta, A., A. Rodriguez, M.A. Esteban and J. Meseguer, 2005. *In vivo* effects of propolis, a honeybee product, on gilthead seabream innate immune responses. Fish and Shellfish Immunol., 18: 71-80.
72. Chu, W., 2006. Adjuvant effect of propolis on immunization by inactivated *Aeromonas hydrophila* in carp (*Carassius auratus*). Fish and Shellfish Immunol., 21: 113-117.
73. Renoux, G., 1980. The general immunopharmacology of levamisole. Drugs, 19: 89-99.
74. Siwicki, A.K., 1989. Immunostimulating influence of levamisole on nonspecific immunity in carp, *Cyprinus carpio*. Dev. Comp. Immunol., 13: 87-91.
75. Mulero, V., M.A. Esteban, J. Munoz and J. Meseguer, 1998. Dietary intake of levamisole enhances the immune response and disease resistance of the marine teleost gilthead seabream, *Sparus aurata* L. Fish Shellfish Immunol., 8: 49-62.
76. Siwicki, A.K. and M. Korwin-Kossakowski, 1988. The influence of levamisole on the growth of carp (*Cyprinus carpio* L.) larvae. J. Appl. Ichthyol., 4: 178-181.
77. Cabaj, W., M. Stankiewicz, W.E. Jonas and L.G. Moore, 1995. Levamisole and its influence on the immune responses of lambs. Vet. Res. Commun., 19: 17-26.
78. Li, P. X., Wang and D.M. Gatlin, 2006. Evaluation of levamisole as a feed additive for growth and health management of hybrid striped bass (*Morone chrysops* x *Morone saxatilis*). Aquaculture, 251: 201-209.
79. Li, P., X. Wang and D.M. Gatlin III, 2004. Excessive dietary levamisole suppresses growth performance of hybrid striped bass (*Morone chrysops×M. saxatilis*) and elevated levamisole *in vitro* impairs macrophage function. Aquaculture. Res., 35: 1380-1383.
80. Tacon, A.G.J. and M. Metian, 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture, 285: 146-158.
81. Smith, C.E., 1979. The prevention of liver lipoid degeneration (ceroidosis) and microcytic anaemia in rainbow trout *Salmo gairdneri* Richardson fed rancid diets: a preliminary report. J. Fish. Dis., 2: 429-437.
82. Moccia, R.D., S.S.O. Hung, S.J. Slinger and H.W. Ferguson, 1984. Effect of oxidized fish oil, vitamin E and ethoxyquin on the histopathology and haematology of rainbow trout, *Salmo gairdneri* Richardson. J. Fish. Dis., 7: 269-282.
83. Wanasundara, U.N. and F. Shahibi, 1998. Antioxidant and pro-oxidant activity of green tea extracts on marine oils. Food Chemistry, 63: 335-342.
84. Nakagawa, H., 1985. Usefulness of *Chlorella*-extract for improvement of the physiological condition of cultured ayu, *Plecoglossus altivelis* (Pisces). Téthys, 11: 328-334.
85. Nakagawa, H. and S. Kasahara, 1986. Effect of Ulva-meal supplementation to diet on lipid metabolism of red sea bream. Nippon Suisan Gakkaishi, 52: 1887-1895.
86. Satoh, K., H. Nakagawa and S. Kasahara, 1987. Effect of *Ulva* supplementation on disease resistance of red sea bream. Nippon Suisan Gakkaishi, 53: 1115-l 120.

87. Ajiboye, O.O. and A.F. Yakubu, 2010. Some aspects of biology and aquaculture potentials of *Tilapia guineensis* (dumeril) in Nigeria. Reviews in Fish Biology and Fisheries, 20: 441-455.
88. Payne, A.I., 1978. Gut pH and digestive strategies in estuarine grey mullet (Mugilidae) and tilapia (Cichlidae). J. Fish. Biol., 13: 627-629.
89. Freitag, M., 2007. Organic acids and salts promote performance and health in animal husbandry, In: Acidifiers in Animal Nutrition - A Guide for Feed Preservation and Acidification to Promote Animal Performance (Lückstädt, C. ed). Nottingham University Press, Nottingham. pp: 1-11.
90. Gildbert, A. and J. Raa, 1977. Properties of a propionic acid/formic acid preserved silage of cod viscera. J. the Science of Food and Agriculture, 28: 647-653.
91. Åsgård, T. and E. Austreng, 1981. Fish silage for salmonids: a cheap way of utilizing waste as feed. Feedstuffs, 53: 22-24.