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Effects of dietary *Nannochloropsis salina* on the nutritional performance and fatty acid profile of Nile tilapia, *Oreochromis niloticus*

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A B S T R A C T

This study assessed the effects of dietary *Nannochloropsis salina* on the nutritional performance and fatty acid profile of Nile tilapia, *Oreochromis niloticus*. Three isonitrogenous and isocaloric diets were formulated to contain 35% crude protein and 13% lipid. In the first diet, protein and lipid was solely from fish meal and fish oil, the second diet was based on soybean meal and soybean oil and the third diet was based on *Nannochloropsis salina* meal. Three replicate groups of fish (initial weight, 12.70 ± 0.03 g) were used for each diet. Fish were fed manually to apparent satiation for 36 days. At the end of the feeding trial, fish fed the *Nannochloropsis* diet had similar weight gain but significantly ($P < 0.05$) better feed conversion ratio than those fed the soybean diet. Moreover, significantly higher protein retention efficiencies (28.86%) were found in fish fed *Nannochloropsis* diet than those fed the soybean meal diet (26.84%). The fatty acid profile of fish body was influenced by the fatty acid composition of the diets. There was no difference between the total n-3 polyunsaturated and n-3/n-6 fatty acid ratio of fish fed the fish meal and *Nannochloropsis* diet. However, both n-3 polyunsaturated and n-3/n-6 fatty acid ratio of fish were higher compared to those recorded in fish fed the soybean meal diet. Generally, this study showed that *O. niloticus* fed *N. salina* based diet was able to have comparably good nutritional performance with fish meal and soybean meal. Furthermore, data on the fatty acid profile showed that *N. salina* could replace fish and soybean oil in the diet of Nile tilapia.

1. Introduction

There is increasing public awareness of the health benefits of eating fish and fish products, in particular, but not entirely because of the high levels of n-3 highly unsaturated fatty acids (HUFA) present in fish [1,2]. The longer chain n-3 HUFA inclusion in the diets is important because the human body has limited capability of elongating short chain fatty acids like α-linolenic, to longer chain 20:5n-3 and 22:6n-3 fatty acids [3].Therefore, there is need for the inclusion of these essential fatty acids in the diet of humans and animals reared for human consumption. The aquaculture sector is emerging as a significant production sector for high protein food. The overall effect of the global growth in aquaculture and the increase in world population means that the average per capita food production from aquaculture annually has increased by ten times [4]. Aquaculture will continue to be relevant in the future as a source of protein. However, the growth in aquaculture has caused major challenges; one of these challenges is the production of practical feeds for the farming of fish and shellfish. Increased fish farming has resulted in increased production of aquafeeds, which depend heavily on fish meal and fish oil as the main source of protein and lipids respectively [5]. Therefore, it has become imperative to assess different protein and lipid sources as appropriate replacement for fish meal and fish oil in aquafeed because of the increasing demand for fish meal and oil due to dwindling fish capture [6]. Aquaculture depends on common input ingredients such as, fish meal, soybean, fish oil, rice and wheat, for which it competes in the marketplace with the animal husbandry sector, as well as with direct human consumption [7]. Furthermore, many of the key ingredients traditionally used in formulating feed for commercial or on-farm aquaculture feeds are internationally traded commodities [8]. Reduction in inclusion level of these conventional feedstuff, especially fish meal and oil, will therefore be important to reduce feed costs and avoid competition with other users [8]. Therefore, there is a need for the intensification of research in alternative feed ingredients in order to reduce the dependence of aquafeed on fish meal and fish oil [8].

Soybean meal is the most extensively used ingredient among the plant protein sources considered in aquafeeds [9]. It is also the most evaluated substitute for fish meal as a result of its availability,

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consistent quality, high protein content and relatively well-balanced amino acid profile compared to other plant protein sources, stable price and supply [10]. Many studies have shown that for many finfish cultured worldwide, soybean meal often constitutes up to 60% of the total formulation and has widely been used as a partial or total substitute for costly protein ingredients, like fish meal with good growth and health performance, while reducing cost of fish production significantly [11,10,3]. However, soybean meal is deficient in methionine, lysine and threonine [3,9]. Furthermore, there are some indications that soybean may induce enteritis in Atlantic salmon [12,13]. The main challenge in replacing fish and soybean meal is to find alternatives that maintain acceptable growth rates, animal health and quality of the final product [14]. Furthermore, they must have vital nutritional characteristics like, good amino acid profile, essential nutrients, high digestibility, palatability, low fibre and anti-nutrient levels [15]. Many methods are under consideration as solutions to fish and soybean meal inclusion in fish feed, most recently, the use of new innovative protein and fat and oil sources, like marine polychaetes and algae, are being considered [9]. Microbial and algal species are being focused on as novel protein sources because of their several benefits, which include their potential in adding essential amino acids, omega-3 fatty acids and reducing or removing anti- nutrients [4]. Algae species compared favourably with other commercially available ingredients for aquaculture. The high production rate and protein content of microalgae, especially in developed, developing and under developed countries, as well as the ability to be produced on an extensive variety of substrates and waste, makes it a possible replacement for fish and soybean meal in aquafeeds [16].

Nannochloropsis is a unicellular microalga with a polysaccharide cell wall, which contains only one chloroplast [17]. Genetic analyses and sequencing was used to recognise six species in the genus *Nannochloropsis*; they are *N. salina*, *N. gaditana*, *N. granulata*, *N. limnetica*, *N. oceanica* and *N. oculata* [17,18]. The rise in cost of fish and its products, which are traditionally being used to enrich poultry and animal product meant for human consumption have paved way for the consideration of more sustainable alternatives, like *Nannochloropsis* and other oleaginous microalgae [18]. *Nannochloropsis* oil has been found to be well tolerated without any negative effects in animals [19]. The use of *Nannochloropsis* as a source of oil in human and animal nutrition is gaining popularity globally, mainly because of the appreciable content of the n-3 HUFA 20:5n-3 and the potential higher yield per hectare compared with conventional oleaginous agricultural crops [20]. Oil from phytogenic sources does not contain the n-3 HUFA, and fish oil which is the conventional source of the n-3 HUFA in livestock and fish feeds is not sustainable. Therefore the choice of oleaginous microalga like *N. salina* as a sustainable alternative to fish oil can be attributed to the significant content of n-3 HUFA like 20:5n-3 [21]. Archibeque et al. [22] reported that *N. oculata* produced favourable nutritional and health performances similar to fish meal and better than soybean meal in the diet of rabbits. Furthermore, in juvenile sea bass, *Dicentrarchus labrax* Haas et al. [23] found that 50% fish oil replacement by *Nannochloropsis* sp. was possible without negative effects on the growth performance and nutrient utilisation. *Nannochloropsis* has been used to enrich the fatty acid profile of human diets by supplementing it in livestock and finfish diets which are meant for human consumption. For instance, Bruneel et al. [24] found that *Nannochloropsis* spp. provides a suitable alternative to fish oil as a source of HUFA to enrich eggs for human consumption. This study, therefore, assessed the potential of dietary *N. salina* in producing beneficial effects in tilapia aquaculture through the enrichment of essential fatty acids as a means of making these available for the final human consumer of the fish. Therefore, the specific objectives of this work were to assess the effect of the total replacement of fish meal, fish oil, soybean meal and soybean oil with dietary *N. salina* on the growth, nutritional performance and fatty acid profile of Nile tilapia.

Table 1
Formulation of the experimental diets (g per kg feed each).

	Diet 1 fish meal	Diet 2 soybean meal	Diet 3 *Nannochloropsis salina*
Fish meal	550		
Nannochloropsis salina			820
Soybean meal		800	
Corn starch	315	0	105
Dicalcium phosphate	0	45	40
Fish oil	100	0	0
Soybean oil	0	120	0
Alginate	30	30	30
*Vitamin and mineral premix	5	5	5
Analysed composition of the experimental feeds (per kg on as fed basis)			
Dry matter	956	963	965
Crude protein	378	362	350
Lipid	148	118	138
Ash	122	95	99
Gross energy (MJ)	19.3	19.0	19.3

Vitamin and mineral premix - Each 1 kg feed contains vitamin A, 1000 mg; vit., D2, 8 mg; vit. E, 7.0 g; vit. K, 0.8 g; vit. B1, 0.49 g, vit. B2, 1.6 g; vit B6 0.6 g; vit. B12 4 mg; Pantothenic acid 49; Nicotinc acid 8 g; Folic acid, 400 mg; Biotin, 20 mg; vit C 40 g; Choline chloride, 200 mg; Copper, 4.0 g; Iodine,0.4 g; Iron, 12 mg; Manganese, 22 g; Zinc 22 g and Selenium 0.04 g – BiotinM, UK.

2. Material and methods

2.1. Fish handling

Oreochromis niloticus fry were reared on a commercial fish feed containing approximately 35% protein and 12% lipid [25] until they reached the appropriate size which was approximately 12.70 ± 0.03 g (initial weight). A total of 15 fingerlings were bulk weighed and stocked in each of the 12 tanks with 3 replicates (15 fish per tank). The trial lasted for 36 days, during which time weighing was repeated every week for the calculation of growth. Fish were fed to apparent satiation manually four times daily, but new pellets were only added when all the feed was consumed from the previous feeding.

2.2. Diet formulation

The formulation of the experimental diets is shown in Table 1. Three experimental diets were formulated to contain 35% crude protein and 13% lipids. In the first diet, protein and lipid was solely from fish meal and fish oil sourced from Cytoplan, (Argentina), the second was based on soybean meal and soybean oil sourced from Merlin Biodevelopments, (North Wales, UK) and in the third diet, the protein and lipid source was exclusively from *Nannochloropsis salina* sourced from Phytolutions GmbH (Germany).

2.3. Biochemical analysis of feed and whole body of fish

Standard and official methods [26] were used to determine the proximate analysis (moisture content, crude protein, ash and energy) of the feed and whole carcass of fish used in the current study. Tilapias were sampled at the beginning and end of the trial. Before the analysis, MS-222 (tricaine methane sulphonate) at a concentration of 200 mg/l was used for terminal anaesthesia of fish. They were then blended to a homogeneous mince (Binatone, Japan) using a meat grinder with a 4 mm diameter orifice plate. A sub-sample of this mince from each tank was taken and stored for estimation of dry matter which, was determined after drying in the oven (Gallenkamp, UK) at 105 °C for 24 h. The remaining fish homogenate was dried in the oven and used for all subsequent analyses. Ash content was calculated by weight loss after incineration in a muffle furnace (Carbolite, UK) for 12 h at 550 °C. A Parr bomb calorimeter was used to calculate gross energy content, this