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Nutritional strategies affect heat increment and ammonia production rate in intensive fish farms

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Abstract

Due to the high toxicity, ammonia threatens fish farms more than ammonium ion. Ammonia is produced as a result of deamination of the consumed amino acids and is released in aquatic environments through gills. Production rate and excretion rate of ammonia are directly related to the level of dietary protein and the ratio of digestible protein to digestible energy of the feed. Increase in the production and excretion of ammonia in fish lead to increase in heat increment in fish.

The current study indicated that increased levels of lipids in the diet compared to the level of dietary protein, can affect the rate of produced ammonia and heat increment and reduced them. On the other hand, the current study showed that the addition of unique compounds such as phospholipids and conjugated linoleic acid (cis-9 trans-12, cis-11, trans-10 isomers) can increase the rate of absorption and catabolism of lipids in the diet. This in turn results in increasing the efficiency of enterocyte absorption of dietary lipids and its consumption in fish, which ultimately leads to saving dietary protein and reducing the rate of production of ammonia.

Keywords: Ammonia, Deamination, Phospholipid, Conjugated Linoleic Acid (CLA)

1. Introduction

Low rainfall and global warming on the one hand and increasing demand of the world population for the use of marine proteins on the other hand have led us towards optimal use of water resources in fish farms. Having clean water (the one in which water-soluble particles and the amount of harmful gases has minimized) in intensive fish culturing projects and circular system fish farms is possible using aeration, adding some chemicals and filtration, all of which require spending high amounts of money. Some parts of the filtration are done mechanically and some parts are done by biofilters.

As the experts in fish nutrition, in the present study we have tried to offer a new idea by the use of which not only the cost of running a fish farm (labor, electricity costs, depreciation of equipment, quantities of water harvesting, etc.) can be reduced, but also increase in production biomass per unit volume of water is accessible.

A change in the energy metabolism or changing the ratio of digestible protein to digestible energy of the diet can help us achieve this goal which ultimately results in a reduction in excreted ammonia resulting from deamination of amino acids and also leads to an increase in protein retention rate.

Therefore, this study pursues the following two objectives:

1. The increase in the retention rate of protein and the growth
PUC (Practical utilization coefficient) = protein retention/ protein intake (Guillaume *et al.* 2001) [12].
2. Reduction in the excreted nitrogen compounds and an increase in storage density of farmed fish per unit volume of water in circular systems

1.1 Water nitrogen compounds in fish culturing systems

Nitrogen has various forms with different chemicals in aquatic ecosystems. The most significant nitrogen compounds discussed in aquaculture include molecular nitrogen N₂, ammonia NH₃, ammonium NH₄, nitrite NO₂ and nitrate NO₃. All of the mentioned compounds are inorganic forms of nitrogen, but the organic form of nitrogen results from analysis. Although nitrogen gas is insoluble in water, but due to its high concentration in the air (about 78% of the atmosphere) it is solved in aqueous environments and has higher

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equilibrium concentration than oxygen. Equilibrium concentrations of nitrogen are reduced by increasing the temperature and salinity of the water. Increasing the amount of soluble nitrogen from the permitted range (10 to 20 mg per liter) is dangerous and causes the gas bubble disease. That is why in intensive fish culturing systems aeration systems should be used to remove nitrogen gas. In circular systems also there is the possibility of an increase in nitrogen concentration due to the fact that the dissolved nitrate can be change into nitrogen (Meade, 1989) [23]. In this case also, using aeration systems can be the solution.

1.2 Total Ammonia Nitrogen (TAN)

Ammonia is excreted in aquatic environments by fish and other invertebrates as the end product of protein degradation and deamination of amino acids (Campbell, 1973 [2], Wood *et al.*, 1989) [33]. Ammonia is combined with water and forms the ammonium ion (NH4+). The combination of ionized ammonia and non-ionized ammonia is called Total Ammonia Nitrogen (TAN).



In natural aquatic environments, ammonia is absorbed by phytoplankton and aquatic plants. That amount of ammonia which is more than the consumption of plants is changed into nitrate in two phases by aerobic bacteria called chemoautotrophic bacteria. The dissolved ammonia in circular culturing systems is converted into nitrate by biofilters and effective activities of Nitrosomonas bacteria and nitro bacteria. Ammonia is a very toxic and dangerous substance especially for salmon fish. Studies show that rainbow trout is also sensitive to low concentrations of this substance (0.02 mg per liter), but the ionized form of this substance doesn't have any danger for this species (Meade, 1989) [23].

With increasing concentrations of ammonia in aquatic environments, gills' ability to excrete ammonia to these environments through emission is reduced. Consequently, the concentration of ammonia in fish blood is increased and this in turn increases fish blood Ph which provides the filed for secondary diseases (growth of fungi and bacteria). On the one hand, gill tissue damage reduces oxygen exchange capacity which causes the need for oxygen to be more evident. This way, the cost of aeration in circular culturing systems will also increase. On the other hand, Mechanik (1957) [24] proved that by reducing the amount of oxygen, protein digestibility rate decreases and as a result growth is affected by this phenomenon.

1.3 Heat increment of feed and deamination of amino acids

As mentioned above, ammonia is the primary excretory waste of fish which is released to the aquatic environment as a result of protein catabolism (Goldstein and Forster, 1970) [10]. Age, species, temperature and pH of the environment are the important factors that affect protein catabolism rate and the amount of ammonia production. According to the energy pathway in fish diets (Figure 1), a significant amount of metabolizable energy is heat increment. Meanwhile, the heat caused by the formation and disposal of excreted materials, especially ammonia is accounted for the bulk of heat increment. Basically, the biochemical basis of heat increment in mammals and birds is the needed energy for deamination and amine nitrogen excretion (Kleiber, 1975) [19]. Of course, this energy loss in aquatic organisms is much less than

terrestrials. The reason is that, unlike terrestrials, fish need for energy is not for the production of urea and uric acid and the produced ammonia from the deamination of amino acids is easily released into the aquatic environments through gills (figure 2) (Cowey, 1975 [5], Martin and Blaxter, 1965) [22].

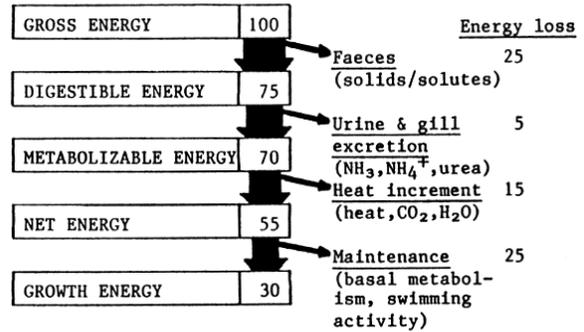


Fig 1: Schematic presentation of the fate of dietary energy for fish

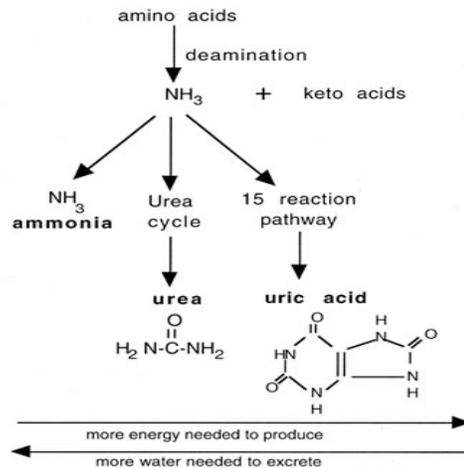


Fig 2: Deamination diagram

1.4. Heat increment and energy metabolism in fish

In the early twentieth century researchers discovered the relationship between the rise in heat increment and protein catabolism in fish and more recently it has been found that the rate of heat increment is indicative of the accumulation rate of proteins in fish. In other words, the dietary protein level affects heat increment (Diagram 1). Generally, heat increment is the total energy losses as heat when converting energy from one state to another in the course of metabolism. (Guillaume *et al.*, 2001) [12].

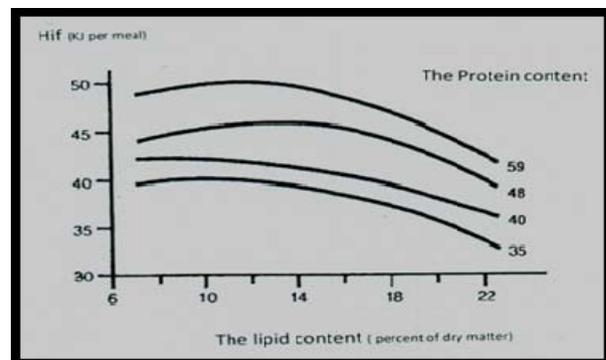


Diagram 1: Effect of perprotein and lipid content on heat increment

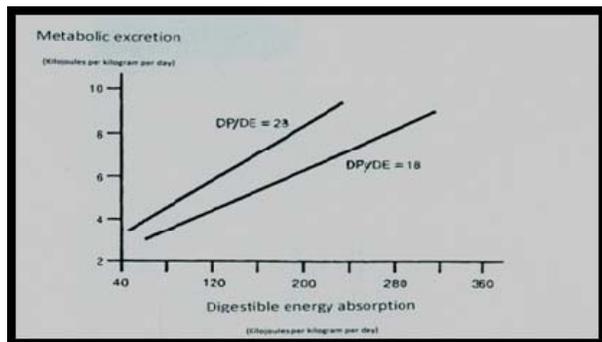


Diagram 2: Effect of DP/DE on metabolic excretion

Heat increment in fish is mainly associated with the consumption of dietary protein and allocates major amounts of metabolizable energy of food to itself. (Cho, 1982) [4] showed that heat increment for rainbow trout at 15 °C is 5% to 15% of the consumed gross energy and is reduced as the ratio of protein to energy is reduced. Although heat increment is in relation to the amount of protein consumption, it is changeable according to the ratio of protein to energy and carbohydrate to dietary protein.

Increased fat catabolism in fish through the use of high levels of protein to fat can reduce the role of dietary protein in supplying body energy and energetic activities and as a result reduces protein deamination rate in fish. This in turn, will reduce heat loss by reducing the amount of excreted ammonia. Therefore, a great amount of metabolizable energy will be changed into net energy and as a result ideal growth and reduction of ammonia emissions in aquatic environments will be achieved. In other words, reduction of heat loss is reduction of dietary protein consumption and this energy production is possible through the precise regulation of dietary fat to dietary protein ratio and achieving the correct energy balance.

There are two proper ways of achieving these goals. The first approach is using some percentage of dietary fat instead of some percentage of dietary protein and reducing dietary protein catabolism to supply energy in fish. Accordingly, Gropp *et al.* (1982) [11] showed that in diets having 30 to 40 percent of protein, 5% of dietary protein can be replaced by fat and this replacement didn't have any negative effect on growth and feed conversion ratio of rainbow trout.

Peragon *et al.* (2000) [27] found that increase in the ratio of dietary fat to dietary protein results in an increase in protein synthesis rate and its retention in fish. They believed that protein synthesis in the body and other physiological activities are directly affected by the ratio of protein to energy and that of carbohydrate to fat.

In brown rainbow trout, increase in feed energy level resulted in an increase in metabolism rate and energy consumption. On the other hand, dietary fat level influenced growth performance (Phillips and Hammer, 1965) [28]. It is worth mentioning that different levels of dietary fat and high amounts of dietary protein, both increase the heat increment, but this increase is more significant in the presence of high amounts of dietary protein (diagram 2). In other words, feed heat increment is mainly affected by the absorption of protein and the ratio of dietary protein to dietary energy. When dietary protein levels are low, increased levels of dietary fat lead to a reduction in heat increment and increase energy retention rate (Cho, 1982 [4], Cho and Kaushik, 1985) [3].

It is worth noting that the different organs of fish show different orientations towards the phenomenon of oxidation

and energy metabolism. For example, the resultant keto-acids of amino acid catabolism in the liver along with the resultant groups of beta-oxidation of fatty acids are considered as the most important energy sources. While in white muscle which has healthy and active fibers, the main source of energy is the conversion of glucose to lactate (Guillaume *et al.*, 2001) [12].

On the other hand, heat increment and metabolic excretion rate has a positive linear relationship with produced ammonia rate of protein. In other words, significant amounts of heat increment () is produced during the deamination of amino acids (Cho, 1982) [4]. The level of excreted ammonia from the gills resulted from the deamination of amino acids, especially in intensive culturing systems, not only creates high toxicity and reduces the production efficiency of farms significantly, but also increases performance of aeration systems and enhances production costs due to increased need for biological filters in these fields.

Although the use of high levels of fat in the diet may increase the net energy, it should be noted that the use of high levels of fat can (depending on fish species) cause fatty liver disease. Therefore, finding unique nutritional ingredients that increase enterocyte absorption of fatty acids and enhance the rate of fat catabolism in fish can be helpful in using high levels of dietary fat and can be considered as the second approach in achieving the above mentioned objectives. Thus, the need for conducting nutritional research in the fields of energy metabolism and investigating the feasibility of bioconversion and utilization efficiency of fatty acids as the main sources of energy seems unavoidable (Geurden *et al.*, 2006) [9].

1.5 Phospholipids and their role in the absorption and metabolism of fat:

Dietary fats are main sources of different forms of various types of lipid molecules (Lipid macromolecules) such as phospholipids which have key roles in the body. Phospholipids such as phosphatidylcholine and phosphatidylinositol play important roles in maintaining the fluidity of the biological membrane. Balancing the exact proportions of the two phospholipids and maintaining the stability of the membrane have a perfect correlation with the concentration of essential fatty acids of the diet. The choline sector of phosphatidylcholine is considered as the source of methyl groups in trans-methylation and is the lipotropic factor which is anti-bleeding in aquatic organisms. So, its presence in the diet is essential for fish to achieve good growth and feed conversion ratio (Ketola, 1976) [18].

Recent studies of marine fish have shown synergistic effects of phospholipids in lipid metabolism. Some researches attributed the increase in the digestion and absorption of fat in the intestine cells (Enterocyte) by phospholipids to the emulsification role of phospholipids in increasing the intestinal absorption of fat (Fontagne *et al.*, 1998 [8], Olsen *et al.*, 1999 [25], Liu *et al.*, 2002) [20]. Hadas *et al.* (2003) showed the positive effect of phosphatidylcholine in absorption and transferring fatty acid C18: 1 (n-9) from enterocytes to Sea bream tissue.

Several researches show that fish and crustaceans cannot make enough amounts of phospholipid needed for their maximum growth. Thus, phospholipid should be added to their diets (Hung, 1989 [14], Ketola, 1976 [18], Poston, 1991 a [29] & b [30], Hung and Lutes, 1988) [15]. The effect of adding phospholipid to diets of fish and evaluating their growth performance has been investigated in several studies. For example, Poston (1991a) [29] showed the positive effect of adding lecithin to the

diets of fish such as *Atlantic Salmo salar* and *Oncorhynchus mykiss* and the same effects were shown by Fontagne (2000) [7] in *Cyprinus carpio*.

Atar *et al.* (2009) [1], who worked on Tilapia larvae, observed the highest protein efficiency and the best feed conversion ratio in fish fed diets containing 2% soya lecithin. Increasing the amount of lecithin as the dietary phospholipid supplement showed significant impact on body weight gain of 3 g Goldfish (*Carassius auratus*) (Lochmann and Brown, 1997) [21]. Similar results regarding an increase in adipose tissue storage were observed in salmon fed diets containing soya lecithin which can be attributed to emulsifier feature of this phospholipid (Hung *et al.*, 1997) [13].

Orthoefer *et al.* (1995) [26], reported that lecithin plays important roles in membrane fabrication, improving digestion and absorption of dietary fat, increasing palatability of food pellets, reducing food leakage in water and acts as a dietary chemical absorbent. On the other hand, supplementing diets with phospholipids can save energy for the production of phospholipids in fish body and improves their growth performance (Craig and Gatlin, 1997) [6].

Therefore, understanding the interaction of dietary fatty acids and the key role of the phospholipids in enterocyte absorption of dietary fatty acids can be beneficial in increasing the efficiency of dietary fat intake and creating changes in fish diet formulation.

Besides phospholipids, another unique combination can dramatically affect energy metabolism in fish is conjugated Linoleic Acid (CLA).

1.6 Conjugated Linoleic Acid (CLA) and its role in lipid metabolism

Several studies have shown the important role of conjugated linoleic acid (CLA) in the absorption and metabolism of fatty acids. CLA refers to a group of positional and geometric isomers of linoleic acid (18: 2 n-6) (Figure 3). In CLA, double bonds are separated by a single bond. This combination is the result of isomerization and biohydrogenation of microbes in the digestive tract of ruminants.

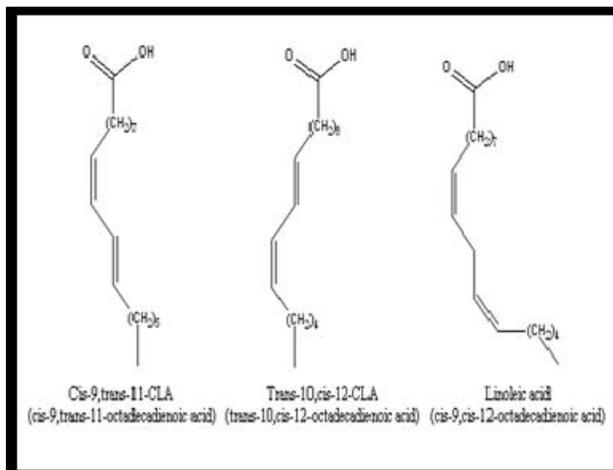


Fig 3: a group of positional and geometric isomers of CLA

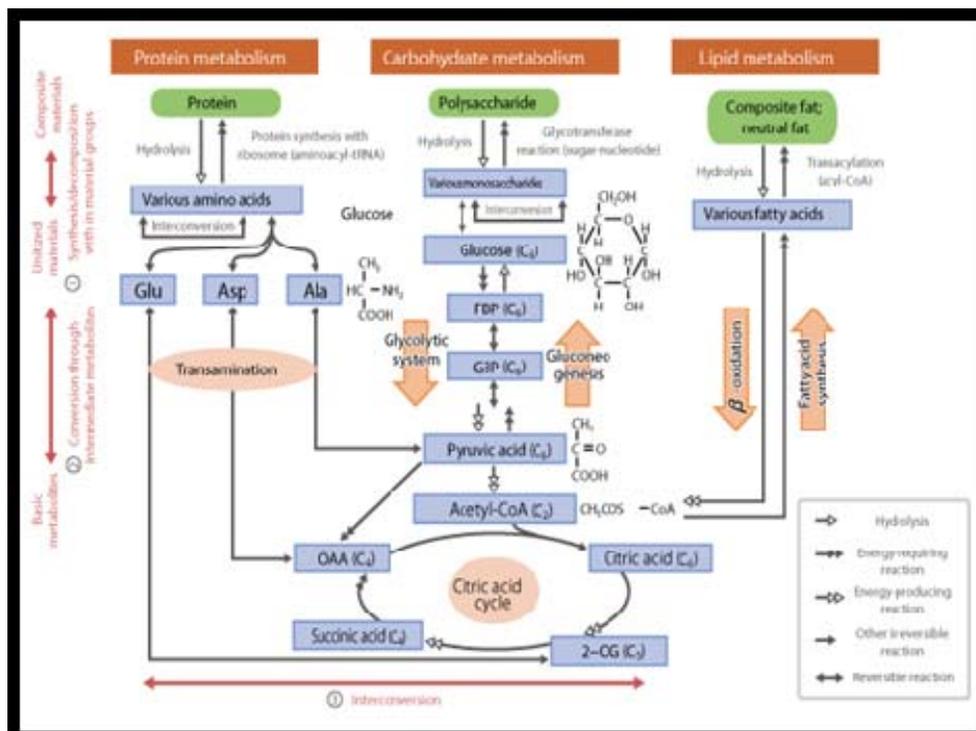


Fig 4: Protein, carbohydrate and lipid Metabolism in fish

Several studies have been conducted regarding the effect of CLA through different mechanisms including lipolysis increase in adipose tissue, increased fatty acid oxidation in muscle cells, reduction in the absorption of fatty acids in the fat cells, increase energy distribution, reduction in the size of fat cells and inhibition of the activities of enzymes involved in

lipid metabolism, but studies on human and animals have shown that CLA can reduce fat tissue. It seems that this anti-adipogenesis performance of CLA is done through regulation of glucose metabolism and fatty acid metabolism in adipose tissue (Figure 4). This feature is visible in certain forms of CLA isomers (trans-10, cis-12) (Kennedy *et al.*, 2005 [17],

Kennedy *et al.*, 2007)^[16]. However, it is worth mentioning that in nutritional studies in aquaculture, among various isomers of CLA, cis-9 trans-11 and trans-10 cis-12 are the two most important (Zhao *et al.*, 2008^[36], Kennedy *et al.*, 2005^[17], Kennedy *et al.*, 2007^[16], Sang *et al.*, 2007^[31], Zanqui *et al.*, 2013)^[35].

Yang *et al.* (2014)^[34], who investigated the effect of CLA on growth, fat metabolism and plasma lipid level of Amur sturgeon (*Acipenser schrenckii*), showed that with increasing levels of CLA from zero to 2%, significant reductions were observed in the amount of fat in muscle and liver as well as serum cholesterol level.

Zue *et al.* (2013)^[37] reported that gene expression of Acyl coenzyme A oxidase and Carnitine acyl transferase I, which had important roles in lipid biosynthesis especially in PUFA in yellow Crocker (*Larmichthys crocea*), decreased as the CLA level increased in the diet (from 0% to 1.7%).

Valente *et al.* (2005)^[32] investigated the effect of dietary CLA on chemical composition of muscle and liver of adult trout. The results of their study indicated that the inclusion of CLA in the diet decreased the activity of malic enzyme and hepatic fat. They also observed that reduction in malic enzyme synthesis resulted in decreased lipogenesis (Figure 4) and indirectly increased the rate of beta-oxidation of fatty acids.

Considering the important roles listed for CLA fatty acid, the need for studies to be conducted regarding the interactions between this composition and phospholipid supplements in increasing the absorption and metabolism of fatty acids seems unavoidable.

2. Results

The results showed that heat increment allocated the bulk of metabolizable energy to itself. Meanwhile, a large amount of heat increment is derived from the deamination of dietary fatty acids or is the result of amino acids which result from breaking down the dietary protein chains.

The high rate of amino acid deamination not only consumes energy and increases heat increment, but also excretes harmful ammonia to aquatic environments. The obtained results from several studies indicated that increase in dietary energy to dietary protein ratio results in saving protein consumption, which is the main source of energy, and reduces the production of excreted ammonia. These will in turn increase growth performance in fish culturing farms. First, protein retention rate and pure energy will increase. Second, reducing ammonia stress will increase growth performance and decrease farmed fish diseases in intensive fish farms. Finally, productivity increase per area unit and water volume in fish farms will be achieved. Therefore, the followings are suggested:

- 1) In order to lower production costs, it is better to use nutritional strategies instead of biofloc system and biofilters which can inhibit ammonia excretion intensive fish farms.
- 2) To reduce the cost of fish feed, depending on the species, certain percentages of fat should replace dietary protein.
- 3) To increase enterocyte absorption of dietary fatty acids, depending on the species, specified level of supplementary phospholipids should be used.
- 4) Depending on the species, using the proposed level of CLA is suggested to increase the efficiency of fat consumption (fat absorbed through food or catabolism of tissue fat).
- 5) Phospholipid supplements and CLA should be used together to lead to higher protein retention rate and net energy rate compared to the time they are used alone.

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