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Review Article

Application of Aquatic Macrophytes in Aquaculture: A Review on Aquatic Macrophyte in Aquafeed and their Effects on Fish

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ABSTRACT

Rapidly rising demand of animal protein together with a high market price of commercially available fish diets urges a necessity of procuring low cost alternative feedstuff for sustainable fisheries. Duckweed, a small family of floating aquatic monocots, a good source of plant protein with a potentiality of replacing costly animal based proteins in fish feed. Various studies have been performed evaluating the nutritive quality of duckweed species and its usage as fish meal replacement for various fish species. The easy adaptability to diverse culture conditions also displaying the possibility of duckweed as a supplementation for feeding purpose in aquaculture. Many study reveals the beneficial role of duckweed on feeding fish at different inclusion level in their diets elicit better growth rate, digestion and other metabolic activities. However, the high inclusion level of plant protein exerts growth depression which can be overcome undergoing different processing techniques. Approaches towards applying molecular tools to understand the involvement of growth factors and gene expression in connection to feed nutrition could be helpful to meet the need of fish production along with its profitability in aquaculture industry.

Keywords: Feedstuff; Aquaculture; Duckweed species; Profitability; Adaptability

INTRODUCTION

The rising demand of edible fish draws the attention of fish industrialists towards proper culturing and high production rate of fish to fulfill the appetite for animal protein. Aquaculture, the fastest expanding production sector of animal food contributed a total production of 3.9% by weight in 1970 raised to 33% in 2005 to global market of crustaceans, molluscs and fish. After cereals and milk, fish has becoming the third major protein source in human nutrition, also represented 7% of all protein consumed and contributing 17% consumption of all animal protein by the population of world by 2015. The United Nations, food and agriculture organization 2018 estimated of feeding nine billion people in the world by 2050. Fish, an ideal candidate for the necessary accomplishment of animal protein because of highly digestible food material with high nutritive value comprising Essential Amino Acids (EAA) like lysine, methionie, arginine etc. and essential Fatty Acids (FA) like long chain polyunsaturated fatty acids, vitamin A, D and minerals. As such fish protein requirement increasing in the global market and aquaculture industry has become the only problem solver. However, feeding alone hugely impact the total operation of the cultured fish in turn greatly influences the growth, its productivity and consecutively the profitability of cultured fish in aquaculture industry. The most effective and widely used protein and lipid sources in aquaculture industry includes Fish Meal (FM) and Fish Oil (FO). But its rising market value urged a need of replacement by more sustainable alternatives. Such curiosity has enforced aquaculturists to search a substitution of protein in fish diets without having an effect on the nutritive quality of fish protein. So for the continual aquaculture development, there lies an essential need of cost effective and eco-friendly substitutes on which fish farmers could rely on. At current situation with a surplus of average 30% growth rates per year, aquafeed is now becoming the fastest growing agricultural industry of the world. Moreover, the expansion in the production of aquaculture sector associated with the quick rising in aquafeed production.

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Many researchers have investigated protein ingredients of plant based to be a suitable substitution of fishmeal because of its usual accessibility and inexpensiveness compared to fishmeal. Research interests is therefore approaching in the direction of assessing and use of unconventional protein sources. Since early days of freshwater fish culture, macrophytes of both terrestrial and aquatic origin have been used as complementary feed in fish farming and still playing an important role in aquaculture as aquafeed. It was observed that freshwater macrophytes recognized as beneficial sources of protein for some fish species to formulate the feed of fish [1]. Duckweed considered as a natural protein source gained resemblances to animal protein as compared to most vegetable proteins. The culture system of duckweed represented a role of importance in obtaining the crude protein content of 40% and above making it a suitable protein source in aquaculture. Many studies showed the suitability of feeding duckweed to various animals. In the 1960's, it was reported of feeding duckweed in pigs and later with waterfowls, cattle, sheep, goats, fish and shrimps specified by Landolt. In some regions of Asian countries duckweed has been used as human food. It's been a long time in using duckweed as food to domesticated animals by allowing them to feed upon duckweed grown water bodies or by addition of their diets with fresh or dried harvested duckweed.

The amino acid composition of duckweeds also qualified it as a good protein source of human nutrition. Also duckweeds possessed fiber in low content (5% in dry matter) and therefore very absorbable. Numerous researches were done focusing on duckweed as aqua feed. Hassan and Chakrabrati did an encouraging work on digestibility test in carps and tilapia using duckweed. Growing interest of utilizing macrophyte as aquafeed in order to substitute the expensive FM, a number of studies was carried out successfully viewing in some cases the appropriateness of duckweed in supplying the protein required by the animals without any ill effects [2]. Appenroth et al. provided an insight of the nutritional beneficiality of each member from each of the five genera of duckweed family showing its utility aspects in human nutrition. Better health status in fish with duckweed supplemented diet has been observed in many studies compared to its duckweed deficit diet, also duckweed used as only animal feed showed reasonable growth in some trials of studies. Inspite of being rich in protein, the presence of Anti Nutritional Factors (ANF) may limit their inclusion in aquafeed. However, detoxification could be done by adopting different techniques before incorporating the plant ingredients in feed for better utilization of nutrients available in plantstuff to obtain better health status of fish. New methods and technology in genetic research have been evolving which might help to retrieve genetic information of various genes related to health status of fish upon feeding supplementary dietary ingredients. However, genomic study is still need to be further investigated in the field of Aquaculture. The present paper aimed to establish the nutritive importance of duckweed as aquafeed and its beneficial role in fish aquaculture [3].

LITERATURE REVIEW

Characteristics of Duckweed

Taxonomy and ecology: Duckweed are monocotyledons of the Lemnaceae family. Duckweed represents a small family with five genera that includes *Spirodela*, *Landoltias*, *Lemna*, *Wolffiela* and *Wolffia* with 37 species reported worldwide. Species of Lemnaceae family belongs to small floating aquatic plants with reduced morphology and fastest growing ones among the species of higher plants. Within 2-4 days, the biomass of duckweeds gets doubled under optimal conditions of temperature, pH, nutrient availability and light. Culturing, harvesting and sun drying of duckweed can be done without much expense of labor, expertise and cost. The water quality parameters influenced the growth of duckweeds which includes temperature, pH, ammonia, phosphates etc. Temperature, the master abiotic factor plays a vital role in duckweed growth ranged between 17°C-18°C and 27-31% as the critical and optimum temperature for the duckweed production [4]. However, Sonta et al. suggested 20°C to 28°C water temperature as the optimum growth conditions for high biomass production and pH between 6.5 and 7.5. Duckweed showed a wide pH tolerance with doubling of biomass production of duckweed at pH 7-8 within 2-4 days. The lux intensity of 4,200-6,700 with 14-16 h photoperiod helped in the optimum production of duckweed. There was production potential of duckweed in freshwater bodies. The ammonia concentration should be maintained between 7-12 mg N/l to obtain the required content of protein in duckweed. Phosphorus, an important factor for the optimum production of duckweed ranged between 4-8 mg/l [5].

Morphological features: Duckweed plants consists of a non-structural tissue frond. The fronds size, shape and thickness varies in each genera of duckweed family. Disc shaped fronds is seen in the genera *Spirodela* and *Lemna* which varies in sizes and thickness among different species. There is also presence of flowers in a number of one or two. *Wolffia* species in holds very minutely sized fronds in the shape of geometric solids which differs among species, absence of roots and seldom presence of single flower. Most diverse morphology observed in *Wolffiela* species [6]. Vegetative budding is the way of proliferation primarily by all the species of duckweed family. Clusters of fronds are shown by the species of *Lemna* and *Spirodela* whereas species of *Wolffia* remains solitary.

Nutritional composition of Duckweed

Duckweeds known to be a high nutritive value plant with more than 40% crude protein when grows in rich culturing system. Minerals and protein concentration in duckweed largely depends on the degree of nitrogen, potassium, phosphorous and other elements availability in its growing media [7]. Duckweed as a replacement for soybean appeared to be cost effective at 40% protein level. Many factors are responsible for the growth, nutritional quality and amount of biomass production of duckweed. Srivastava et al. suggested the use of Organic Manures (OM) included mustard oil cake, poultry wastes and cattle manures for the mass production of live food organism, also facilitated the outdoor facility production of zooplanktons.

The culturing technique plays an essential role for the obtainment of nearly 40% crude protein which is equivalent to the nutritional value of animal source protein and for the fulfillment of the nutritional requirement of fish. Therefore, nutritional composition of duckweed largely depends on its culture system [8].

Proximate composition: Proximate composition analysis of duckweed varied while grows on in different culture medium. The crude protein content in duckweed varied between 9%-20% in low nutrient water conditions and in high nutrient water, protein content ranged between 21%-41%. The protein content found to be 36.07 ± 0.18 and 27.12 ± 0.4% in *Lemna minor* and 35.82 ± 0.14 and 30.50 ± 0.03% in *Spirodela polyrhiza* cultured in OM and Inorganic Fertilizer (IF). Appenroth et al. suggested the protein concentration among various species of duckweed in between 20%-35%. Also reports indicated protein content between 18.9%-36.5% in duckweed. The content of lipid and ash in duckweed, *L. minor* found between 7.15% to 8.45% and 19.42% to 21.41% while in *S. polyrhiza*, it ranged between 7.11%-7.19% lipid content and 18.53%-20.64% ash content. Appenroth et al. reported 4% to 7% lipid content in different species of duckweed. In the contrary, the content of carbohydrate was low in macrophytes cultured in OM compared to culture in IF. Appenroth et al. found the starch content between 4%-10% in six investigated duckweed species and 11% starch content in four investigated species of duckweed. Low starch content obtained in duckweed species compared to protein content when cultured under favorable conditions [9].

Amino acid and fatty acid: The amino acid composition of duckweed grown in OM culture system consisted of the essential amino acids of 39.20% in *L. minor* and 37.4% in *S. polyrhiza* and 53.64% in *L. minor* and 58.2% *S. polyrhiza* non-essential amino acid. The amino acids which are essential includes histidine, phenylalanine, tryptophan, methionine, valine, lysine, leucine, isoleucine and threonine which were found in good quantity in duckweed as investigated. The nutritional quality of duckweed further improved due to the presence of some amino acids of non-proteinogenic (7.13%) including citrulline, sarcosine, hydroxyproline and taurine. Appenroth et al., studied the constituents of amino acids in six species belonging to five genera of duckweed, with the highest level of tyrosine +phenylalanine, lysine, cysteine+ methionine in *Wolffia hyaline* and *Wolffia* microscopic among all the investigated species [10]. Legumes like lupine or pea and chickpea constituted of amino acids comparable with duckweed's amino acid composition.

OM cultured *L. minor* possessed higher proportions of fatty acids contents including both saturated and unsaturated in *L. minor* cultured in OM compared to IF. *L. minor* composed of 60%-63% content of PUFA (Polyunsaturated Fatty Acid) which was dominated over other fatty acids comprising at around 41%-47% of α-Linolenic Acid (ALA) and Linoleic Acid (LA) at 17%-18%, then by saturated fatty acids of 23%-26% and monoenes of 11%-12%. There was also reports of rich fatty acid content in *S. polyrhiza*, where PUFA accounted 47%-53%, primarily 36%-39% of ALA and 11-14% LA followed by 32%-39% of total saturated fatty acids and monoenes at 9%-11%. Different species of duckweed ranged between 48%-71% of total fatty acids consitituted PUFA content and rich in ALA which is an omega-3 fatty acid thereby enhancing the nutritive value of Duckweed.

Other nutrients: The concentration of mineral content in duckweed plant also varies using a suitable nutrient medium. Therefore nutrient rich media helps in accumulating trace minerals like phosphorus, potassium etc., pigments like xanthophylls, carotene elevating the suitability of using duckweed meal as a good substitute to FM. Duckweed is capable of absorbing many macronutrients and micronutrients which includes nitrogen, carbon, hydrogen, calcium, manganese, phosphorus, copper, chlorine, sodium, silicon, potassium, boron, aluminium, iron, magnesium and zinc. The association of duckweed plants to aquatic ecosystem makes it vulnerable to certain toxic elements whose might seems harmful for consumption. But according to the report of FAO, the content of certain elements like lead, zinc, strontium, cadmium, copper, aluminium and gold imparted no certain risk to animals after feeding [11]. Since the content of the above mentioned elements in duckweed showed no such threat to human and animals as such highlighting more the usage of duckweed as feed product in aquaculture, livestock and poultry farming. Appenroth et al., reported the presence of varied quantity of carotenoids like lutein, β-carotene, zeaxanthin and α-tocopherol in the species of Wolffia. Moreover, the findings in the presence of phytosterol such as stigmasterol, β-sitosterol and campesterol in various Wolffia species increasing the nutritive property of duckweed.

Effect of Duckweed on fish health as dietary supplement

Fish growth performance: Goswami et al. reported highest growth in Rohu fingerlings (*Labeo rohita*) fed *L. minor* diet. Elevation in final body weight and high specific growth rate was recorded in duckweed, *L. minor* fed fish compared to the fish fed almond oil cake (*Terminalia catappa*), water fern (*Salvinia molesta*) and a combined diet of *L. minor*+ almond oil cake+water fern. Asimi et al., also reported duckweed fed fish with high growth rate in terms of Feed Coversion Ratio (FCR) and Specific Growth Rate (SGR). He replaced FM in the diet of Cyprinus carpio (*C. carpio*) by duckweed (*L. minor*) at 0%, 15%, 30% and 45% level maintaining 32% crude protein. The best result was however observed with partial FM replacement of 15% duckweed diet [13]. A similar result was also observed with improvement in growth performances at 15% duckweed diet in *Labeo rohita* (*L. rohita*). However, *Heterobranchus longifilis* fingerlings showed better results at 10% inclusion of duckweed meal in comparison to the diets with duckweed inclusion level of 20% and 30%. Fasakin et al., indicated progressively declination in growth performances and nutrient utilization in fish at higher inclusion of duckweed in diets resembled the work of Asim et al., who observed lower growth of *C. carpio* at higher inclusion of duckweed diet (30% and 40%). Wanderi and Olendi, studied duckweed diet effect on tilapia (*Oreochromis niloticus*) with formulated feed of dry duckweed at 100% (duckweed alone), 50% (equal proportion duckweed and fishmeal), 0% (control, without duckweed). It was observed that 50% feed showed a positive impact on fish growth as compared to 100% duckweed diet, indicating the appropriateness of duckweed plant as fish feed. It was therefore observed in various studies as reported by several authors that feeding fish species as well other animals with low and partial duckweed supplemetation favored better growth performances and nutrient utilization. Fresh Duckweed contains high moisture (96%) and air pockets in the leaves which facilitates its floating in water thus decreasing its palatability whereas dry duckweed's nutrient content may vary depending in its culture system which accordingly either hinders or promote the growth performances of fish [12].

Duckweed, *L. minor* fed Grass carp, *Ctenopharyngodon idella* (1.81 ± 0.42 g) showed a high growth performance in terms of gut length and weight as well as the relative gut length and weight with chironomid larvae fed fish. Carp species, *L. rohita* principally herbivorous with some extent of omnivory feeding habit, always have a preference of feeding on plant material Pradhan et al., stated *W. globosa*, an absolute feed for nurturing *L. rohita* fry which was successfully cultured under semi-intensive system, confirmed better growth in live *Wolffia* feeding rohu, markedly showed high conclusive mean length and weight, daily growth index as well as specific growth pace in comparison to commercial formulated diet fed rohu. Duckweed, *Wolffia* sps. possessed physiological and biological convenience over other formulated feed of fish. Growth promoting factors occurrences in *Wolffia* might have an effect on growth of fish in its early stage. The relatively smaller size of *Wolffia* (0.34 nm) is easily consumed by the fish at nursery stages. Sinking nature of formulated diets and floating nature of *Wolffia* duckweed plant also suggested as influencing factor in feeding behavior of fish. Bhatnagar and Dhillon illustrated an improvement in growth performances and feed utilization with supplementation of lysine and methionine together with Duckweed (*L. minor*) diet in *L. calbasu* fingerlings as compared to diets with no methionine and/or lysine addition. Decreased growth rate observed in *C. carpio* affected by low water temperature while feeding marketable carp diet (control diet) in the experiment comparison to duckweed meal. Assumption was made that the presence of essential amino acid, lysine supported high growth rate and feed utilization in common carp fed duckweed contained fish feed. Such findings corroborated by the work of Viola et al., who established 80% supplementation of soybean meal with addition of L-lysine (4-5 g) and oil (100 g/kg) ameliorate health status of carp gained weight and better utilization of protein rates in relation to fish meal fed carp. Thus, it can be said that lysine presence was critical in fish diet to achieve the needed growth and efficiencies in feed utilization by feeding upon plant inclusion diet. Duckweed has attended special recognisation as natural sources of food either in fresh forms or in combination with other feedstuff for various culturable fish. Fasakin et al., also observed higher growth rates in fish given duckweed (*S. polyrrhiza*) inclusion diet than water fern, (*Azolla africana*) fed fish and 30% duckweed inclusion diet found to be the most cost efficient diet. Almazan et al., attributed reduced growth performances in Nile tilapia fed *Azolla* diets due to deficiencies in EAA, especially tryptophan and threonine (Table 1). van Hove reported a strain of water fern had reduced levels of cysteine, methionine and lysine. However, the EAA content in duckweed comparable to soybean meal and considerably elevated that of peanut [13].

Table 1: Showing duckweed as supplementation of diet on growth performances of diverse fish species.

S. no.	Duckweed species	Species studied	Substituting level and its effect
1	*Spirodela polyrhiza*	*Onchorynchus mykiss* (rainbow trout), fry (Average 0.28 ± 0.01 g body weight)	6.25%: SGR(N), FCR(I), AWG(D), SR(D)
			12.5%: SGR(N), FCR(I), AWG(D), SR(D)
2	*Lemna minor*	*Channa striatus* (striped snakeheaded), fingerlings (Average 3.18 ± 0.25 g body weight)	25%: SGR(I), FCR(D), AWG(I), SR(I)
			50%: *SGR(I), FCR(D), *AWG(I), SR(I)
			75%: SGR(I), FCR(I), AWG(I), SR(I)
			87%: SGR(I), FCR(N), AWG(N), SR(I)
3	*Lemna minor*	*Cyprinus carpio* (common carp), fry (Average 0.29 g body weight)	5%: SGR(N), FCR(N), AWG(N), SR(I)
			10%: SGR(N), FCR(D), AWG(N), SR(I)

			15%: SGR(D), FCR(I), AWG(D), SR(N)
			20%: SGR(N), FCR(N), AWG(N), SR(N)
4	*Spirodela polyrrhiza*	Nile Tilapia (*Oreochromis niloticus*), fingerling (Average 13.9 ± 0.1 g body weight)	5%: SGR(N), FCR(I), AWG(D), SR(I)
			10%: SGR(N), FCR(I), AWG(D), SR(N)
			20%: SGR(D), FCR(I), AWG(D), SR(I)
			30%: SGR(D), FCR(I), AWG(D), SR(N)
			100%: SGR(D), FCR(I), AWG(D), SR(N)
5	*Lemna minor*	Common carp fry, (Average 1.61 ± 0.21 g body weight)	15%: GR(D), AWG(I), SR(N)
			30%: GR(D), AWG(D), SR(D)
			45%: GR(D), AWG(D), SR(D)
6	*Lemna minor*	Silver barb (*Barbodes gonionotus* Bleeker), Average 1.5 ± 0.2 g body weight	10%: SGR(N), FCR(I), AWG(D)
			20%: SGR(N), FCR(I), AWG(D)
			30%: SGR(D), FCR(I), AWG(D)
			35%: *SGR(D), FCR(I), AWG(D)
7	*Lemna minor*	Tilapia (*Oreochromis niloticus*), fingerlings (Average 1.45 g body weight)	5%: SGR(N), FCR(I), AWG(D)
			10%: SGR(D), FCR(I), AWG(D)
			15%: SGR(D), FCR(I), AWG(D)
			20%: SGR(D), FCR(I), AWG(D)
			25%: SGR(D), FCR(I), AWG(D)
8	*Spirodela polyrrhiza*	Tilapia (*Oreochromis niloticus*), fingerlings (Average 13.09 ± 0.13 g body weight)	5%: SGR(N), FCR(I), AWG(D), SR(D)
			10%: SGR(N), FCR(I), AWG(D), SR(D)
			20%: SGR(D), FCR(I), AWG(D), SR(N)
			30%: SGR(D), FCR(I), AWG(D), SR(N)
			100%: SGR(D), FCR(I), AWG(D), SR(D)

9	*Lemna minor*	Common carp (*Cyprinus carpio*), fry (Average 16 g body weight)	15%: SGR(D), FCR(N), AWG(D), SR(D)
			30%: SGR(D), FCR(I), AWG(D), SR(D)
			45%: SGR(D), FCR(N), AWG(D), SR(D)
10	*Lemna polyrhiza*	Rohu (*Labeo rohita*), fingerling (Average 6.4 ± 0.41 g body weight)	10%: *SGR(I), *FCR(D), *AWG(I)
			20%: SGR(I), FCR(D), AWG(I)
			30%: SGR(I), FCR(D), AWG(I))
			40%: SGR(D), FCR(D), AWG(D)
11	*Lemna* sp.	African catfish (*Clarias gariepinus*) fingerlings (Average 3.84 ± 0.43 g body weight)	20%: SGR(D), FCR(I), AWG(D)
			50%: SGR(D), FCR(D), AWG(D)
			75%: SGR(D), *FCR(I), AWG(D)
			100%: SGR(D), FCR(I), AWG(D)

Note: SGR: Specific Growth Rate; FCR: Feed Conversion Rate; AWG: Average Weight Gain; GR: Growth Rate; SR: Survival Rate. Maximum effect (*). Letters indicate an Increase (I), Decrease (D) and No effect (N) on duckweed substitution level.

Digestive physiology: Intestinal digestion and absorption capacity to different diets by various fish species conveys the status of fish health. Digestive enzyme activities varied among fish species, in a similar way the activity of the same digestive enzyme differs within a fish in various regions of the intestine. Secretion of digestive enzymes by the exocrine pancreas of fish in the anterior region of the intestine made it more active than its posterior region concerning digestion and absorption of nutrients. Wu and Zhu investigated intestinal digestive enzymes activity on various fish species fed different diets showed highest protease and lipase activity in carnivorous fish which was followed by activity showed in omnivorous and herbivorous fish. On the contrary, fish of herbivory feeding behavior with highest amylase activity followed by omnivorous and carnivorous fish. Similar results observed in mandarin fish (*Siniparca chuatsi*), with highest protease and lipase enzyme activity and lower level of amylase activity. Goswami et al., reported significantly higher digestive enzyme activity of trypsin, amylase, chymotrypsin and less protease and lipase activity in duckweed, *L. minor* fed rohu. Better enzyme activities might result in better FCR in lemna fed rohu compared to other feed. Zhao et al. suggested duckweed fed common carp with less trypsin and lipase enzyme activity. It was found that the foregut region of the intestine of common carp showed maximum activity of digestive enzymes especially the trypsin enzyme compared to the other region of the intestine, while duckweed diet resulted relatively higher α-amylase and lipase activity along the intestine of common carp. Protein digestion and absorption happened primarily in the foregut of intestine, starch and cellulose digestion throughout the entire intestine took place. Hindgut region of intestine showed higher activity of cellulose when compared with the other region of the intestine. The higher enzymatic action of cellulase in the hindgut compared to foregut and midgut. Different heights and widths of intestinal folds reflected the intestinal absorption area indicating the intestinal absorption capacity of fish. Different types of diets brings about changes in the intestinal absorption areas with different digestive mechanism which might resulted in differences in the activity of digestive enzymes. Common carp successfully showed its adaptability to changes in diets by adopting different digestion and absorption strategies.

He et al., reported the findings of the activity of digestive enzyme fed with duckweed and larvae of chironomid separately in fish, grass carp. At 30 days of experimental period, liver and intestine of grass carp showed significantly higher activity of trypsin enzyme and decreased activity of enzyme amylase and lipase while fed duckweed. Bhatnagar and Dhillon, reported increased digestive enzyme activites (proteolytic, amylolytic and cellulolytic) implied high digestibility and nutrient utilization in duckweed fed Labeo calbasu fingerlings supplemented with lysine and methionine. The presence of a balanced amount of proteins in the diet helped in absorption and assimilation of the required amino acids leading to higher growth and muscle protein accumulation. Digestive enzyme activities increased similarly in *Mughil cephalus* and *Chanos chanos* reporting effective digestion and absorption when fed on lysine and methionine supplemented soybean diet. Plant based feeding in fish showed significantly higher enzymatic activities due to efficient enzyme system of nutrients digestion and absorbtion. The dietary replacement of FM by alternative protein sources also affected gene expression concerned to digestion in fish. The activity and expression of gut and liver genes in different sizes of Gibel carp

(*Carassius auratus gibeli*) were affected while feeding soybean diet substituting FM.

The physiological and pathological status in fish can also be determined by the evaluation of haematological parameters, a potent implementation to know the health condition of fish. Pradhan et al., stated *Wolffia* as an immune enhancer. Packed cell volume, plasma protein levels, blood glucose, total RBC and WBC count was significantly improved while fed *Wolffia* in *L. rohita* fry. *Wolffia* feeding elevated plasma protein levels associated with enhancement in innate immune mechanism of *L. rohita* fry. However, few reports were available on feeding of live duckweed or fresh addition of it as ingredient in feed of fish. Although study has been carried out showing improvement in the immune system of fish fed other plant ingredients in the diets as for instances there was increased in non-specific immunity of *L. rohita* while fed *Achyranthes aspera* in their diet and also lessen the mortalities of fish infected with a bacterial pathogen, *Aeromoas hydrophila*. Similar immune modulatory role observed as reported by Harikrishnan et al. in *C. carpio* infected with *Aeromonas hydrophila* while fed diet consisting of leaf extract of *Azadirachta indica*. As such more research may be needed in identifying the immune augmenting properties of duckweed and its effect on the immune mechanism of fish.

Dietary effect of duckweed on growth factors and gene expression of fish

Most applicable technology to fish aquaculture includes sequencing, DNA markers, genome mapping and microarrays. Recently researchers were approaching towards the next generation sequencing technique comprises RNA sequencing and transcriptomic analysis, new DNA based marker for the discovery of differential gene expression of RNAs involved in various phenomenon of biological processes like fish growth. Growth is a somatic function and environmental influential trait controlled by different genes related to growth. The most important factors includes Growth Hormone (GH) and Insulin-Like Growth Factor-1 (IGF-I) determines the health status of fish and other organisms. These are the key dietary and environmental influential factors. The acceptance of EAA and FA in substituted diets of fish meal able to activates the factors like GH and IGF-I only upto a certain level of their tolerancy. There were several reports indicating replacement of fish meal above certain level as such 60% fish meal replacement by soyameal resulted in minor declination of EAA along with complete replacement resulted in deprivation of both EAA and FA affecting its growth rate. The synthesis and secretion of IGF-I is regulated by the GH. The high FM substitution by alternative diet resulted in GH desensitization where severe rise in GH exhibits decrease in the expression of IGF-I also with growth rate which brings probable declination of Growth Hormone Receptor (GHR) production in hepatic cells. It is because of the limited feedback loop of IGF-I on GH, antagonistic function of IGF-I come to an end with increase in the level of GH. Gomez-Requeni et al., reported such phenomenon in fish species like *Sparus aurata* and *Oreochromis mykiss*. Therefore high or total replacement of fishmeal severely effects on growth factors whose degree vary along fish species and could be harmful for fish health. Studies in other systems also revealed the involvement of new factors like miRNAs that controls gene expression playing significant roles in growth. MicroRNAs (miRNA), small non-coding RNAs consist of approximately 22-long nucleotides that regulates transcription and post-transcriptional process of mRNA. There are possiblities in the application of miRNAs in aquaculture industry as selection markers. There has been bulk interest of researcher in investigating such genes in the recent past.

A research progression by RNA sequencing in fish such *Danio rerio* (Zebra fish), *Salmo salar* (Atlantic salmon), *Ctenopharyngodon idellus* (Grass carp) and *Lateolabrax maculates* (perch) was done to unveil unknown gene regulation mechanisms. Several miRNAs reported to have a role of importance in skeletal muscle growth of teleost. Huang et al., reported in fast-growing and slow-growing Nile tilapia (*Oreochromis niloticus*) about the involvement of skeletal muscle growth related miRNAs and its differential expression. An investigation was also performed in Chinese perch (*Siniperca chuatsi*) on growth related miRNA profiles. Such studies will help in the enhancement of knowledge in understanding the involvement of miRNAs and the related molecular pathways of different biological processes including growth in fish. Authors also reported some studies on dietary effect of duckweed inclusion on genes related to feeding and growth of fish species, which further supported the beneficial property of duckweed and its application on fish aquaculture.

Epidermal Growth Factor (EFG) is a cell proliferating factor secreted by the salivary and the brunners glands of duodenum stimulating the gastrointestinal growth as well as *in vitro* multiplication of various cell lines. Duckweed fed young grass carp showed better growth performance with an increased expression of Efg which might involve in gut growth. In addition to Efg, there were also reports of presence of other factors like Npy Y8b, Npy Y8a, Npy, and Ob-r which leads to growth in gut of fish fed duckweed. It was revealed in some studies on the expression of high mRNA levels of NPY and its regulatory role on intake of food in different fish species. Study in European sea bass, *Dicentrarchus labrax* showed differential expressed genes related to feeding upon plant based diet, also observed 4435 differential expressed genes related to signaling pathways in appetite and digestive mechanism upon feeding on animal and plant diets in grass carp by the application of transcriptomic approach [14]. Transcriptomic analysis was done to know the status of transcription at a specific time of the biological sample. Moreover, Zhao et al. suggested a relation between the types of diets fed in common carp and involvement of the differential expressed genes in the signaling pathways of digestive physiology like digestion and absorption of protein, secretion of bile and immune regulatory role in intestine, also hematopoietic cell lineage whose role was observed in different diet utilization. Further study will put more light on the relation between plant based diets and its utilization in fish body through analysing the expression of differential genes related to different physiological mechanism of fish health [15].

Zhao et al., reported downregulation of the Solute Carrier (SLC) genes in common carp fed duckweed diet alone compared to mixed diet (earthworm+duckweed) suggesting a declination in transportation of amino acids and bicarbonate ion into small intestinal epithelial cells, whereas in mixed diet containing both animal (earthworm) and plant (duckweed) diet, the SLC gene was up-regulated to transport amino acids and bicarbonate ion. SLC family are the membrane transport proteins which involved in digestive processes. Besides there was up-regulation of gene expression engaged in cholesterol metabolism, which converts cholesterol to bile acid in duckweed fed common carp compared to earthworm+duckweed and alone earthworm diet indicating that duckweed diet might have the property of reducing cholesterol in the liver by converting it to bile acid [16].

DISCUSSION

Anti-Nutritional Factors (ANF in Duckweed

ANF also known as anti-nutrients are endogenous compounds that present in plant based FM replacement feed which by itself or by producing metabolic products in living systems, exerts obstruction in metabolic activities like digestion and nutrients absorption, reduction in feed utilization and feed efficiencies, withholding of N and losses of energy, resulted in reduced growth rate. Presence of various types and concentration of ANF in plants exerts an effect on the complete usage of plant based feed as FM replacement [17]. Different types of ANFs commonly found in various plant species includes phytic acid, tannin, saponins, trypsin inhibitor, lectins, glucosinolates, phytoestogens, alkaloids, cyanogens, non-starch polysaccharides etc. Existence of low doses of ANF such as phytic acid, tannins and cyanide in duckweed, *S. polyrhiza* might affect the utilization of nutrient and growth rate at higher inclusion of duckweed in fish feed. An observation of poorest performances of growth in fish fed with duckweed alone (100%), similar results was also reported by several authors. Therefore, it was stated that the presence of ANFs, low EAAs and PUFA consequences in growth reduction of fish fed with higher inclusion of duckweed. Soybean meal, the most alternative protein source to FMl was restricted by anti-nutritional substances. However, the less content of phytic acid in duckweed than in soybean meal made duckweed a suitable alternative source to FM. Oxalic acid, the only reported toxic component in duckweed which found to be unfavorable to animal health. Oxalic acid interferes the gut lining and prove to be toxic at high doses. It was indicated that cooking and blanching techniques could be applied for the reduction of oxalic acid content in plant material [18].

Application of various types of processing technique having the potentiality of inactivating or eliminating the harmful properties of ANFs present in plant stuff used in fish diet to improve the health status of fish. Some of these techniques includes heat treatment to inactivate the proteinaceous ANFs, de-hulling treatment to eliminate the ANFs in the seed coat, solvent extraction of removing ANFs having solubility in organic and inorganic solvents, bacterial fermentation and supplementation of exogenous enzymes like carbohydrases and phytases leads to enzymatic action on ANFs to bring its breakdown. A role of significance plays by the gut microbiota in fish degrading ANFs by their ability to produce extracellular enzymes in order to increase the nutrient availability in plant materials. Extracellular enzyme degrading activities of gut bacteria may indicated the beneficial role of gut microbiota in proper digestion and utilization of plant based diets by fish and other animals. Such enzyme producing bacteria possesses the ability to utilize as probiotics in aquaculture for better digestion and nutrients utilization of plantfeed stuff. Bairagi et al., studied the fermentation technique application in *L. polyrhiza* leaves with fish gut bacteria, *Bacillus* sp. significantly lowering the content of phytic acid and tannin as well as crude fiber while the amino acid and fatty acid levels in *L. rohita* fingerlings increased showing better feed utilization and growth performances in compared to raw fed duckweed meal. Other processing method include soaking, germination, boiling, autoclaving, extrusion and genetic manipulation. Goswami et al., also found better growth rate by feeding rohu with raw duckweed supplemented diet after applying extrusion technique for feed preparation. However, further research is necessary on the application and searching of efficient probiotics to increase the nutritive value of plants to improve fish health and production in aquaculture along with proper elimination method without altering the nutritive value of plant products [19].

Duckweed in wastewater treatment

The emergence of duckweed in wastewater treatment is not a new concept. Its been about more than two decades of duckweed application for wastewater treatment and the first duckweed appearance in the scientific world was traced in between the year of early 1960's and late 1970's. The research on duckweed revolves around two broad areas of feeding and biological wastewater treatment. The properties of altering the contaminated water by using duckweed sp. have made this plant wide application for biological wastewater treatment in the world. Lemnaceae family is exploited in the field of biotechnology, dietetics, phytotherapy as well as in ecotoxicology. Several authors reported in the application of duckweed as a means of ethanol and biogas production. Additional application in heavy metals absorption by duckweed from the water bodies was also reported. Speedy growth rate of duckweed plants made it utilizing many nitrogen compounds available in its growing medium as a result naturally responsible for the high uptake rate of nutrients. Among the nitrogen compounds, duckweed prefers ammonium ion (NH_4^+) the most and utilized it for growth purpose. Elevation in eutrophication occurs due to high accumulation of ammonium ion which converted into nitrates on groundwater, so it's highly essential to absorb ammonium from wastewater. Duckweed, *S. punctata* was used in swine wastewater for the removal of phosphorus and nitrogen with high efficiencies. The same property was shown by duckweed, *S. olygorrhiza* and *Landoltia punctata*. Such observation made researchers to study more about the involvement of duckweed in wastewater treatment. Stadtlander et al. reported duckweed's potential utilization of dissolved organic N (<0.2 μm size particles) which includes amino acids, proteins, peptides, urea, bacteria and viruses. Phosphorus, an important component for the growth of duckweed accumulated around 1.5% in dry matter. In addition to phosphorus, little quantity of potassium also required for the normal growth of duckweed [20]. Duckweeds ability to uptake high nutrients and removal of ammonia, phosphorus as well as other pollutants allowing its wide application to phytoremediate natural, agricultural, domestic and industrial wastewater.

CONCLUSION

Strict legislation, the scarcity and high prices have generated the interests of researcher of employing plant protein sources in order to substitute fractionally or entirely FM for the continual progression of aquaculture industry. An equilibrium of indispensable and dispensable amino acids resultant by degrading the complete protein content of fish diets is essential for the synthesis of protein to achieve optimum growth and feed efficiency by fish body. Therefore an enhancement in the property of feed considering mostly its protein content is assumed to be beneficial since the growth pattern is based on the weight of the fish. In this case, locally available duckweed stand out as one of the plant protein that could be incorporated in fish diet due to its availability in different climates. Duckweed possesses various advantageous quality which makes it a good candidate for using as feed ingredient in aquaculture. Duckweeds are high protein rich plants have high nutrient uptake capabilities, low content of lignin and fiber, speedy growth and easy to harvest, as a means of phytoremedition and many others practical application. The richness in amino acid content of duckweed was not found in adequate quantity in other plant proteins. It included the EAA like lysine, methionine and arginine playing an essential role for the nutrient fulfillment required in animal feeds as well. The foremost limiting amino acids in aqua feed are lysine and methionine augmenting the importance of unvalued plant and concerned to beta oxidation of long chain fatty acyl group by serving as precursors to carnitine. Moreover, the content of antioxidant like tocopherol, lutein and zeaxanthin and also phytoesterol adds to the useful properties of duckweed. Therefore, duckweeds proved to be an excellent potential substitute in fish diets as compared to animal derived fish feed. Many factors like light intensity, salinity, regional temperature, culture medium and crowding degree of the plants influenced the distribution and growth of various members of duckweed. Various species of duckweed successfully accomplished as a suitable alternative sources to FM. Species like *Lemna, Wolffia, Spirodela* and others showed better growth rate and nutrient utilization efficiencies in fish like rohu, tilapia, common carp etc. High protein content and tenderness in duckweed (*Lemna* sp.) made it a nutritious vegetable food in comparison to other aquatic weeds and considered nutrient rich feed ingredient for carp, Rohu. Nutritional evaluation of all duckweed species needed for their effective usage in fish feed. Viability and reproduction of an organism is solely depends on its diet which must able to fulfill the nutritional requirement of the body. Survivality of an organism also risen up with its ability to adapt different diets. The health status of fish could be known by the active participation of digestive enzymes towards different diets. Digestive enzymes like protease, α-amylase, lipase etc. showed variation in its activity depending on the types of feed eaten by fish. There were reports supporting duckweed as fish diet, showing positive effects on growth performances and physiology of various fish species. However presence of some secondary metabolites restricts its utilization with full potential. Therefore, more duckweed research in identifying and evaluating the risks in its production, attainment and processing to sustain its nutritive value. More studies needed to understand the structural, functional and detrimental role of different secondary metabolites present in duckweed. Different genetic factors controlling gene expression learned in other system have been emerging with the discovery and advancement of researches in aquatic sciences. Discovery of small RNAs like miRNA unfolding a new way of research in gene analysis, its expression and molecular networks. miRNA seems to be an important tool in studying their regulatory metabolic function giving more emphasis on growth of fish. The evolving technologies and discovery in aquatic sciences could help to decipher research on nutritional aspect of gene regulation that will impact to improve fish health and achieving sustainable fish productions for the coming generations.

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