Egyptian Journal of Basic and Applied Sciences xxx (2017) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

Egyptian Journal of Basic and Applied Sciences

journal homepage: www.elsevier.com/locate/ejbas

Full Length Article

# Evaluation of blended virgin coconut oil and fish oil on growth performance and resistance to *Streptococcus iniae* challenge of Nile tilapia (*Oreochromis niloticus*)

Andrews Apraku, Liping Liu *, Xiangjun Leng, Emmanuel J. Rupia, Christian Larbi Ayisi

*Key Laboratory of Freshwater Fishery Germplasm, Ministry of Agriculture, Shanghai Ocean University, 999 Huchenghuan Road, Shanghai 201306, PR China*

ARTICLE INFO

*Article history:*
Received 4 May 2017
Received in revised form 11 June 2017
Accepted 13 June 2017
Available online xxxx

*Keywords:*
Virgin coconut oil
Fish oil
Performance
*Streptococcus iniae*
*Oreochromis niloticus*

ABSTRACT

Five isolipidic experimental diets (32% crude protein) were formulated to contain 3% fish oil (FO) and virgin coconut oil (3VCO) as sole lipids or blends of FO + VCO in ratios of 75:25% (0.75VCO), 50:50% (1.5VCO) and 25:75% (2.25VCO). Triplicate groups of *O. niloticus* were fed one of five diets to apparent satiation, twice daily for 8 weeks. It was observed that fish fed diet 3VCO exhibited the best performance with respect to feed intake (492.1 g), final weight (214.60 g) and weight gain (154.90 g). Significant effects of dietary fatty acid profile were reflected in fish fed the diets in whole body, muscle and liver C12:0 and C14:0. However, eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) were significantly different ($P \geq 0.05$) compared to their respective diets while liver n-3: n-6 ratio significantly increased and recorded low levels in whole body and muscle. Statistically, least values of mortality were recorded as VCO levels were elevated when fish were subjected to *Streptococcus iniae* infection while plasma metabolite indicators among treatments were not altered. The inclusion of VCO at 3% in the diet gave excellent performance, indicating that it could wholly replace FO and as such represents a better alternative lipid source for feeding *O. niloticus*.



## 1. Introduction

Dietary lipids are the only source of essential fatty acids and also provide highly digestible energy while facilitating the absorption of fat-soluble nutrients necessary for proper functioning of physiological processes and to some extent maintaining biological structure [30,55].

Fish oil and fish meal is considered as the main protein components of feed [36,57] in the aquaculture sector and as such influences the cost of production. It is therefore expected that, higher demand in these components will raise the cost of feed production and thereby affect production rate and the ability of the industry to maintain its pace and stability in growth. Also, the higher demand for fish oil has endangered some fish species (herring, sardine, anchovy, capelina, etc) which are considered to have low economic value and less for human consumption used in the production of the oil as they have been overexploited [10,17,49,58,75].

It is based on these facts that researchers have focused on reducing the cost of feed by producing practical diets that are available and cheaper at all times [24,33]. As such, dietary alternatives to FO has become the best option because they are currently incorporated at higher levels as FO is becoming costlier and less available [66].

Several studies have shown that sources and levels of dietary lipid affect the growth performance and also health of fish [31]. Other investigations have also shown the cost effectiveness in protein sparing effect of lipids to fish [22,60].

Vegetable oils have been successfully incorporated in aqua-feed for various fish species with similar or improved growth results as fish oil [9,41,47,63,72]. Vegetable oils have thus been considered as a suitable replacement of marine fish oil [11,25], especially for fish species with preference for n-6 fatty acids (FAs) unlike n-3 FAs [53] of which there are more to explore and enhance this substitution.

Nile tilapia (*O. niloticus*), a widely cultured fish species has been fed on a series of alternative lipids and blends with fish oil (FO) at various levels. These include soybean oil, sunflower oil, linseed oil and palm related oils, among many others [26,40–42] with a variety of desirable characteristics ranging from environmental tolerance to physiological changes [3]. Nile tilapia can accept higher levels of linoleic (n-6) series FAs (18:2n-6 and 20:4n-6) with normal growth and reproduction [41,71] than other warm-water fish that require more linolenic (n-3) FA series (18:3n-3; 20:5n-3 and 22:6n-3) in the diet [54].

* Corresponding author.
*E-mail addresses:* apranium@myself.com, lp-liu@shou.edu.cn (L. Liu).

http://dx.doi.org/10.1016/j.ejbas.2017.06.002
2314-808X/

Please cite this article in press as: Apraku A et al. Evaluation of blended virgin coconut oil and fish oil on growth performance and resistance to *Streptococcus iniae* challenge of Nile tilapia (*Oreochromis niloticus*). Egyp. Jour. Bas. App. Sci. (2017), http://dx.doi.org/10.1016/j.ejbas.2017.06.002

Consumers' health in terms of immune response and disease resistance has been shown to be influenced by the choice of lipid FAs [10,44]. A deficiency in linolenic FA has been shown to have an adverse effect on antibody production and macrophage killing ability [27] while causing mortality in excessive amount [20].

Irrespective of widely available reports on vegetable oils (VO) as lipids in growth performance and disease resistance [2,7,12], few studies have evaluated virgin coconut oil as a dietary lipid for *O. niloticus* [4,34,46,35,70].

Virgin coconut oil (VCO) has the ability to increase antioxidant enzymes while reducing lipid peroxidation [39]. According to [15] and [73], the abundant MUFAs (65%) of VCO did not participate in the biosynthesis and transport of cholesterol and as such allowed for mobilization of protein for body protein synthesis. It has also been shown to maintain normal levels of lipid parameters in serum and tissues and inhibit LDL-oxidation [14,38] while having the ability to destroy pathogenic gram-negative bacteria with appropriate chelator [14–15].

This study was conducted to evaluate the effects of replacing fish oil with varying levels of virgin coconut oil on the growth performance, fatty acid composition and immune response to a *Streptococcus iniae* challenge in Nile tilapia (*Oreochromis niloticus*).

## 2. Materials and methods

### 2.1. Experimental diet preparation

Five isolipidic experimental diets were formulated to contain different lipid sources which included fish oil (FO) (Nonghao Feed Company, Shanghai, China), and Virgin Coconut oil (The Philippines) as the sole lipid source, blends of FO + VCO (50:50%), or in partial replacement of FO at increasing levels of VCO at 25 and 75% as represented by FO, 3VCO, 0.75VCO, 1.5VCO, and 2.25VCO respectively.

Dietary ingredients were finely ground and sieved (40 mm mesh) before the addition of oil and approximately 200 ml of deionized water/kg diet. Extruded pellets were produced by an extrusion mill (SLP-45, Chinese Fishery Machinery and Instrument Research Institute of the Chinese Academy of Fishery Sciences, Shanghai, China) and air-dried at room temperature to a moisture content of 13%. Pellets were then sieved to obtain appropriate sizes and were stored frozen in air-tight plastic bags at −20 °C for subsequent use. Triplicate diet samples were analyzed to confirm the proximate composition according to standard methods for the determination of dry matter, protein and ash content of animal feeds [6]. Lipid content was determined following the method of [19] and the diets were analyzed for fatty acid composition.

### 2.2. Fish and facilities

*O. niloticus* used in this experiment were obtained from Hainan Xinji Aquatic Science & Technology Co. Ltd, China, transported to concrete tank facilities at the Shanghai Ocean University Aquaculture Farm (Binhai, Shanghai, China), and acclimated for two weeks. Fish were fed commercial fish pellets (Tongwei Company Limited, Chengdu, China) during acclimation. 750 fish ranging between 53 and 56.5 g (55.35 ± 3.22 g; mean ± SD) were randomly stocked in 15 cages (2.0 × 1.0 × 1.0 m, L × W × D) in indoor concrete tanks at a density of 50 fish per cage. The tanks were supplied with a constant flow of well water and continuously aerated with air stones. Water samples were taken at 20 cm below the water surface. The water temperature monitored during the feeding trial ranged from 29.11 °C to 29.90 °C, pH from 7.37 to 7.55, dissolved oxygen from 7.07 to 7.70, nitrogen from 0.02 to 0.04 and ammonia ranging from 0.13 to 0.30 mg/l. Triplicate groups of fish per treatment were fed one of five experimental diets twice daily (008:30-009:00 and 16:00-16:30) to visual satiety with feed intake recorded by the difference in weight prior to and after feeding. Fish in each group were batch-weighed and counted to monitor growth, feed utilization survival once every two weeks whiles tanks were cleaned. Diet was withheld 24 h prior to sampling days and offered once after sampling.

### 2.3. Harvest, sample collection and growth performance

Fish were starved for 24 h prior to harvest after completion of the trial period. All surviving fish within each tank were counted and batch-weighed. Fifteen fish at the initial stage of the experiment and 75 at the end of the trial were randomly sampled, euthanized with an overdose of tricaine methane sulfonate (MS-222 at 200 mg/L in culture water), weighed individually, pooled and stored at −20 °C for subsequent determination of proximate composition and analyses. Each sample was analyzed in triplicate for whole body proximate composition following standard methods [5]. A muscle sample (5 × 2 × 3 cm without skin) was taken from the left back, 3 cm below the dorsal fin, from three fish per tank [21]. Livers were dissected to calculate hepatosomatic index. The following parameters were calculated as such:

1. Total weight gain (WG)
   WG = FW (g) − IW (g).
   FW and IW for final weight and initial weight, respectively.
2. Specific growth rate (SGR) (%)
   SGR (%) = (ln FW (g) − ln IW) (g)/T × 100.
   T for total number of culture days.
3. Feed intake (FI) for the total feed consumed (g) during the entire trial.
4. Feed conversion ratio (FCR)
   FCR = FI (g)/WG (g).
5. Protein efficiency ratio (PER)
   PER = WG (g)/PI (g).
   WG and PI for body weight gain and protein intake, respectively.
6. The hepatosomatic index (HSI)
   HSI = [LW/BW] × 100
   LW and BW stand for liver weight (g) and total body weight (g), respectively.
7. Survival rate (SR)%
   SR = [TF/TFT] × 100
   TF and TFT for total number of fish stocked and total number of fish at termination point, respectively.
8. Condition factor (K)
   K = Total BW (g)/TL (cm) 3 × 100.
   TL for total length.

### 2.4. Assays of water, ash and protein content

The whole body, dorsal muscle and liver from all groups were analyzed in triplicate for moisture and protein content according to standard methods [6]: moisture was determined by oven drying at 105 °C to constant weight; ash content was determined by incinerating dry matter samples in a muffle furnace at 550 °C for 12 h, crude protein was determined by the Kjeldahl method and by multiplying the nitrogen content by 6.25. Ash and water content were expressed as percentage content, and protein was expressed as% dry weight, (DW).

### 2.5. Analyses of lipid content and fatty acid composition

Dried tissues were ground individually to a powder before each assay was performed. The total lipid (TL) of each sample was



Please cite this article in press as: Apraku A et al. Evaluation of blended virgin coconut oil and fish oil on growth performance and resistance to *Streptococcus iniae* challenge of Nile tilapia (*Oreochromis niloticus*). Egyp. Jour. Bas. App. Sci. (2017), http://dx.doi.org/10.1016/j.ejbas.2017.06.002