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Replacing Fish Meal with Plant Proteins and Amino Acids: Effects on Growth and Physiology

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Abstract: The objective of this work is to compare the capacity of fish meal to be replaced by plant protein sources; soy protein concentrate (SPC) and wheat gluten meal (WGM) with essential amino acids (EAAs). The first objective was to assess the effects of this replacement on growth rate, feed conversion, metabolic shift, and sustainability of the aquaculture industry. The results indicated that EAAs supplemented plant diets resulted in comparable gain in body weight, SGR and FCR as those obtained with fish meal. The supplementation with EAAs enhanced protein utilization hence supplementing amino acid deficiencies in the plant products used in the formulation improving feed conversion rate. Biochemical and hematological analysis of the fish showed that the fish was normal in its biochemical parameter, immunity and stress, which implies that feed formulated from plant source did not in any way threaten the health status of the fish. On the whole, the outcomes clearly indicate the notion that the utilization of the plant-originated protein through the utilization of the required amino acids will be beneficial for elevating high level reactions in the immune systems as well as improving the overall physiology of the aquaculture species. In addition, the research emphasizes the ecological and economic impact of fish meal with substitute plant proteins since it minimizes the dependency on marine stocks, and reduces feed costs, as a result it enhances the efficiency of the aquaculture systems. Therefore, this study concludes that for SPC and WGM when supplemented with essential AA are possible replacers of fish meal and the technique seems to hold bright future in sustainable fish production. This means that; the future studies require the accumulation of more detailed data on protein formulations in the plant that improves feed quality besides considering the endogenous impacts on health, fertility, growth, and resistance to diseases in fish. Consequently, it is possible to state that the outcomes derived belong to the degree determining the readiness to use plant-based diets within the framework of increasing consumers' selfish consumption and developing the practice of ecoal friendly aquaculture methods.

Keywords:

Fish meal replacement, Plant proteins, Soy protein concentrate, Wheat gluten meal, Essential amino acids, Aquaculture sustainability.

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Introduction

The following is a brief discussion of the development of aquaculture as a viable sub-sector of food production currently meeting a global essential need for seafood. Fish meal is spotlight in the feed ingredients of aquaculture due to its quality proteins, essential amino acid profile, biological values and palatability of fish. However, use of fish meal has the following drawbacks: Here are however the following sustainability issues coming out of the fish meal; high pressure on the fish, pollution, high cost of feed and competition with other animals deemed it fit to look for other source of protein in aquaculture feeds [1]. Soy protein concentrate and wheat gluten meal of plant origin are the observed protein sources they have capability to replace fish meal in diets for aquaculture fisheries. These are easy to obtain ingredients that will not cost much to obtain and the added bonus is that green in nature. However, substitution of fish meal with plant proteins has some issues because such proteins have disadvantages, such as low EAA digestibility, the presence of ANFs, and low acceptability. However, if not well managed, such aspects may lead to low growth efficiency, feed conversion and other patho-physiological effects on the fish [2].

To overcome these challenges; the supplementation of plant protein base diets with essentiality has been researched for widely. The EAA, as Methionine, Lysine and threonine are very essential in fish as are in other animals in the synthesis of their proteins and in their growth and metabolic activities. Therefore, it is possible to supplement plant protein diets with these amino acids in order to obtain growth promoting nutrients that are in line with fish meal and enhance feed conversion ratios [3]. This paper examines the concrete and beam performance of full-depth precast pre stressed concrete pavement bridge decks SLABs with varying concentrations of soy protein concentrate and wheat gluten meal with essential amino acids as fish meal substitute for fish intake, nutritional efficiency, and physiology of the fish. Soy protein concentrate at high protein and containing much lower ANFs than raw soybean meal; wheat gluten meal contributes additional amino acids and digestive energies [4]. One of the implications of this kind of plant protein sources is that it can, together with formats above, offset the position of fish meal as the future diversified resource. In relation to the above stated objectives, the following study aims at assessing the impacts of these diets on growth rates feed converter ratio, feed digestibility and other key factors

that are characteristic of fish physiology in order to establish the feasibility of using PP sources in diet formulations of fish species that are grown under aquaculture systems. Further, it fosters the attempt trying to optimize feed formulation and application procedures, which help to make aquaculture positive to the environment and give relatively cheap but high stock yields [5].

Materials and Methods

This section outlines the design of the experiment, the development of the diets, management of the fish and method applied to assess the effects of replacing fish meal with PP and EAA supplemented diets.

Experimental Design and Diet Formulation: As the part of current research experimental diets were prepared to feed the fish with partially or wholly replacing fish meal with soy protein concentrate (SPC) and wheat gluten meal (WGM). The protein sources were also enriched with essential amino acids (EAA) where methionine, lysine and threonine incorporated per the fish meal amino acid profile to increase the dietary nutritional value [6]. The control diet included fish meal as the major protein source in order that relative impacts of the experimental diet might be assessed. These diets were prepared with different percentage of fish meal replaced (for instance; 25%, 50%, 75% and 100%) to evaluate the impact of replacement level of fish growth and metabolic rate. All diets were of similar nitrogen and energy concentrations in order to make the various diets fed to the animals to contain similar protein and energy. The Proximate analysis on feeds determined that feed ingredients subjected to fine grinding, mixed properly and pelleted according to the preferred sizes of the experimental fish [7]. The feed pellets were also dried and kept in appropriate environmental condition in order to maintain proper feed quality for different phases of the experiment.

Fish Species, Rearing Conditions, and Feeding Trial: It was conducted on a commercially important finfish species that is farmed, such as Nile tilapia or rainbow trout. Both harvest juveniles of similar average body length and good physical health were employed and acclimatized to the test conditions before the start of the experiment. Fish used in each of the treatments tanks or cages had replicate groups of fish under similar environmental conditions of dissolved oxygen,

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Temperature, pH and ammonia levels [8]. The fish were also divided under different treatment groups and each diet type was added to several tanks as a comparison to the others. Fish were administered feed at standard feeding ratio either in percent body weight feed or several times a day. Feed intake throughout the duration that the feed was consumed and any remaining after the time was also taken while calculation feed conversion ratios [9].

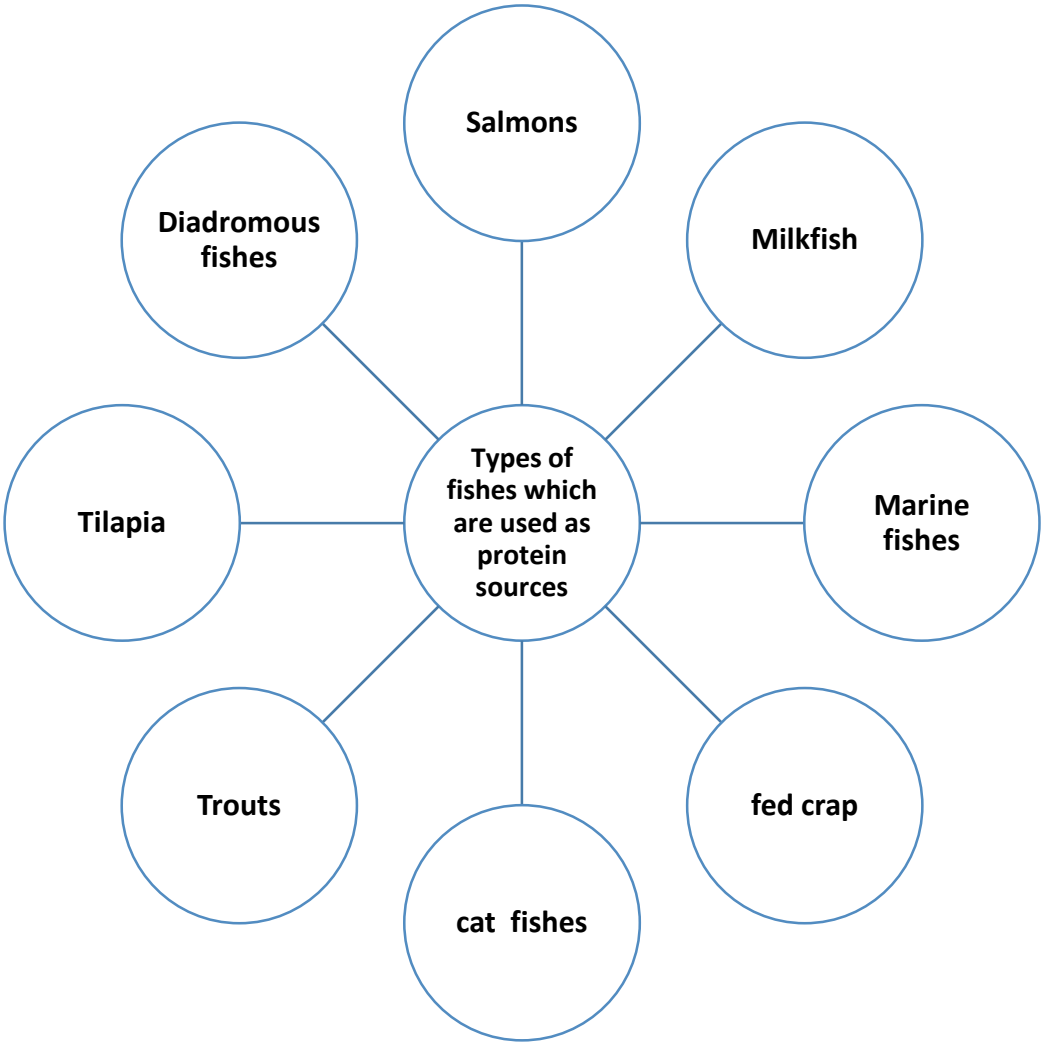

Figure: 1 showing types of fishes which are used as protein sources

Sampling and Data Collection: Growth rate in terms of weight, standard length, and condition factor, as well as survival rate during the feeding trial of fish was noted from time to time. At the

end of the trial, the fishes were fed to determine the nutrient metabolic ability, protein productive value, blood chemistry, hematology and immunology. The nutrient utilization of the diets and fish tissues was therefore established by proximate composition analysis [10].

Statistical Analysis: All the data gathered were analyzed statistically in order to establish the significance of the aforementioned various treatments offered. All collected data were statistically analyzed by applying correct statistical tests, namely analysis of variance (ANOVA) and comparisons of means, specifying with a significance level of $p < 0.05$. All data are expressed as mean ± standard error [11].

Growth Performance

Growth performance is thus a major component that defines the level of nutrition in experimental diets used in fish farming. The impact of fish meal replaced by the blend of SPC and WGM with EAAs on fish growth rate and weight gain were also considered based on five major growth performances trial inclusive weight gain, SGR and survival rate [12].

Weight Gain and Specific Growth Rate (SGR): Increase in weight is a sure signal of the kind of utilization of the provided diets for growth in fishes. It indicates that diet can afford the nutrients that fulfill fish physiological requirement as far as protein and energy and some essential nutrients. The other species of fish that were fed on diets formulated at higher fish meal replacement levels of 75 %, 100 %, had growth constraint due to deficiencies of amino acids or anti –nutritional factors (ANFs) in plant based materials. These deficiencies however can be prevented by supplementing plant based diets with the EAAs required for protein synthesis and energy for growth in fish [13]. The business-like growth rate, or the specific growth rate (SGR) of the body weight change per day gives a direct handle in comparing the growth under different treatments. In the present investigation, such diets containing plant proteins sources and supplemented with essential amino acids were examined on S.G.R of fish by comparing with fish meal fed group. Therefore if there is a good replaceable strategy the SGR values of the experimental and control group should be almost equal [14].

Feed Conversion Ratio (FCR): FCR stands for feed conversion ratio It is one of the best measures that is used to estimate the feed efficiency. It is stated in term of a unit of feed consumption relative to an increment in body weight. Altogether a lower FCR value represents better feed conversion ratio, feed cost effectiveness and actual feed conversion for the bird. FCR improves when high fish meal replacement is obtained due to poor nutrient digestibility or feed palatability which are characteristic to low quality feeds. However, supplementation of Essential Amino Acids is aimed at enhancing FCR through enhancing protein utilization and reducing the N output [15].

Survival Rates: Other parameter that may define the wellbeing and stability of fish under experimental conditions is survival rate. Every effect seen in fish fed with PP diets has a positive outcome which further confirms that the replacement strategy has no impact whatsoever on the physiological state and stress tolerance of the fish. Where feed formulated with high fish meal replacement results in poor survival this may be due to a wrong nutrition balance or anti-nutritional efficacy of the food source [16].

Key Findings: The growth performance data from this work helps in the evaluation of the capacity to replace fish meal with plant protein source. An optimum growth achieved with the aid of EAA supplementation will aid in improves or further weight gain, SGR, FCR and survival to diets containing fish meal. The knowledge provided in this article can be useful for dispositions of economic, rational and inexpensive aquaculture [17].

Feed Utilization

In evaluating the efficiency of an aquaculture diet, feed utilization is the most effective way of determining growth performance and the general production costs of the diet. This parameter consist of factors like nutrient utilization, feed to meat or feed to gain ratio, and protein quality. Thus, this study case investigated how effectively fish could assimilate nutrients and transformed ingredients into body weight when fed on diets where fish meal was replaced with SPC-WGM blend, supplemented with EAAs [18].

Nutrient Digestibility: This is the extent to which nutrients that go into the fish are assimilated by the fish and assimilated by the different tissues in the fish. It depends with the soluble and insoluble proteins in the diet, the facility of the fish to digest and absorb nutrients, as well as the actions of ANFs in diets with high plant protein content. Soy protein concentrate and wheat gluten meal are acknowledged to possess high digestibility value when compared with raw material of plant origin but they also contain some anti-nutritional factors (such as trypsin inhibitors and lectins) which may affect the bioavailability of nutrients available to the animal [19]. Since the true digestibility coefficients of the nutrients could not be ascertained in this instance, the immediate practical approach was used and the apparent digestibility coefficients (ADC) of the diets for protein, lipid, and carbohydrate nutrients were therefore calculated. The ADC determines the amount of nutrient which gets assimilated and gets used by the fish, and therefore gives indication of the nature of diet that is being fed to the fish and how effective it is in delivering the nutrient to the fish. This work intend to highlight whether supplementing essential amino acids would overcome possible adverse impacts of plant protein on feed digestibility and conversion heterogeneities [20].

Protein Efficiency Ratio (PER): The PER stands as an important factor that defines the ability that the fish have to convert the protein feed into body tissues. The surrogate for dietary protein is weight gain, and it is measured per portion size of protein consumed. Generally fish fed on plant protein diets perform poorly in PER than those fed on fish meal based diets, which could be attributed for in differences in amino acid composition. Though, incorporating essential amino acids like methionine, lysine and threonine into plant based diets is used to achieve optimal utilization of amino acids in the body [21]. This means that the higher the PER better off the fish are because they are efficiently using all the available protein for growth especially where fish meal is partially or fully replaced. In this study PER was determined for fish fed diets with different levels of fish meal replacement (25%, 50%, 75%, and 100 %) with special reference to the diets enriched with essential amino acids. This enabled a direct comparison in protein utilization between the various dietary treatments [22].

Feed Conversion Ratio (FCR) and Efficiency: FCR, more specifically feed conversion ratio, makes it possible to have an understanding of how effectively fish use feed to gain weight. FCR

mean low feed conversion ration hence less feed is needed to produce a unit of growth as compared to FCR. Fish meal is usually replaced with plant proteins and this leads into high FCR values mainly because the availability, digestibility and amino acid profile of the plant ingredient may not match that of fish meal. However, since plant-based diets lack certain amino acids locally, it is believed that FCR could be enhanced making plant-base diets more economical. From these multiple diets, FCR was determined and of prime interest was the FCR of fish fed the diets containing plant protein and EAA compared to fish fed the diets containing fish meal. A decrease in FCR for the supplemented diets indicates an implication of essential amino acids in improving feed conversion ratio [23].

Implications for Sustainable Aquaculture: Efficient feed use through proper choice of diets is central for survival and profitability of aquaculture, 60. Optimal feed conversion improves the feed to gain food ratio in the animal and therefore decreases the feed expenses in production hence easing the problem of high feed wastage and the pollution of watersheds by leaching of nutrient from consumed feed [24]. The findings of this study highlight the potential strategies for enhancing the efficiency of feed for fish production by substituting fish meal by affordable sources of plant proteins' and subsequently supplementing the resultant substandard diets with essential amino acids so as to make the process of Aquaculture more sustainable and economically viable. Through increasing nutrient digestibility, protein utilization and feed conversion efficiencies, this study presents avenues by which the socially responsible growth of aquaculture may be realized. The findings could be useful in reducing the reliance on fish meal; hence, assist in protecting the aquatic resources while ensuring that an increased production and value of fish live up to anticipation [25].

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Figure: 2 showing implications for sustainable aquaculture

Physiological Responses

In this study, physiological responses are important biomarkers to evaluate the performance of fish to various dietary formulations because they provide information on the health state, stress, immune system and metabolism of the fish. In this study emphasis was placed on recording the changes in the growth physiology as affected by the replacement of fish meal with SPC and WGM fortified with EAAs. This understanding therefore forms the basis of assessing the postulated plant based diet on fish welfare and broader aquaculture production outcomes in the long run [26].

Biochemical and Hematological Parameters: Analysis of blood serum composition, enzyme activity as well as concentrations of different metabolites in fish provide important information about metabolic processes proceeded in fish organism. A shift in the biochemical composition can show how the fish are adapting to alteration in diet as per their ability to assimilate nutrients and energies, metabolic condition basically [27]. For instance, plasma protein levels, glucose and lipid

profiles, are some of the parameters used to determine nutritional state in fish. If a fish has an optimal performance and the nutrients that have been supplied could be properly assimilated then the biomarkers should be standard. These parameters were first scrutinized with a view of establishing any side effects arising from replacement of fish meal with plant proteins. Also, the enzyme indicators of mortality of liver cells, alanine-aminotransferase and aspartate non-aminotransferase were determined, which allow assessing the state of liver functioning and overall oxidative stress. This would suggest that substantial variations in normal enzyme levels might be caused by stress or liver disorders stemming from dietary disturbances or anti-nutritional factors in plant proteins [28].

Additional hematology data such as erythrocyte count, hemoglobin, and leukocyte count were also determined to give information about the immune condition of the fish. A lower than normal RBC count or hemoglobin levels mean the body might be anemic due to low nutrient intake and nutrient deficiencies while high levels of white blood cells would mean the immune system is stressed or there is an infection. Thus, comparing these parameters in the course of different diet treatments revealed possible adverse effects of plant protein-based diets on fish health [29].

Immune Responses: Another befitting aspect of physiological function whose intensity is affected by diet is the immune system. Fish are always under pathogen challenge in aquaculture systems; therefore, immune health is critical to provide defense against diseases. There are many ways to assess immune responses some of them being through degrees of activity of strategic immune cells such as macrophages and lymphocytes, and levels of immune proteins yeast Immunoglobulins and cytokines. Here, immune responses were measured to determine the impact of plant diets on immune competence of fish [30]. The addition was thought to keep immune system robust through sufficient synthesis of proteins and immune mediators based on intake of essential amino acids as ingredients in the diets. This would mean that fish fed on plant protein based diets lacking the EAAs would have lower immune function which will have an implication of them getting diseases and stress easily. Moreover, the protein oxidation by products, the level of pro- and antioxidants were asses in order to determine the state of the oxidative equilibrium in fish fed the experimental diets, including the activity of antioxidant enzymes (SOD, catalase). Hyper-oxidative states

defined by the ROS and antioxidant system ratios suggest cellular stress from dietary induced metabolic and inflammatory changes [31].

Stress Indicators: Stress is a physiological reaction to unfavorable environmental stimuli and may be precipitated by poor diet or imbalances in nutrient intake. High stress levels in the human body cause stunted growth, hampered immune system, as well as a predisposition to fall ill. Pinch indicators including cortisol levels were evaluated to determine the level of stress the fish developed due to different diets. Stress has many definitions, but in this study, stress is defined as any factor that causes a rise in cortisol level in fish. This physiological stress might be indicated by increased cortisol levels in fish fed on diets with higher levels of fish meal replacement and could result from reduction in nutrient profile, anti-nutritional factors, and imbalanced amino acid. On the other hand, diets containing DAA are anticipated to cut short stress consequences through enhanced growth and metabolism [32].

Organ Health and Histopathology: Effects on crucial organs including the liver, kidney and gut to assess the physiological changes that accrue from plant-base diets were also assessed. Liver and kidney tissues were collected and histo pathological changes in the tissue were checked for toxicity, inflammation or organ damage for effect of dietary composition. The gut is another organ responsible for nutrient absorption, and since anti-nutritional factors or poor protein quality in diets can cause structural changes in the intestinal tissue, we were able to make this determination using histological evaluation of the tissue samples [33].

Implications for Aquaculture: The physiological effects, namely changes in the biochemical profile, immunoc ompetence, and stress and tissue health in fish demarcate the impact of dietary alterations. This information is of significance for appraisal of consequences of the indicated diets in relation to healthiness and celebrity of farm animals, belonging to the aquaculture category [34]. It is thus possible to avoid adverse impacts and at the same time promote growth and reproduction, only if replacement amino acids are incorporated into plant ingredients in a way that would not limit the fish's development in enclosures during aquaculture. The results of this study may be

useful in manipulating feed formulation for fish to more efficient, lower cost, and nutritious diets to foster healthier aquaculture [35].

Discussion

The result of the present study is useful to understand the possibility of using new formulated diets in which fish meal is replaced with plant protein sources namely soy protein concentrate (SPC) and wheat gluten meal (WGM) blended with essential amino acids (EAAs). In later years as the demands throughout the world for seafood increases, concern towards sustainability to fish meal is a very big issue and so finding out for other options are very important. An extended discussion is made on the consequences of these findings in relation to growth performance, feed conversion ratio, physiology, and overall future trends in aquaculture [36].

Comparison with Fish Meal-Based Diets: Fish meal has been the main ingredient used worldwide on aquaculture feeds because of its high digestibility, balanced amino acid and overall nutritional value. However, when fish meal sources diminish due to increased fishing pressures, and production costs rise, it remains crucial to seek plant-based ingredients. The findings of this work reveal that it is possible to replace fish meal in the aquaculture diets using SPC and WGM when these ingredients are fortified with the essential amino acids without negatively affecting growth characteristics or feed conversion [37]. Indeed, growth parameters like weight gain, SGR and FCR were also similar with the fish meal based control diet and the plant based diets, particularly the diets supplemented with essential amino acids. This proves that with the right supplementation plant derived protein sources can substitute fish meal. But as we said earlier, the level of fish meal replacement affected performance though not in the same magnitude as the first trial. Fish fed on the diets where fish meal was fully replaced had some relatively lower growth rate than the fish in the other intermediate level replacement diets. This indicates that despite being adequate for growth, plant proteins do not mimic exactly the amino acid balance, and digestibility of fish meal even after supplementation. Methionine and lysine which come along with amino acids have a special role on growth and the supposed physiological balance between them is

stressed and general supplementation to achieve similar growth rates to fish meal based diets is emphasized here [38].

Essential Amino Acids and Protein Utilization: One of the interventions in this work was the addition of indispensable amino acids into plant diets which could often be lacking in plant proteins. It should be quite evident from the above results that supplementation with essential amino acids effectively enhanced the status of protein utilization as per the two index the PER and FCR. Low PER and high FCR in supplemented plant based diets dictate the significance of EAAs in enhancing dietary protein utilization. This serves to underscore the idea that although plant proteins are cheap and bountiful, many of them need to be amino-acid enhanced of benefit in the preparation of fish [39]. This was complemented by the effects of the supplementation on counteracting the effects of potential growth limiting factors which include the ANFs and suboptimal amino acid profiles in plant based ingredients. While SPC and WGM supplied much needed protein, the addition of essential amino acids permit optimized nutrient utilization, protein deposition as well as growth? Hence, the need to support the notion that diets containing plant protein may provide equivalent nutrients to fish meal if supplemented properly [40].

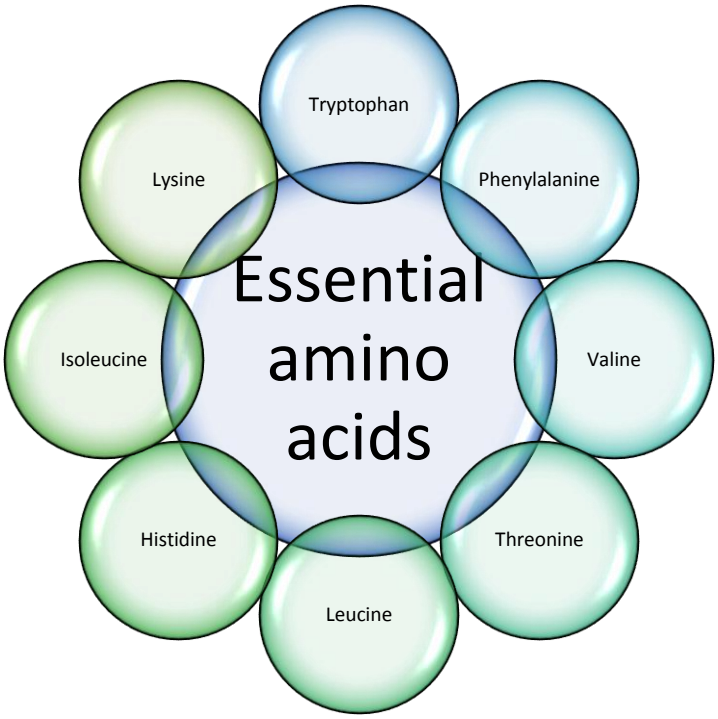

Figure: 3 showing essential amino acids

(Shoaib & Shakeel. 2024)

Physiological Implications: The overall immunological and stress performance of the fish fed the formulated plant protein based diets with selected EAAs were not significantly compromised and the overall health of vital organs was not significantly affected. There were no relevant differences of hematological and biochemical variables, which are RBC count, serum glucose level and liver enzymes activity with the control group; this explains that the plant based diet that is properly balanced do not cause nutritional stress or a threat to the general health of the fish. These findings are relevant for promoting plant protein containing diet for growth as well as fish well-being in aquaculture production [41]. Surprisingly, no differences existed in the immune responses between the fish fed on the plant diets and those fed on the fish meal diets, an implication that EAAs are essential in immune function. This is especially relevant in aquaculture since diseases may cause severe loses to the producers businessmen. The identity of the plant proteins used can be optimized to the corresponding desirable amino acid makeup and thus provide high immune competency even under a fish meal-less diet [42].

Sustainability and Economic Implications: The reason of this study can be attributed to the fact that presently, the dietary ingredients used in feeding fish in aquaculture are very expensive. The production of fish meal has been cited to have major effects on the environment such as over fishing, habitat destruction and high resource use. Using SPC and WGM instead of fish meal helps to liberate pressure on the marine resources and improve the sustainability of aquaculture system. Moreover, plant proteins are more cheaply compared to fish meal since their prices vary depending on the availability; therefore reducing cost of production in aquaculture [43]. The results of this study, therefore, provide evidence for the feasibility of widely embracing plant-feed-based diet in commercial aquaculture system. By supplementing these diets with indispensable amino acids, fish farmers are likely to enhance the overall growth performance and tangible feed conversion ratios that reduce feed costs thus enhancing productivity. Also, the inclusion of plant-based proteins in fish farming can offshoot desires of protein foods by offering plant-based proteins which are in plenty and do not harm the aquatic life as it is the case when we over-fish the seas [44].

Future Directions: However, as the study shows the applicability of SPC and WGM for fish meal replacement it also marks out some opportunities for future research. Further studies could be directed toward the assessment of a long-term fitness impact of plant-protein containing diets on reproduction, disease susceptibility and general health of fish. Furthermore, improving upon the plant protein blends with a view of optimizing nutrient density and minimizing anti-nutritional effects might increase the effectiveness of these diets. Research could look at supplementation of other plant derived protein sources like pea protein, canola meal and microalgae which could further categories the sundry to fish meal [45]. In particular, further research must be done to advance feeding technologies so that as the growing industry expands, aquaculture can feed fish both efficiently and sustainably to meet the global demand for seafood. This work defines the possibilities for using plant proteins with added essential amino acids in the diets of aquaculture fish stocks and, therefore, opens vast opportunities for making fish farming more efficient and less environmentally burdensome.

Conclusion

The results of the present investigation establish that fish meal can effectively be replaced with plant protein sources such as soy protein concentrate (SPC) and wheat gluten meal (WGM) in feeds of aquaculture with EAAs. From the research, there are several crucial areas of concern that would lead to the chances of coming up with sustainable and economic means in fish meal in formulation of fish feed. Fish fed the plant based diets with the added EAA were as growth performing and feed efficient as fish fed a traditional fish meal based diet. Growth rate, measured by weight gain, SGR and FCR showed also that replacing fish meal did not affect the growth rate except that the diets have to be adequately fortified with essential amino acids. This implies that, if well formulated, plant proteins can supplement fish growth and feed conversion well thus can be an excellent source instead of fish meal.

Supplementation with essential amino acids, which corrected for deficiencies compare to animal proteins identified in plant based proteins, was a key factor in improving the rate of protein deposition and proper balance of amino acid profile in the body. This discovery increases the need

to accurately prepare plant-based meals for fish such that their nutrient needs are met and points to the fact that incorporating amino acids is essential for the best results. As per the physiological changes, it was observed that plant based diets incorporated with EAAs had no adverse impacts on the biochemical, hematological or immune status of the fish. Approximately 50, 254, and 26 % of the fish fed the experimental diets returned values for blood serum composition, liver enzyme activity, immune cell activity, and stress indicators into the normal range, which means that the fish were able to acclimate well to the dietary changes. Thus, it can be suggested that if sufficient essential amino acids are provided to the fish through plant protein based diets, the fish health and immune status are at optimal level necessary to fight diseases and to survive the conditions in aquaculture.

These findings indicate that fish meal can be replaced by plant proteins in the diets without any adverse effect on the physiological health of fish if the diet is properly formulated and supplemented with nutrients required for satisfactory growth and development of the fish. This is more apparent in the view of intensive commercial fish farming where the health of fish is a critical factor in a profitable venture. Perhaps, the greatest of the implications of this study is on the sustainability of aquaculture. Fish meal is one of the food inputs that have undesirable impacts on the marine environment, they exert pressure on the fishing industries which leads to over fishing. It was also found that the substitution of fish meal by plant proteins like SPC and WGM not only makes aquaculture which is least impacting the environment but also more certain nutrition supply. In addition, plant-based proteins are evidently cheaper to fish meal with an indication that reduction of feed costs will increase the economic efficiency of fish farming systems.

Plant protein sources in aquaculture can also be expected to address the increasing worldwide demand for seafood in order not to endanger fish populations in the seas, as well as contribute to a solution to the problem of feeding the world's population. With the advancement in production technology and globalization, aquaculture is gradually moving up to feed the growing fish products market, plant based diets with appropriate fortifications are sustainable and cheaper than fish meal. However, the outcome of this study encourages more research to be done concerning the impacts of plant based diets on fish growth, fertility and disease survival rates. Further, the practical aspects

such as enhancement in the rate of plant protein digestibility and nutrient utilization and reduction or control of anti-nutritional factors should also act as a reason for further research and development on plant protein formulations. Other sources of plant proteins like peas, lentils or microalgae also seems to offer more options to fishmeal and are also worth exploring.

However, future research could focus on the effects of distinct amino acids composition and supplement approaches of fish and pouches as the nutrient needs may comprise variations across multiple fish species. Appreciating the nutritional value of plant-derived ingredients and responding to species' particular requirements entails great opportunities for the improvement of the sustainability of aquaculture production systems. The study confirms the possibility of the use of plant protein sources with essential amino acids as a replacement for fish meal in feeds without affecting the growth, feed conversion and the general physiological well-being of the fish. This approach offers a relatively unexplored strategy to deal with environmental penalties of aquaculture and increase the economic efficiency of fish production. These dietary change approaches could be instrumental in meeting the global demand of seafood with environmental sustainability and efficient economic returns to aquaculture industries. Therefore, further research and development studies will be necessary in the future to standardize plant-based diets to satisfactorily feed fish while at the same time promoting sustainable food production for the future.

References

[1]. Bulbul M, Koshio S, Ishikawa M, Yokoyama S, Kader MA. Performance of kuruma shrimp, Marsupenaeus japonicus fed diets replacing fishmeal with a combination of plant protein meals. Aquaculture. 2013; 372:45-51

[2]. Cabral EM, Fernandes TJ, Campos SD, Castro-Cunha M, Oliveira MB, Cunha LM et al. Replacement of fish meal by plant protein sources up to 75% induces good growth performance without affecting flesh quality in on growing Senegalese sole. Aquaculture. 2013; 380:130- 138.

[3]. Rossi W, Moxely D, Buentello A, Pohlenz C, Gatlin DM. Replacement of fishmeal with novel plant feedstuffs in the diet of red drum Sciaenops ocellatus: an assessment of nutritional value. Aquaculture Nutrition. 2013; 19(S1):72-81.

[4]. Rodiles A, Herrera M, Hachero-Cruzado I, Ruiz-Jarabo I, Mancera JM, Cordero ML et al. Tissue composition, blood biochemistry and histology of digestive organs in Senegalese sole (Solea senegalensis) juveniles fed diets containing different plant protein ingredients. Aquaculture nutrition. 2015; 21(6):767-779.

[5]. Suprayudi MA, Inara C, Ekasari J, Priyoutomo N, Haga Y, Takeuchi T et al. Preliminary nutritional evaluation of rubber seed and defatted rubber seed meals as plant protein sources for common carp Cyprinus carpio L. juvenile diet. Aquaculture research. 2015; 46(12):2972- 2981

[6]. Bonaldo A, Di Marco P, Petochi T, Marino G, Parma L, Fontanillas R et al. Feeding turbot juveniles Psetta maxima L. with increasing dietary plant protein levels affects growth performance and fish welfare. Aquaculture nutrition. 2015; 21(4):401-413.

[7]. Yu DH, Gong SY, Lin YC, Yuan YC. Partial replacement of fish meal by several plant proteins with or without iron and lysine supplement in diets for juvenile Chinese sucker, Myxocyprinus asiaticus. Aquaculture nutrition. 2014; 20(2):205-212.

[8]. Minjarez-Osorio C, Castillo-Alvarado S, Gatlin DM, González-Félix ML, Perez-Velazquez M, Rossi W. Plant protein sources in the diets of the sciaenids red drum (Sciaenops ocellatus) and shortfin corvina (Cynoscion parvipinnis): A comparative study. Aquaculture. 2016; 453:122-129.

[9]. Espe M, Lemme A, Petri A, El-Mowafi A. Can Atlantic salmon (Salmo salar) grow on diets devoid of fish meal? Aquaculture. 2006; 255(1):255-262.

[10]. Bulbul M, Kader MA, Ambak MA, Hossain MS, Ishikawa M, Koshio S. Effects of crystalline amino acids, phytase and fish soluble supplements in improving nutritive values of high plant protein based diets for kuruma shrimp, Marsupenaeus japonicus. Aquaculture. 2015; 438:98-104.

[11]. Zhang Y, Øverland M, Shearer KD, Sørensen M, Mydland LT, Storebakken T. Optimizing plant protein combinations in fish meal-free diets for rainbow trout (Oncorhynchus mykiss) by a mixture model. Aquaculture. 2012; 360:25-36.

[12]. Papatryphon E, Soares JH. Optimizing the levels of feeding stimulants for use in high-fish meal and plant feedstuff-based diets for striped bass, Morone saxatilis. Aquaculture. 2001a; 202(3):279-288.

[13]. He S, Liang XF, Li L, Sun J, Shen D. Differential gut growth, gene expression and digestive enzyme activities in young grass carp (Ctenopharyngodon idella) fed with plant and animal diets. Aquaculture. 2013; 410:18-24.

[14]. Silva JM, Espe M, Conceição LE, Dias J, Costas B, Valente LM. Feed intake and growth performance of Senegalese sole (Solea senegalensis Kaup, 1858) fed diets with partial replacement of fish meal with plant proteins. Aquaculture Research. 2010; 41(9):20-30.

[15]. Fontainhas-Fernandes A, Gomes E, Reis-Henriques MA. Replacement of fish meal by plant proteins in the diet of Nile tilapia: digestibility and growth performance. Aquaculture International. 1999; 7(1):57-67.

[16]. Kader MA, Bulbul M, Koshio S, Ishikawa M, Yokoyama S, Nguyen BT et al. Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea bream, Pagrus major. Aquaculture. 2012; 350:109-116.

[17]. Nagel F, Danwitz A, Schlachter M, Kroeckel S, Wagner C, Schulz C. Blue mussel meal as feed attractant in rapeseed protein-based diets for turbot (Psetta maxima L.). Aquaculture research. 2014; 45(12):1964-1978.

[18]. Zhou Y, Thirumurugan R, Wang Q, Lee CM, Davis DA. Use of dry hydrolysate from squid and scallop product supplement in plant based practical diets for Pacific white shrimp Litopenaeus vannamei. Aquaculture. 2016; 465:53-59.

[19]. Hari B, Madhusoodana Kurup B. Comparative evaluation of dietary protein levels and plant–animal protein ratios in Macrobrachium rosenbergii (de Man). Aquaculture Nutrition. 2003; 9(2):131-137.

[20]. Zhang H, Yi L, Sun R, Zhou H, Xu W, Zhang W et al. Effects of dietary citric acid on growth performance, mineral status and intestinal digestive enzyme activities of large yellow croaker Larimichthys crocea (Richardson, 1846) fed high plant protein diets. Aquaculture. 2016; 453:147-153.

[21]. Wu G, Bazer FW, Burghardt RC, Johnson GA, Kim SW, Knabe DA et al. Proline and hydroxyproline metabolism: implications for animal and human nutrition. Amino acids. 2011; 40(4):1053-63.

[22]. Li X, Rezaei R, Li P, Wu G. Composition of amino acids in feed ingredients for animal diets. Amino acids. 2011; 40(4):1159-1168.

[23]. Yamamoto T, Akimoto A, Kishi S, Unuma T, Akiyama T. Apparent and true availabilities of amino acids from several protein sources for fingerling rainbow trout, common carp, and red sea bream. Fisheries science. 1998; 64(3):448-458

[24]. Cheng ZJ, Hardy RW. Protein and lipid sources affect cholesterol concentrations of juvenile Pacific white shrimp, (Boone). Journal of Animal Science. 2004; 82(4):1136-1145.

[25]. Zhang K, Ai Q, Mai K, Xu W, Liufu Z, Zhang Y et al. Effects of dietary hydroxyproline on growth performance, body composition, hydroxyproline and collagen concentrations in tissues in relation to prolyl 4- hydroxylase α (I) gene expression of juvenile turbot, Scophthalmus maximus L. fed high plant protein diets. Aquaculture. 2013; 404:77-84.

[26]. Eisemann JH, Van Heugten E. Response of pigs to dietary inclusion of formic acid and ammonium formate. Journal of Animal Science. 2007; 85(6):1530- 1539

[27]. Jongbloed AW, Mroz Z, Van der Weij-Jongbloed R, Kemme PA. The effects of microbial phytase, organic ~ 175 ~ International Journal of Fisheries and Aquatic Studies acids and their interaction in diets for growing pigs. Livestock Production Science. 2000; 67(1):113-122.

[28]. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiological Reviews. 2001; 81(3):1031-1064.

[29]. Hamer HM, Jonkers DMAE, Venema K. The role of butyrate on colonic function. Alimentary Pharmacology & Therapeutics. 2008; 27(2):104-119.

[30]. Gao Y, Storebakken T, Shearer KD, Penn M, Øverland M. Supplementation of fishmeal and plant protein-based diets for rainbow trout with a mixture of sodium formate and butyrate. Aquaculture. 2011; 311(1):233-240.

[31]. Aksnes A, Hope B, Høstmark Ø, Albrektsen S. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, Gadus morhua. Aquaculture. 2006b; 261(3):1102-10.

[32]. Holme MH, Zeng C, Southgate PC. The effects of supplemental dietary cholesterol on growth, development and survival of mud crab, Scylla serrata, megalopa fed semi-purified diets. Aquaculture. 2006; 261(4):1328- 1334.

[33]. Song, Z. D., Li, H. Y., Wang, J. Y., Li, P. Y., and Zhang, L. M. (2016). Effects of replacement fishmeal with soy protein hydrolysates on growth, blood biochemistry, body composition of juvenile starry flounder Platichthys stellatus pallas. Acta Hydrobiol. Sin. 40, 165–172. doi: 10.7541/2016.23

[34]. Sun, H., Jiang, W. D., Wu, P., Liu, Y., Jiang, J., Yang, Q. H., et al. (2019). Betaine supplementations enhance the intestinal immunity of on-growing grass carp (Ctenopharyngodon idella): partly related to TOR and NF-κB signaling pathways. Aquaculture 2019:734846. doi: 10.1016/j.aquaculture.2019.73 4846

[35]. Tibaldi, E., Tulli, F., and Amerio, M. (1999). "Feed intake and growth responses of sea bass (D. labrax) fed different plant-protein sources are not affected by supplementation with a feeding stimulant," in Recent Progress in Animal Production Science: I. Proceedings of the A.S.P.A. XIII Congress, Piacenza, Italy, 21–24 June 1999, eds

[36]. G. Piva, G. Bertoni, S. Satoh, P. Bani, and L. Calamari (Piacenza: Assn. Sci. Anim. Production), 752–754.

[37]. Tibbetts, S. M., Mann, J., and Dumas, A. (2017). Apparent digestibility of nutrients, energy, essential amino acids and fatty acids of juvenile Atlantic salmon (Salmo salar L.) diets containing whole-cell or cell-ruptured Chlorella vulgaris meals at five dietary inclusion levels. Aquaculture 481, 25–39. doi: 10.1016/j.aquaculture. 2017.08.018

[38]. Tu, Y. Q., Xie, S. Q., Han, D., Yang, Y. X., Jin, J., and Zhu, X. M. (2015). Dietary arginine requirement for gibel carp (Carassis auratus gibelio var. CAS III) reduces with fish size from 50 g to 150 g associated with modulation of genes involved in TOR signaling pathway. Aquaculture 449, 37–47. doi: 10.1016/j. aquaculture.2015.02.031

[39]. Uddin, M. N., Nishio, N., Ito, S., Suzuki, H., and Isobe, K. (2012). Autophagic activity in thymus and liver during aging. Age 34, 75–85. doi: 10.1007/s11357- 011-9221-9

[40]. Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3:research0034.1. doi: 10.1186/gb-2002-3-7-research0034

[41]. Verhoeven, J., Salvo, F., Hamoutene, D., and Dufour, S. (2016). Bacterial community composition of flocculent matter under a salmonid aquaculture site in Newfoundland, Canada. Aquac. Environ. Interact. 8, 637–646. doi: 10.3354/ aei00204

[42]. Wang, T. T., Xu, M. M., Wang, J. T., Wan, W. J., Guan, D. Y., Han, H. J., et al. (2020). A combination of rapeseed, cottonseed and peanut meal as a substitute of soybean meal in diets of Yellow River carp Cyprinus carpio var. Aquac. Nutr. 26, 1520–1532. doi: 10.1111/anu.13099

[43]. Watanabe, T., Verakunpiriya, V., Watanabe, K., Viswanath, K., and Satoh, S. (1998). Feeding of rainbow trout with non-fish meal diets. Fish. Sci. 63, 258–266. doi: 10.2331/fishsci.63.258

[44]. Wu, M. J., Lu, S. D., Wu, X. Y., Jiang, S. T., Luo, Y., and Yao, W. (2017). Effects of dietary amino acid patterns on growth, feed utilization and hepatic IGF-I, TOR gene expression levels of hybrid grouper (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂) juveniles. Aquaculture 468, 508–514. doi: 10.1016/j. aquaculture.2016.11.019

[45]. Wu, Y., Han, H., Qin, J., and Wang, Y. (2015). Replacement of fish meal by soy protein concentrate with taurine supplementation in diets for golden pompano (Trachinotus ovatus). Aquac. Nutr. 21, 214–222. doi: 10.1111/anu.2015.21. issue-2