The Use of Chemicals in Aquafeed

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ABSTRACT

Various chemicals and additives used in fish and shrimp feeds may have impacts on animal health, product quality and the environment. This paper reviews the use and effects of vitamins (vitamins C and E), essential fatty acids, carotenoids, immunostimulants, hormones and attractants added to feeds for cultured fish and shellfish.

INTRODUCTION

The rapid growth of the fish and shrimp culture industries, especially in Asia, has been accompanied by the negative impacts of disease. Although parasitic (e.g., *Zoothamnium*) and bacterial diseases (e.g., *Vibrio* spp.) sometimes result in heavy losses in hatcheries, viral diseases are the most serious problem in grow-out facilities. Chemotherapy is now recognized as an increasing source of problems in aquaculture. The presence of residual antibiotics in sediments and in the tissues of shrimp may have public health implications, and these substances are regarded as dangerous for humans. Effective vaccines for shrimp are not available; such vaccines may not be practical because of the non-self-recognition immune system of these animals. Several appropriate measures could be employed to reduce losses from disease. One preventive measure is to ensure optimal diet quality. In mammals, one of the first signs of malnutrition is immunosuppression. This review paper emphasizes chemicals such as vitamins, fatty acids, carotenoids, immunostimulants, hormones and attractants used in feed which impact on health, product quality and the environment.

VITAMINS

So far, four fat-soluble and 11 or 12 water-soluble vitamins are known to be required by fish and shrimp, respectively. Vitamin deficiencies lead to aberrant biochemical functions and consequent cellular and organal disfunctions which gradually manifest as clinical deficiencies. The weak integrity of skin and epithelial tissues consequently predisposes fish to infections. Moreover, cells involved in the generation of both specific and nonspecific immune responses are metabolically active and are also likely to be affected by vitamin deficiency. Most studies on the positive correlation between vitamin and immune response of fish are confined to the antioxidant vitamins C and E.

Vitamin C

The clinical signs of ascorbic acid (AA) deficiency in fish were described by Halver *et al.* (1975). They include lordosis, scoliosis, distortion of support cartilage, hyperplasia of gill tissue, shortened opercles and petechial hemorrhages. External signs of AA deficiency have been described in rainbow trout (*Oncorhynchus mykiss*) (Kitamura *et al.* 1965; Halver *et al.* 1969; Hilton *et al.* 1977, 1978), brook trout (*Salvelinus fontinalis*) (Poston 1967), coho salmon *(O. kisutch*) (Halver *et al.* 1969), cherry salmon (*O. masou*) (Halver *et al.* 1975), mrigal (*Cirrhina mrigala*) (Mahajan and Agrawal

1980), common carp (*Cyprinus carpio*) (Dabrowski *et al.* 1988), guppy (*Poecilia reticulata*) (Halver *et al.* 1975), yellowtail (*Seriola quinqueradiata*) (Sakaguchi *et al.* 1969), channel catfish (*Ictalurus punctatus*) (Lovell 1973, Lim and Lovell 1978, Miyazaki *et al.* 1985), Japanese eel (*Anguilla japonica*) (Arai *et al.* 1972), lake whitefish *(Coregonus clupeaformis*) (Zitzov and Millard 1988), plaice *(Pleuronectes platessa*) (Rosenlund *et al.* 1990), Zill's tilapia (*Tilapia zilli*) (Anadu *et al.* 1990), Mayan chichlid (*Cichlasoma uropthalmus*) (Chavez de Martinez 1990), Atlantic salmon (*Salmo salar*) (Sandnes 1991), seabass (*Lates calcarifer*) (Boonyaratpalin *et al.* 1989) and tiger shrimp (*Penaeus monodon*) (Boonyaratpalin and Phongmaneerat 1995). It is generally agreed that the clinical signs of deficiency seen in fish are caused by impaired collagen and support cartilage formation (Wilson and Poe 1973, Halver *et al.* 1975, Lim and Lovell 1978). In *P. monodon*, the signs of deficiency include soft shelling, reduced activity, opaque whitish muscle, big head with flipped gill cover, incomplete molting and mortality (Boonyaratpalin and Phongmaneerat 1995).

The role of vitamin C in disease resistance has been studied in several fish. Durve and Lovell (1982) found that a dietary supplementation of 30 mg vitamin C/kg supported normal growth and prevented deficiency signs in channel catfish. Increased resistance against infection by *Edwardsiella tarda* was observed at a supplementation level of 150 mg/kg at water temperature of 21 °C; however, at 33 °C, increasing the supplemented level of vitamin C had no significant effect on resistance against infection. Feeding channel catfish a megadose (3000 ppm) of vitamin C enhanced disease resistance against *E. ictaluri* significantly.

Fish fed a vitamin C-deficient diet showed suppressed specific antibody production against *E. ictaluri* serum complement activity and non-specific phagocytic index (Li and Lovell 1985). Liu *et al.* (1989) reported that when pond-reared catfish were immunized with *E. ictaluri* and challenged 1 mo later with virulent *E. ictaluri*, a dietary AA level of 1,000 mg/kg of diet significantly decreased mortality. Higher supplementation of vitamin C (2,000 and 4,000 mg/kg diet) did not further decrease mortality. This lack of a positive effect of megadose of vitamin C on complement activity does not agree with the findings of Li and Lovell (1985). In contrast to Li and Lovell (1985), Li *et al.* (1993) observed that high vitamin C diets appear to be ineffective as a prophylactic treatment to enhance disease resistance against *E. ictaluri* in channel catfish.

Yano *et al.* (1990) found that megadose levels of L-ascorbic acid and L-ascorbyl-2 phosphate Mg (10,000 mg/kg diet) increased the phagocytic index of pronephros cells of red seabream but did not influence the complement activity.

In salmonids, the effects of AA on immune response vary widely, and results are less conclusive. Blazer (1982) showed that rainbow trout fed a diet with 120 mg/kg AA had significantly lower phagocytic index compared to fish fed a diet containing 1200 mg/kg AA. Blazer and Wolke (1984a) challenged rainbow trout with sheep red blood cells or *Yersinia ruckeri*. Those fed diets containing higher than standard levels of AA and vitamin E had increased thymus-dependent cells (T cells) and bursa-dependent cells (B cells), as well as enhanced specific antibody titers.

Wahli *et al.* (1986) studied the effect of dietary AA on the disease resistance of rainbow trout infected with the holotrichous ciliate *Ichthyophthirius multifiliis*. Trout fed 0 and 5,000 mg AA/kg diet for 5 d were infected with 8,800 and 11,500 tomites per fish. Fifty days after infection no parasites could be detected on fish that survived the infection. At the lower infectious dose, survival rate increased from 48% (0 mg AA/kg) to 98% (5,000 mg AA/kg), while the respective values were 0% and 82% in trout infected with the highest number of ciliates. Serum from experimentally infected fish was examined for tomite-immobilizing activity, IgM and specific antibodies against *I. multifiliis* (Wahli *et al.* 1986). The authors concluded that the results definitely indicated the presence of an immune response against this parasite, and that this response may have been enhanced by dietary AA.

Navarre and Halver (1989) studied the effects of high levels of dietary AA on disease resistance and humoral antibody production against *Vibrio anguillarum* in rainbow trout. Young fish were fed purified experimental diets supplemented with 0, 100, 500, 1000 and 2000 mg AA/kg for 28 wk. Resistance to bacterial infection was related to dietary AA, and was reflected in improved survival of challenged fish fed diets with 500, 1000 and 2000 mg AA; higher antibody production was observed. Navarre and Halver (1989) concluded that rainbow trout may survive bacterial challenge and improve antibody production when the diet contains 500-1000 mg AA/kg. Verlhac *et al.* (1991) found impaired phagocytosis and antibody response in vitamin C-deficient rainbow trout. The variation in phagocytosis and antibody production of fish fed 1000 ppm calcium L-ascorbate-2 monophosphate was not significantly different when compared to those fed normal requirement level (100 ppm). Verlhac *et al.* (1996) showed that high dietary level of ascorbate polyphosphate (1000 ppm) enhanced the phagocytic index of rainbow trout but had no antibody response effect.

Lall *et al.* (1989) found no conclusive evidence that vitamin C affects the susceptibility of Atlantic salmon to vibriosis or furunculosis, the production of antibodies, or the bactericidal activity of serum from normal or immunized fish.

Olivier *et al.* (1989) fed a practical diet supplemented with 0, 50, 100, 200, 500, 1000 and 2000 mg AA/kg to Atlantic salmon for 22 wk. For all diets, no effects on non-specific resistance factors were found. Groups of fish fed 0, 100, 500 and 2000 mg AA/kg were immunized against *Aeromonas salmonicida* and *Vibrio anguillarum*. One month after vaccination, analysis for complement activity and humoral antibody response revealed no effects of dietary AA.

In Atlantic salmon parr, Sandnes *et al.* (1990) studied the production of antibodies against a soluble artificial antigen (NIP_{11}-LPH) following a feeding period of 6 wk when fish were fed different equivalent level of AA and AS in the diets (0, 500 and 5000 mg AA/kg). The antibody response was somewhat reduced in fish deprived of vitamin C, but there were no differences between fish fed 500 and 5000 mg/kg, irrespective of chemical form. The results indicated that antibody production is not dependent on a high physiological status of AA (Sandnes *et al.* 1990).

Hardie *et al.* (1991) found that serum complement activity of Atlantic salmon was significantly reduced in AA-depleted fish. On bath challenge with a virulent strain of *A. salmonicida*, a significant increase in mortality was seen in AA-depleted fish. However, specific and certain non-specific cellular defense-related responses including respiratory burst (superoxide anion) production and erythrophagocytosis were unaffected by dietary vitamin C.

Erdal *et al.* (1991) fed Atlantic salmon three diets with varying contents of AA and AS and measured general resistance after challenge with *V. salmonicida*, development of specific immune response after vaccination against *Yersinia ruckeri*, and survival after challenge. No significant differences in general or specific resistance were found; however, the experimental design renders interpretation of the results difficult. Thus, the experimental diets were supplemented with either 90 mg AA/ 218 mg As/kg feed (diet 1), 2980 mg AA/218 mg AS (diet 2) or 90 mg AA/4470 mg AS (diet 3).

In Pacific salmon, higher levels of dietary vitamin C showed no positive effect on immune response or disease resistance. Bell *et al.* (1984) reported that dietary ascorbate had no effect on the development of bacterial kidney disease (*Reinbacterium salmoninarum*) in sockeye salmon (*Oncorhynchus nerka*).

In summary, (1) there is evidence that high dietary dose of vitamin C protects against infectious diseases in channel catfish; (2) no clear conclusion can be drawn with regards to salmonids; and (3) there is general agreement that vitamin C-depleted fish are more susceptible to disease.

The role of vitamin C in reducing the negative impacts of environmental stress on health and on disease resistance has also been studied. Stress increases susceptibility to infection (Maule *et al.* 1989) and is increased by handling, vaccination and transport of fish. Often, acute and chronic stress causes hypersecretion of corticosteroids, particularly cortisol (Robertson *et al.* 1987), which may induce some secondary effects on the immune system (Bennett and Wolke 1987, Ellsaesser and Clem 1987). Prolonged elevation of cortisol significantly increased disease susceptibility in brown trout (*Salmo trutta*) (Pickering and Pottinger 1985). Stress-induced ascorbic acid depletion from the anterior kidney in coho salmon and rainbow trout has been observed (Wedemyer 1969). Channel catfish fed vitamin C-free diets are more sensitive to physiological stressors such as ammonia and low oxygen levels than when fed diets with vitamin C (Mazik *et al.* 1987). Ascorbic acid reduces the effects of toxicity of waterborne copper (Hilton 1989) and nitrite (Wise *et al.* 1988), and also protects against the adverse effects of intakes of the organochlorine pesticide Aldrin (Agarwal *et al.* 1978) and the insecticide Toxophene (Mayer *et al.* 1978).

Ascorbic acid has antioxidant properties in feed (Cort 1982, Takahashi *et al.* 1986). The antioxidant effect of ascorbic acid and ascorbyl palmitate is more effective in combination with vitamin E, as vitamin E reacts with the free radicals first. Ascorbic acid and ascorbyl palmitate regenerate vitamin E until all ascorbate is consumed (Lambelet *et al.* 1985).

Vitamin E

Reduced growth, muscular dystrophy and anemia (red blood cell lysis) caused by oxidative damage of components in the cell membranes were observed in vitamin E-deficient fish (Moccia *et al.* 1984, Cowey 1986, Furones *et al.* 1992, Mcloughlin *et al.* 1992). Studies on the protective effect of vitamin E are limited.

Wise *et al.* (1993a) fed channel catfish fingerlings with purified diets either unsupplemented and deficient in both selenium and vitamin E, deficient in either selenium or vitamin E, adequate in both selenium (0.2 mg/kg) and vitamin E (60 mg/kg) or excessive in both nutrients (four times the recommended levels) for 120 d. The results indicated that higher than recommended levels of one or both nutrients may enhance macrophage function; selenium and vitamin E did not complement each other. Wise *et al.* (1993b) fed channel catfish fingerlings with purified diets containing α-tocopherol acetate to provide 0, 60 and 2500 mg vitamin E/kg for 180 d. The susceptibility of red blood cells to oxidative hemolysis decreased with increasing levels of dietary vitamin E, and the ability of macrophages to phagocytize virulent *E. ictaluri* was enhanced by vitamin E, but agglutinating antibody titers were not effected by dietary vitamin E levels.

Blazer and Wolke (1984b) reported that α-tocopherol deficiency suppressed all aspects of humoral and cellular immunity as well as the phagocytic index of rainbow trout. Macrophages from fish fed a deficient diet showed significantly reduced ability to engulf latex beads as compared to those from fish on a control diet.

Ndoye *et al.* (1989) observed a gradual increase in antibody production in trout with an increase in dietary vitamin from 23 mg to 60 and 600 mg/kg diet. In trout fed diets supplemented with 0, 45 and 450 mg vitamin E/kg for 23 wk, Verlhac *et al.* (1991) observed that antibody titre against redmouth disease at 40 d after vaccination was higher for fish fed 450 mg vitamin E/kg as compared to those fed at 45 mg/kg. However, the beneficial effect on stimulation was time dependent, and after 120 d of feeding no difference was observed. Verlhac *et al.* (1991) also concluded that vitamin E deficiency impaired antibody response and lymphoproliferation.

Rainbow trout fed diets containing 7, 86 or 806 mg vitamin E/kg for 22 wk were exposed to virulent *Yersinia ruckeri* by bath or injection. Mortalities were always least among those fed the highest concentration of vitamin E, but serum antibody production was not affected by vitamin E

levels (Furones *et al.* 1992).

Studies conducted on Atlantic and chinook salmon (*Oncorhynchus tshawytscha*) have not confirmed these observations. Lall *et al.* (1988) found that vitamin E had no effect on the non-specific resistance of Atlantic salmon challenged with *A. salmonicida*. Neither humoral response nor the complement system was affected 6 wk after vaccination with formalin-killed *A. salmonicida* cells. Hardie *et al.* (1990) reported that secretion of superoxide anion, serum lysozyme, lymphokine production and humoral immune response in Atlantic salmon were not affected by dietary vitamin intake (7, 86, 326 and 800 mg vitamin E/kg), but the complement system was compromised in fish fed a low level of vitamin E. Waagbø *et al.* (1993a) found that the interaction between dietary lipid, vitamin E and water temperature was apparent for disease resistance of Atlantic salmon challenged with *Vibrio salmonicida* by injection. Fish fed sardine oil supplemented with vitamin E showed best survival at low water temperature (8 °C), while the capelin oil diet with vitamin E was superior to the soybean oil and sardine oil diets at 13 °C. Data from all treatments showed higher total antibody level in fish fed vitamin E-supplemented diets, an opposite effect to the differences found as regards to specific antibodies.

In coho salmon, Forster *et al.* (1988) showed that disease resistance as determined by the rate of mortality induced by exposure of nonvaccinated fish to challenge with *Vibrio anguillarum* or *V. ordali* was likewise uneffected by dietary vitamin E level (30 and 1030 IU/kg).

Blazer (1991) found that increasing the concentration of vitamin E to 2500 mg/kg of diet depressed the killing ability of macrophages and suggested that very high levels of vitamin E may have depressed other killing mechanisms such as superoxide anion and peroxide production. Lall and Olivier (1991) recommended that higher dose of vitamin E (>2000 mg/kg) should not be used in studies on immune response of fish because of problems due to hypovitaminosis.

These results indicate that the effects of vitamin E on the immune function of fish are not clear. The discrepancies between studies are difficult to explain, but they could be due to different methodologies, environmental conditions or feed compositions used and experience in determining the non-specific and specific immuno-response parameters. Since the science of fish immunology is still at the developmental stage, more research is needed to determine proper doses and durations of application to enhance the health of fish and shrimp.

As to the role of vitamin E on product quality, increasing supplements of vitamin E in the diet of channel catfish may provide additional protection against lipid oxidation in fillet tissue (Gatlin *et al.* 1992). This would mean improved quality of fillet and shelf life. This information will probably be most useful in countries where raw fish is a preferred food and freshness brings a premium price.

ESSENTIAL FATTY ACIDS

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contained in fish and crustaceans are essential fatty acids for marine animals, and these highly unsaturated fatty acids are also required by humans. Omega-3 highly unsaturated fatty acids (Ω–3 HUFA) are quantitatively the dominant fatty acids in marine fish. Fish cannot synthesize essential Ω-3 fatty acids (EFA), and therefore, these fatty acids must be provided in the feed. Requirements of salmonids for Ω-3 fatty acids are estimated to be approximately 1% of the diet in low fat diets (Castell *et al.* 1972, Yu and Sinnhuber 1979). Two highly unsaturated fatty acids (HUFA) of the Ω-3 family, namely eicosapentaenoic acid (20:5 n-3) (EPA) and docosahexaenoic acid (22:6 n-3) (DHA), are of particular importance (Gunstone *et al.* 1978, Ackman and Takeuchi 1986). Omega-3 HUFA may have a higher EFA value, and requirements for rainbow trout are satisfied by a level of 0.5% of the diet or 10% of total dietary lipids (Watanabe 1982).

The significance of Ω-3 fatty acids in various tissues, other than on growth-promoting effect, has not been determined in detail. Membrane-bound lipids are important for temperature adaptation in cold-blooded animals (Hazel 1979). At low temperatures, a selective increased incorporation of Ω-3 fatty acids into the cell membrane takes place (Hazel 1979). It is assumed that this enhances the fluidity of the cell membrane. Further, Ω-3 fatty acids are important precursors in the synthesis of eicosanoids (Bakhle 1983), which are, in turn, important mediators in inflammatory reactions and, partly, in the regulation of the immune response. The bactericidal activity of macrophage cells *in vitro* was reduced when the diet was EFA deficient. A maximum number of *Aeromonas salmonicida* survived in this group of salmon. Macrophages of fish receiving lenolenic acid (LNA) or n-3 HUFA were more effective in killing the bacteria *in vitro* (Kiron *et al.* 1995).

The effectiveness of n-3 series fatty acids in intracellular killing has been demonstrated in a study by Sheldon and Blazer (1991) on channel catfish. Using purified diets containing either menhaden oil, soybean oil or beef tallow at 7%, they correlated enhanced bactericidal activity to increasing levels of HUFAs, results that were independent of the rearing temperature. Waagbø *et al.* (1993a) observed in Atlantic salmon that the bacterial killing activity of macrophages at 12 °C was reduced in fish fed on sardine oil which contained more n-3 polyunsaturated fatty acid (n-3 PUFA) as compared to fish receiving capelin oil which had lower n-3 PUFA content. However, there was no difference in the macrophage activity at a higher temperature (18 °C). Kiron *et al.* (1995) reported that in rainbow trout, the macrophage activity *in vitro* was superior in all the groups receiving EFA (LA, LNA and n-3 HUFA) as compared with the deficient group receiving palmitic acid. In this study, the macrophages of rainbow trout receiving the PUFAs responded better against the bacteria. The immunomodulation by dietary lipid is effected by changes in the plasma membrane lipid structure of the lymphocyte subpopulation. However, caution has to be exercised in such dietary manipulations, as it has been shown that high fat concentration in the diet, particularly PUFA, can suppress lymphocyte functions when EFA requirements are met.

Erdal *et al.* (1991) illustrated the relationship between n-3 fatty acids and the immune response in Atlantic salmon. Fish fed diets with various types of oils containing increasing amounts of n-3 fatty acids (from 2.9 to 3.6-5.8) exhibited an immune suppressive effect. Waagbø *et al.* (1993a) showed that increasing the amount of n-3 PUFA from 3.5 to 6.0% in diets of Atlantic salmon was again the reason for a reduction in specific antibody production against *Vibrio salmonicida*.

Henderson *et al.* (1992) reported that antibody production was not affected by increased dietary n-3 PUFA in rainbow trout vaccinated with *Y. ruckeri*. However, Kiron *et al.* (1995) suggest that optimal use of EFA (0.5% according to Watanabe (1982) and 1.0% according to Castell *et al.* (1972) enhances antibody activity. This indicated that EFA above the required level might have impaired the defense mechanisms. Waagbø *et al.* (1993a) showed an interaction between dietary lipid, vitamin E and water temperature for disease resistance of Atlantic salmon challenged with *V. salmonicida* by injection. Dietary vitamin E requirement increases as dietary n-3 PUFA increase. Dietary n-3 PUFA requirement level decreases as water temperature increases.

The increase in clotting time observed with increasing level of dietary n-3 PUFA and vitamin E (Salte *et al.* 1987, 1988; Waagbø *et al.* 1993b) resembles the classical effects of marine lipids reported in humans and experimental animals, where n-3 PUFA-rich diets prevent the development of cardiovascular diseases by decreasing the incidence of thrombosis (Herold and Kinsella 1986, Weber 1989). An awareness of the influence DHA, contained in the mammalian brain, on intelligence and the protective role of n-3 PUFA against the development of cardiovascular disease has prompted the promotion of fish consumption. Fish rich in n-3 PUFA would seem to give the most benefit. In general, marine fish will have higher n-3 PUFA then freshwater fish; however, lipid contents, nutritional status, total lipid and phosphoglyceride DHA levels of gillhead seabream were directly correlated with dietary DHA levels (Mourente *et al.* 1993). For most fish, body lipid composition is affected by dietary lipid composition. In this connection, fish nutrition, though

feed manipulation, would play an important role in the production of fish that are rich in n-3 HUFA for the benefit of human health.

CAROTENOIDS

Coloration

One of the most obvious functions of carotenoid in feed is in coloration of aquatic animals. Carotenoid use in fish culture is mainly associated with astaxanthin and canthaxanthin pigmentation of the flesh of salmonids, or astaxanthin in the shells and flesh of shrimp and lobsters. Next to freshness, the pigmentation of the flesh of Atlantic salmon and rainbow trout is regarded as the most important quality criteria. The market demand for Atlantic salmon is for a flesh concentration of astaxanthin above 6-8 mg/kg. Because shell and flesh color of shrimp and fish whet the appetite and enhance enjoyment, food color acts as an optical seasoning. Ornamental fishes change their hues in response to background coloration to avoid being preyed upon, and also display color response during excitement and courtship (Moyle and Cech 1982).

Fish and shrimp are not able to synthesize carotenoids "*de novo*" and depend completely on the presence of necessary carotenoids in their feed. Salmonids absorb and deposit astaxanthin and canthaxanthin in the muscle during the grow-out period. At the time of sexual maturation, they mobilize stored carotenoids to the ovaries and finally, on to the progeny. This active transfer of carotenoids from the mother to the eggs has led to the hypothesis that carotenoids are vital for egg and larval development (Negre-Sadargues *et al.* 1993).

Shrimp (*Penaeus monodon*) absorb cantaxanthin, which is transformed into astaxanthin before being deposited in flesh and shell. Therefore, astaxanthin is 2-3 times more efficiently utilized by *P. monodon* than is cantaxanthin because no transformation is needed. Citranaxanthin had no effect in coloration of *P. monodon* (Boonyaratpalin *et al.* 1994). It appears that astaxanthin is more efficiently used in pigmentation of *P. monodon* than is β-carotene (Chien and Jeng 1992). This result supports the work done by Katayama *et al.* (1972) on the metabolic pathway of β-carotene to astaxanthin in shrimp. Foss *et al.* (1984) and Torrissen (1986) also found that astaxanthin was more efficacious than canthaxanthin in pigmenting the flesh of rainbow trout, but astaxanthin and canthaxanthin were not interconverted.

Growth and Survival

Torrissen (1984) found a positive effect of 30 mg/kg canthaxanthin or astaxanthin supplementation on growth during the start of the feeding period, and no significant differences were found between fish fed the astaxanthin and canthaxanthin-supplemented diets. Goswami (1993) showed that a supplement of β-carotene and canthaxanthin in the diets of Indian major carps resulted in better survival and growth as compared to conventional diets without carotenoids. Improved survival rates were also reported for kuruma shrimp (*Penaeus japonicus*) (Chien and Jeng 1992, Negere-Sadargues *et al.* 1993) by astaxanthin supplementation or supplementation with a combination of astaxanthin and canthaxanthin (1:1), but no differences were observed in growth or molting.

A study on the quantitative requirement for astaxanthin in Atlantic salmon showed increased growth and survival, and protein utilization in the range of 0.7 to 5.3 mg/kg with a constant, high value for diets supplemented above 5.3 mg/kg (Torrissen and Christiansen 1994). Thomson *et al.* (1995) found no marked differences in food conversion efficiency or growth of rainbow trout fed diets with and without astaxanthin.

Reproduction

Hartmann *et al.* (1947) reported that astaxanthin had a function as a fertilization hormone by stimulating and attracting spermatozoa, and Deufel (1965) found that fertilization increased in trout given a canthaxanthin-supplemented diet. This is supported by Longinova (1977), who reported higher survival of highly pigmented rainbow trout eggs as compared to pale ones.

In red seabream (*Chrysophrys major*), the percentage of buoyant eggs was found to be improved by a diet fortified with carotenoids (β-carotene, canthaxanthin or astaxanthin). The hatching rate was not influenced, but abnormality in number and position of the oil globules was reduced. Consequently, the total number of normal larvae was higher when the broodstock was supplied carotenoids (Watanabe *et al.* 1984). β-carotene was confirmed to be non-transferable, resulting in poor egg quality (Watanabe and Miki 1991). The freshwater fish *Heteropneustes fossilis* fed a carotenoid-free diet showed atrophied gonads with damaged germinal epithelium (Goswami 1988).

In contrast, Torrissen and Chistiansen (1994) produced Atlantic salmon eggs with astaxanthin concentrations varying from 0.1 to 20 mg/kg by keeping salmon in filtered sea water on a carotenoid-free diet from smolt stage to spawning. Comparison of the progeny with brood from fish given identical treatment but a diet fortified with 100 mg/kg astaxanthin showed no significant differences in rate of fertilization, survival during the green egg stage, from eyed egg until hatching or of the yolk-sac fry. No differences in size, deformities, general performance or tolerance to oxygen depletion could be detected. Harris (1984) and Tveranger (1986) found no effect of dietary astaxanthin or canthaxanthin supplementation on fecundity, fertilization rate or hatching rate of rainbow trout and Atlantic salmon. Torrissen (1986) found no effect of egg pigmentation on survival during the embryonic stages.

Health and Immunology

Except for the consensus that carotenoids enhance the performance of fish and their brood, there is little information on the direct effects of carotenoids on fish health. Thompson *et al.* (1995) found that astaxanthin does not appear to have any marked effect on innate or specific immunity, and therefore has little potential as immunostimulant for cultured trout.

Conclusions

As coloration is a positive selection criterion for the consumer, synthetic astaxanthin is routinely added to the diets of farmed salmon. Available data show that the amount of dietary astaxanthin required for growth is 5.3 mg/kg; for survival and egg quality, it is 20 mg/kg. However, normal dosage in commercial salmon feed is between 30 to 70 mg/kg, starting with a high dose and decreasing as fish grow. Astaxanthin feed was used through the whole production cycle (OJ Torrissen pers. comm.). Astaxanthin's effects on immune response and disease resistance need further investigation.

IMMUNOSTIMULANTS

As a number of fish and shrimp diseases are associated with intensive aquaculture, control of disease has become an urgent need. Efforts to protect fish by vaccination have found that immunity is short-lived and that there are difficulties with administration (Plumb 1988a, b). Arthropods have a simple nonspecific defensive mechanism, such as non-self recognition of foreign particles. These mechanisms involve the phagocytosis or encapsulation of large particles (Gotz 1986), blood clotting, nodule formation, the prophenaloxidase activity system and release of opsonizing factor (Perrson *et al.* 1987).

Many chemicals have been used as immunostimulators for fish and shrimp. Intraperitoneal injections of channel catfish with β-1, 3 glucan (derived from baker's yeast) greatly reduces mortality from experimental infection with *Edwardsiella ictaluri*. The results indicate that β-1,3 glucan potentially could be utilized prophylactically as an immunomodulator in channel catfish (Chen and Aingworth 1992). Verlhac *et al.* (1996) showed that the antibody response of trout can be enhanced by feeding glucan for 2 wk, and that some effects lasted 2 wk after fish has been switched back to a control diet. β-glucan, β-1,3 and 1,6 linkage polysaccharide are structural elements of fungal cell walls. *Saccharomyces cerevisiae* can enhance nonspecific disease resistance and growth in *Penaeus monodon* post-larvae via immersion, however, the protective effect of glucan treatment lasted only 14 d (Sung *et al.* 1994). Boonyaratpalin *et al.* (1993) reported that *P. monodon* fed a diet supplemented with peptidoglycan (a chemical substance from the cell walls of a bacterium, *Brevibacterium lactofermentum*) at 0.01% showed better growth, survival, disease resistance, salinity stress resistance and hemocyte phagocytic activity, whereas a higher concentration of peptidoglycan (0.1%) in feed showed an adverse effect. Sung *et al.* (1994) found similar results for high concentration of glucan, and concluded that the adverse effects were caused by gill tissue change. The mechanism of better growth probably relates to the disease resistance of shrimp. Itami *et al.* (1993) have also reported that peptidoglycan can enhance disease resistance of *P. japonicus* and increase the phagocytic activity of prawn hemocytes. Oral administration of a β-1,3-glucan derived from *Schizophyllum commune* (SPG) has produced enhanced disease resistance against vibriosis in kuruma prawn. SPG was administered orally at a level of 50 mg or 100 mg/kg body weight/d in the feed. High phagocytic activities were observed in the hemocytes of prawn fed 100 mg SPG for 3 d or 50 mg SPG for 10 d (Itami *et al.* 1994).

Based on this information, β-glucan and peptidoglycan apparently have the potential to be used prophylactically as a short-term immunostimulant for fish and shrimp. However, success of administration depended on dose and timing. Therefore, further studies are needed to find out the most practical and efficacious way of administration. The results confirm the important role of β-glucan in fish health.

HORMONES

17 α methyltestosterone

The ability to control the sex of fish populations would be advantageous to producers of economically important species, and is especially suited for prolific species such as tilapias. Androgens, 17 α methyltestosterone (MT) induced masculinization when fed to tilapia (Guerrero 1975, Nakamura 1981, Shelton *et al.* 1981); rainbow trout (Johnstone *et al.* 1978, Okada *et al.* 1979) and salmon (Fagerlund and McBride 1978). Culture of mixed sex tilapia may result in limited growth, 30-40% of the harvested fish being under marketable size. However, the use of sex steroids has sometimes yielded inconsistent results (Schreck 1974, Hunter and Donaldson 1983), presumably due to differences in duration, dose, temperature, timing of treatment, availability of natural food and species studied.

The culture of monosex tilapia has been widely practiced in Thailand, the Philippines, Indonesia and North America. Oral administration of 17 α methyltestosterone at 40-60 mg/kg feed from first feeding for a duration of 3-4 wk will produce about 98-100% male tilapia. If the fry are cultured in green water where plenty of natural food is available, then 60 mg MT/kg diet is required. This concentration can be reduced to 40 ppm when fry are cultured in clear water. The duration of treatment increases as water temperature decreases. The optimal duration treatment is 3 wk at temperature above 28 °C and 4 wk when water temperature is below 28 °C.

Concerns exist over residues of the androgen remaining in fish destined for human consumption.

Radioactivity in the carcass and viscera was evaluated in juvenile blue tilapia (*Oreochromis aureus*) (Goudie *et al.* 1986), Mossambique tilapia (*O. mossambicus*), rainbow trout (Johnstone *et al.* 1983) and coho salmon (Fagerlund and McBride 1978) fed steroid-incorporated diet (tritium and carbon^{14}-labelled MT). Radioactivity was detected in the carcass within 1 h after initial feeding and reached the highest level by 6 h. Most of the radioactivity (>90%) was in the viscera during the period when the radiolabelled diet was being fed. Radioactivity was eliminated, exponentially decreasing by 90% within 24 h after the last feeding. By 72 h, only 4% of the original radioactivity remained, and was distributed evenly between carcass and viscera. Although less than 1% of the original radioactivity remained in *O. mossambicus* 100 h after withdrawal of radiolabelled diet (Johnstone *et al. 1983*), this level was not reached in *O. aureus* until 21 d after withdrawal (Goudie *et al. 1986*). This persistence may reflect differences in experimental protocols (continued radioisotope exposure for 21 d in *O. aureus* versus a single radioisotope exposure in *O. mossambicus*).

The short-term (about 4 d) elimination patterns for both tilapia and rainbow trout were similar. The observed low levels of residual radioactivity at the conclusion of the sex reversal period, as well as anticipated dilution through growth during the culture of fish to marketable size (250-300 gm), support the conclusion that no potential health hazard exists for people who eat fish that have been fed 17 α methyltestosterone as juveniles (<1gm). At the end of the study (21 d after last feeding of radioisotope diet), only 5 ng MT/gm remained in tissue. If all the residue persisted until the fish reached marketable size, tissue levels would be only 20 pg/gm. Therefore, the use of MT for sex-reversal was recently approved by the US FDA.

ATTRACTANTS

Attractants are mainly used to enhance feed intake and growth. In farmed fish, encouraging feed consumption can increase survival and shorten production intervals, while reducing wastage of feeds that also fouls the water. Also, effective attractants would encourage the use of bland ingredients that normally would go unused. Attractants are needed in semipurified diets in vitamin, mineral or fatty acid requirement studies. Attractants will have a greater effect on feed intake and survival of young fish at the "start feed" stage. Attractants added to feed may also help offset reduction in feed intake during times of disease or stress.

It has been demonstrated that amino acids, nucleic acid-related compounds, lipids, and nitrogenous-base and sulfur-containing organic compounds are potential attractants for abalone, fish and shrimp. The effective compounds have a wide difference among the test animals. It was ascertained for all test animals that the attraction activities were present in L-amino acids but not in D-types. When effective compounds were combined, the activities for all the test animals became higher in the order two, three and four combinations. Results showed that animals were strongly attracted by these combinations rather than by individual compounds (Harada 1985a, b, 1986; Harada and Akishima 1985; Nakajima *et al.* 1989). Nakajima *et al.* (1989) found that the sulfur-containing organic compound dimethyl-β-propiothetin (DMPT) strongly prompted the sticking behavior in goldfish and common carp when included in a synthetic diet (cellulose alone). The high-potential attractants for different test animals are compiled in Tables 1-4.

Conclusions

Feed acceptance generally is not a problem in cultured fish and shrimp except:

- fry feed
- high soybean meal feed
- semipurified diet for marine carnivorous fish
- medicated feed

Table 1. L-amino acids having high attraction activity in abalone, fish and shrimp (from Harada 1985a, b, 1986; Kanazawa 1991).

Species	Chemical	Activity
Abalone	Hydroxyproline	high
(*Haliotis discus*)	Ornithine	moderate
	Lysine	low
	Glycine	moderate
	Cystine	moderate
	Tyrosine	moderate
	Hydroxylysine	moderate
Weather fish	Histidine	high
(*Misgurnus anguillicaudatus*)	Arginine	high
	Lysine	high
	Ornithine	high
	Glycine	moderate
	L-alanine	moderate
	Homocysteine	moderate
Yellowtail	Histidine	high
(*Seriola quinqueradiata*)	Arginine	moderate
	Lysine	moderate
	Ornithine	moderate
	Glycine	high
	Valine	moderate
	Threonine	high
Kuruma shrimp	Proline 0.7%	high
(*Penaeus japonicus*)	Alanine+proline	high
Atlantic salmon	L-proline	
(*Salmo salar*)	L-alanine	
	L-lysine	
Rainbow trout	L-alanine	
(*Oncorhynchus mykiss*)	L-leucine	
	L-tryptophan	
	L-phenylamine	
Brown trout	L-proline	
(*S. trutta*)	L-methionine,taurine	
	L-cystine	
	L-glutamic acid	
	L-glycine	
	Glutathione	

Table 2. Nucleic acid-related compounds recorded as potential attractants in abalone, fish and shrimp (from Harada 1986, Carr and Thompson 1983, Carr *et al.* 1984).

Species	Chemical	Activity
Abalone	AMP+xanthine+cytosine	highest
(*Haliotis discus*)	AMP+xanthine	higher
	AMP	high
	Cytosine	moderate
	Xanthin	low
Japanese eel	AMP	none
(*Anguilla japonica*)	IMP, UMP	low
	GMP	high
Yellowtail	Cytosine	high
(*Seriola*	GMP	high
quinqueradiata)	Guanine	moderate
	Adenine	moderate
	AMP	high
	Cytosine + AMP	higher
	Cytosine + AMP - guanine	highest
	Cytosine + AMP + dGMP	highest
Weather fish	Adenine	high
(*Misgurnus*	Guanosine	moderate
anguillicaudatus)	GMP	low
	Adenine + cytosine + GMP	higher
	Adenine + cytosine + GMP + guanosine	highest
Marine shrimp	AMP	high
(*Palaemonetes pugio*)	ADP, XMP, GMP, CMP, UMP	moderate

Attractants might be used in such feeds to improve taste or mask adverse taste. Two attractant formulations have been recommended for marine fish and shrimp in Japan (Tables 5 and 6). Betaine is one of the more commonly used chemo-attractants because it is commercially available. Finnsugar researchers have shown betaine to be an especially effective attractant for Atlantic salmon and rainbow trout (Gill 1989). Finnsugar has developed a betaine-based attractant called "Finnstim" which is a mixture of betaine and amino acids that is claimed to be very effective. The recommended dose is 1.5-2.0%.

Table 3. Nitrogenous bases which were potential attractants for fish and shrimp (from Harada 1985b, Harada and Akishima 1985, Pierce and Laws 1986).

Species	Chemical	Activity
Abalone (*Haliotis discus*)	Monomethylamine	highest
	Monoethanolamine	moderate
	Pyrrolidine	moderate
	R-aminobutyric acid	highest
	Choline	moderate
	Ammonium acetate	moderate
Weather fish (*Misgurnus anguillicaudatus*)	Monomethylamine	high
	Dimethylamine	high
	Trimethylamine	high
	R-aminobutyric acid	high
	Trimethylamine oxide	moderate
Yellowtail (*Seriola quinqueradiata*)	Ammonia	high
	Trimethylamine	moderate
	Pyrrolidine	low
Sole (*Solea solea*), Japanese eel (*Anguilla japonica*)	Betaine	high
Giant prawn (*Macrobrachium rosenbergii*)	Trimethylammonium hydrochoride (TMAH)	hig

Table 4. Lipid as attractants for abalone (from Harada and Akishima 1985).

Species	Chemical	Activity
Abalone (*Haliotis discus*)	Phosphatidyl inositol	high
	Tristearin	moderate
	Lecithin (bean)	low

Table 5. Composition of amino acid mixture as an attractant for marine fish (from Yone 1976).

Amino Acid	gm/100 gm diet
L-Phenylalanine	0.6
L-Arginine-HCl	1.3
L-Cystine	0.7
L-Tryptophan	0.2
L-Histidine HCl H_2O	0.2
DL-Alanine	1.3
L-Aspartic acid Na	1.0
L-Valine	0.7
L-Lysine HCl	0.6
Glycine	0.4

Table 6. Composition of attractant for marine shrimp (from Kanazawa 1991).

Chemicals	gm/100 gm diet
Inosine 5-monophosphate	0.1
Taurine	0.3
Betaine	0.3
L-Glutathione	0.1
Glycine	0.2

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