Research Article

Effect of Pumpkin Seed Oil (*Cucurbita maxima*) Supplementation to Nile Tilapia (*O. niloticus*) Diets on Performance, Feed Utilization and Body Composition

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Abstract | Feed additives can be used as functional feed and applied in aquaculture for improving performance and feed utilization. This study aimed to examine the effect of pumpkin seed oil (PSO) supplementation to Nile tilapia (*Oreochromis niloticus*) fingerlings diets on growth performance, feed utilization and body composition. Nile tilapia (4.12g $fish^{-1}$) were fed the basal diet contained 30.36% crude protein and 3879 kcal kg^{-1} gross energy with different levels of PSO (0.0 (control), 25%, 50% and 75% from the source of vegetable oil that was added to the basal diet). Fish (300 male) were distributed randomly into four groups (4 groups x 3 replicate x 25 fish of each). All groups were fed diets twice a day during the trial period (56 day) at 5% of body weight for the first two weeks thereafter at 4% for the last period. The results showed that the best values of specific growth rate, final weight, weight gain, feed intake and feed conversion ratio were obtained with fish fed diet supplemented 75% PSO followed by 50% PSO. The best values of protein efficiency ratio and protein productive value was obtained with fish fed 75% PSO followed by 50% and 25 % PSO supplementation. The maximum value for energy retention that was recorded in fish group fed 75% PSO, while the other groups had no significant differences among them. No significant difference was detected among all treatments in whole body composition. From these results it could be concluded that using PSO up to 75% from the ordinary source of vegetable oil in Nile tilapia fish diets enhances growth performance and feed utilization and without negative effect on whole body composition which may lead to improve fish production.

Keywords | Pumpkin seed oil, Nile tilapia, Performance, feed utilization, Body composition.

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INTRODUCTION

Phytogenic compounds are a class of feed additives that have drawn a lot of attention to it in the feed industry. There is a wide range of herbs, spices, and the products derived from them mainly oils (Hashemi and Davoodi, 2010). Vegetable oils, commonly known as essential oils, are fragrant, oily liquids derived from plants (flowers, seeds, leaves, fruits and roots). It is a complex blend of organic compounds such as terpenes, alcohols, esters, aldehydes, ketones, and phenols. Phytogenic feed additives have been selected to improve productive performance and immune responses for animals (Peterson et al., 2014; Harikrishnan et al., 2011), increasing feed nutrient digestibility (Abo-State et al., 2017) and can effect on parameters of reproduction (Mangiagalli et al., 2010).

Pumpkin (*Cucurbita* spp.) has great attention in nowadays for its nutritional and health protective properties (Montesano et al., 2018), as well as clinical application (Tanaska et al., 2020). The oil yield and quality extracted from pumpkin seed oil were influenced by solvents polarity used in the excretion process. It ranged from 4.12% to 27.93%, with the highest yield recorded in petroleum ether and lowest in ethanol (Chatepa and Masamba, 2019; Can-Cauich et al., 2021). Also, variations in fatty acid profiles and amounts of pumpkin seed oil were dependent on the origin of particular cultivars, climatic conditions, and postharvest management (Rabrenović et al., 2014). Pumpkin seed oil consisted of many fatty acids including linoleic acid (C18:2), oleic acid (C18:1), palmitic acid (C16:0) and stearic acid (C18:0) (Applequist et al., 2006). Habib et al. (2015) found that pumpkin seed oil contains the highest amount of oleic acid 40.58% while stearic acid, palmitic acid and Linoleic acid content are found to be 27.06, 17.39 and 14.97%, respectively. Pumpkin seed oil has a greater percentage of unsaturated fatty acids (55.55%) than saturated fatty acids (44.45%). Bardaa et al. (2016) showed an excellent quality of pumpkin oil with high content of polyunsaturated fatty acids (Linoleic acid: 50.88±0.106 g/100 g of total fatty acids), tocopherols (280 ppm) and sterols (2086.5±19.092 ppm). Pumpkin seeds contain omega-6 and -3 fatty acids with a very high concentration of vitamin E (Hashemi, 2013; Ryan et al., 2007), it is important for growth performance and feed utilization for Nile tilapia (Bardaa et al., 2016; Medjakovic et al., 2016; Wang et al., 2017) have emphasized the therapeutic benefits of pumpkin seed oil in the treatment of variety of illnesses and organ damage, including hypertension, diabetes, and cancer. It is also antimicrobial, antioxidant and anti-inflammatory (Yadav et al., 2010; Gutierrez, 2016; Petropoulos et al., 2021). In animals for livestock (Valdez-Arjona and Ramírez-Mella, 2019), hens (Herkel et al. 2014), broiler chicken (Wafar et al., 2017), roosters (Lotfi et al., 2021) and rabbits (Bakeer et al., 2021). There are numerous studies that have used different sources of vegetable oils such as soya oil, groundnut and palm oil for different kinds of fish species. On the other hand, there are not sufficient studies about using PSO on different fish species such as Japans sea bass (*Latealabrax japonicas*) (Xue et al. 2006), African catfish (*Clarias gariepinus* juveniles) (Olukunle, 2011) and Nile tilapia (Erondu and Akpoilih, 2020), which is the focus point of our study.

Pumpkin seed oil (PSO) has been known as functional food oil due to some bioactive components contained such as phenolics and tocopherols with beneficial effects to improve health and production. However, application of pumpkin seed oil as functional feed in different species in aquatic animals is still far from. Therefore, the following study aimed to assess the pumpkin seed oil effect on growth performance, feed efficiency and body composition of Nile tilapia (*Oreochromis niloticus*) fingerlings.

MATERIALS AND METHODS

Experimental fish, housing and diets

Nile tilapia fingerlings (male) (4.19g) were obtained from a private tilapia hatchery company. The fish were assigned at random to four treatment groups with three replicates for each group and 25 fish per replicate. At the beginning, 50 fish were taken randomly, weights were recorded and then frozen at -20°C for chemical analysis. Water temperature, dissolved oxygen and pH were adjusted almost 26.45, 6 mg/L and 7.45 respectively in all groups.

The basal diet was formulated to include 30.36% protein, 5.79 fat, 6.7 fiber, 14.2% ash and 3879 kcal kg^{-1}gross energy (Table 1). Pumpkin seed oil was produced from a local market and inclusion in the basal diet at 0.0, 25, 50 and 75% from the source of oil that added to the basal diet as the functional diet. Fish were fed these diets twice a day for 56 days (5% of body weight for the first 2 weeks and then 4% for the subsequent weeks). The mean weight of fish was recorded every 14 days and daily rations were readjusted accordingly.

Table 1: Formulation and proximate composition (%) of the basal diet.

Ingredients	%
Yellow corn	35
Soybean meal	30
Corn gluten meal	19
Wheat bran	11
Vegetable oil	4
Vitamin and mineral premix*	1
Proximate composition (%DM)	
Crude protein	30.36
Crude fat	5.79
Crude fiber	6.70
Crude ash	14.20
Gross energy kcal/kg	3879

*Vitamin and mineral premix: containing the following per kg of feed: A= 4500 I. U, D= 11252 I.U, E= 71 I.U, K 3= 2 mg, B 12= 0.015 mg, panthothenic acid= 5 mg, nicotinic acid= 14 mg, folic acid= 0.4 mg, biotin= 0.04 mg, choline= 150 mg, cobalt= 0.2 mg, copper= 4.5 mg, iron= 21 mg, manganese= 20 mg, iodine= 0.6 mg, selenium= 2.2 mg, zinc= 20 mg, antioxidan = 2 mg.

Chemical analysis of diets and whole body fish

The examined diet and 15 fish collected from each treatment at the start and the end of the trial were weighted and kept in deep freeze until analyzed for moisture, protein, fat and ash according to the criterion methods of AOAC (2006). The moisture content of the fish was determined before freeze-drying the samples. This was performed using a forced air oven at 105°C for five hours and then repeated for 30 minutes interval until a constant weight was obtained. The loss in weight was calculated as the moisture content. The protein content of dry samples was determined by Kjeldahl apparatus, crude fat was assayed by Soxhlet apparatus with diethyl ether as solvent and the ash content was determined by a muffle furnace at 600°C for 6 hrs.

Calculations of fish performance

The parameters used to measure the performance and feed efficiency of Nile tilapia fish were:

Weight gain (WG) = final weight – initial weight.
Specific growth rate (SGR) = 100 (Ln W_2 – ln W_1) / days of feeding

W_1 and W_2 were the initial and final weights, respectively.

Feed conversion ratio (FCR) = dry feed intake (g) / fish live weight gain (g).
Protein efficiency ratio (PER) = 100 (weight gain (g)/ protein intake (g)).
Protein productive value (PPV) = 100 (protein gain (g)/protein fed (g)).
Energy Retention (ER) = Retained energy in carcass (Kcal)/ energy intake (Kcal)×100.

Statistical analysis

One-way analyses of variance (ANOVA) were used to analyze statistical data of 95% dependability (SPSS, 2007). Duncan's Multiple Range test (Duncan, 1955) was used to detect the differences among means at significant (P<0.05).

RESULTS AND DISCUSSION

Growth performance of Nile tilapia

Table 2 shows the initial, final, gain of body weight and specific growth rate of Nile tilapia fingerlings fed varied amounts of pumpkin seed oil supplementation. The starting weight was almost the same in all replicates. Body weight gain (BWG), final body weight (FBW) and specific growth rate (SGR) with the best growth performance (P<0.05) was obtained with 75% PSO; being 15.16 g, 10.98 g, and 2.30 respectively, followed by 50% PSO being 13.06 g, 8.90 g, and 2.04, respectively. Meanwhile FBW and BWG did not significantly affect with 25% PSO and without significant difference with the control group.

Feed utilization

Table 3 displays the findings of the parameters of performance and feed efficiency. The findings clearly indicated the beneficial effect of 75% pumpkin seed oil (PSO) on feed utilization had the greatest feed intake (FI) and best feed conversion ratio (FCR), being 16.80 g, and 1.53 followed by 50% PSO, being 15.65g, and 1.76 respectively. There were no significant differences were detected between the group fed on 25% PSO and the control group in feed intake. Results of protein efficiency ratio (PER) showed a statistically significant difference (P<.05) among dietary treatments. Fish fed diet with 75% PSO gave the best value of PER (2.16). No significant differences were detected between groups fed on 50% PSO and that fed on 25% PSO (1.87 and 1.82). Otherwise, group fed diet without PSO gave the lowest PER (1.74). Likewise, the protein productive value (PPV) was significantly (P<.05) affected by PSO addition. The highest (P<.05) PPV value were obtained for fish fed 75% PSO (28.60) then the other three groups 50%, 25% PSO and the control being 25.45, 23.91, 23.77 respectively, with no significant differences among them. Moreover, the greatest value for energy retention (ER) (30.01) was recorded in fish group fed 75% PSO, while the other groups had no significant differences among them 50%, 25% PSO and control 24.70, 23.70, 25.67, respectively.

Table 2: Growth performance parameters of Nile tilapia fed different levels of Pumpkin seed oil (PSO).

Item	IBW (g)	FBW (g)	BWG (g)	SGR
Control (0.0%PSO*)	4.20	12.05^c	7.85^c	1.88^d
25% PSO	4.15	12.35^c	8.20^c	1.95^c
50% PSO	4.16	13.06^b	8.90^b	2.04^b
75% PSO	4.17	15.15^a	10.98^a	2.30^a
SE of mean	±0.02	±0.37	±0.37	±0.05

Values in the same column with different superscripts are significantly different at P<0.05. Whereas: Initial body weight (IBW), Final body weight (FBW), Body weight gain (BWG), Specific growth rate (SGR). *PSO levels (0.0, 25, 50, and 75%) from the source of vegetable oil that was added to the basal diet.

Table 3: Feed utilization parameters of Nile tilapia fed different levels of Pumpkin seed oil (PSO).

Item	FI (g)	FCR	PER	PPV	ER
Control (0.0%PSO*)	14.77^c	1.88^a	1.747^c	23.767^b	25.67^b
25%PSO	14.86^c	1.81^b	1.817^b	23.913^b	23.88^b
50% PSO	15.65^b	1.76^b	1.87^b	25.450^{ab}	24.70^b
75% PSO	16.80^a	1.53^c	2.16^a	28.60^a	30.01^a
SE of mean	±0.25	±0.04	±0.01	±1.25	±1.77

Values in the same column with different superscripts are significantly different at P<0.05. Whereas: Feed intake (FI), Feed conversion ratio (FCR), Protein efficiency ratio (PER), Protein productive value (PPV), Energy Retention (ER). *PSO levels (0.0, 25, 50, and 75%) from the source of vegetable oil that was added to the basal diet.

Table 4 presents the body composition results. There was no different (P>0.05) among all measures of body composition (moisture, protein, fat and ash %) content across all treatments.

Fish growth is influenced by nutrition, which is particularly evident in fish produced in intensive aquaculture systems. Pumpkin seed oil have several beneficial effects on growth and immune status, it is reported to be beneficial in immune response, treatments for a wide range of diseases, production and improves metabolism of fats (Achilonu et al., 2018).

Table 4: Body composition of Nile tilapia fed different levels of Pumpkin seed oil (PSO).

Item	Dray matter	Crude protein	Ether extract	Ash
Initial fish	22.11	53.70	16.20	14.83
Control (0.0%PSO*)	26.69	48.69	33.02	10.27
25%PSO	25.10	50.69	31.03	10.63
50%.PSO	25.39	51.47	30.36	10.77
75%PSO	25.98	49.65	34.15	9.60
SE of mean	±0.91	±1.36	±2.24	±0.60

*PSO levels (0.0, 25, 50, and 75%) from the source of vegetable oil that was added to the basal diet.

Lipids are a source of fatty acids, phospholipids, sterols, and fat-soluble vitamins that are required for the normal functioning of physiological processes and to a lesser extent, the preservation of biological structure and cell membrane function (Ghanawei et al., 2011). Furthermore, adequate of fatty acids are significant factors for influencing various biological processes, such as growth, feed utilization, reproduction and immune responses (Chatepa and Masamba, 2019; Montesano et al., 2018). However, an overabundance of dietary lipids can limit feed uptake and the use of other nutrients, resulting in a slower development rate (Ghanawei et al., 2011) as well as improved efficiency of fat (Hillestad and Johnsen, 1994). As a result, the lipid or fatty acid requirements for farmed fish should be considered functional when creating their meals (Ayisi et al., 2017).

Tilapia, like other warm-water fish, need more linoleic fatty acids (18:2 n-6) or (20:4 n-6) than n-3 fatty acids for ideal performance (Aziza et al., 2013), they only need about 1% n-6 fatty acids in feed (Bazaoglu and Bilguven, 2012). Plant oils high in n-6, n-3, or both fatty acid series such as linseed oil have been found to be an excellent supply of lipids for Nile tilapia (Erondu and Akpoilih, 2020).

The information on the nutritional value of pumpkin oil as functional feed is inconsistent. From the previous discussion we can concluded that different lipids which include distant fatty acids have an impact on several growth and feed utilization parameters due to pumpkin oil. Analysis of pumpkin seed oil implied, it has a high degree of unsaturation, making it a good drying agent, and a low free fatty acid concentration. The results of fatty acid composition show that PSO has contain the highest fatty acids. PSO has a greater percentage of unsaturated fatty acids (55.55%) than other oils which is useful and necessary for Nile tilapia nutrition (Habib et al., 2015).

Improving growth performance and feed utilization parameters with using pumpkin oil in Nile tilapia fingerlings diet may be due to contain more amount of vitamin E and fatty acids. Hashemi (2013) reported that pumpkin seeds oil contains omega-6 and omega-3 fatty acids (Murkovic et al., 2004) and very high concentration of vitamin E (Ryan et al., 2007), it is important for performance and utilization of feed for Nile tilapia. The current study revealed that different levels of pumpkin oil had significantly effect on FBW, BWG, SGR and FCR of *Oreochromis niloticus* fingerlings. These results findings consistent with earlier research on Nile tilapia (Ochang et al., 2007b) who reported that growth performance of fish was influenced with different levels of lipid. On the other hand, using different levels of palm oil in the tropical bagrid Catfish Mytus (Ng et al., 2000) or Clariesgariepinus (Ochang et al., 2007a) had no effect on FBW, BWG, SGR, or FCR.

Feed intake and another feed utilization parameters were improved when pumpkin oil is added in the diet up to 75% of whole oil in the basal diet. Ayisi et al. (2017) found that protein efficiency ratio in Nile tilapia was significantly affected with palm oil level which in agreement with our study although this differs from the results of Elekasheif et al. (2011).

The chemical composition of body tilapia fingerlings fed elevated levels of pumpkin oil were not significantly affected by different treatments. In contrast Ayisi et al. (2017) found that addition of dietary palm oil to fish diet significantly increased the protein levels of whole body. Also, Song et al. (2009); Wang et al. (2005) suggesting that high lipid consumption may result in fat accumulation in the liver and visceral cavities.

Addition of vitamin E in the diet enhances juvenile sea bream development and decreasing lipid peroxidation products in tissues (Tocher et al., 2002). Both nutrients (vitamin E and polyunsaturated fatty acids) are thought to have a synergistic impact on immune responses in bastard halibut *(Paralychthys olivaceous)*. Bai and Lee (1998) discovered that higher amount of linoleic (18:2 n-6), linolenic (18:3 n-6) and alpha-linolenic acid (18:3 n-3) were related with higher amounts of vitamin E as long more in arachidonic acid (20:4 n-6) linked with increased vitamin E levels, which is agreement with the results of Navarro et al. (2012), discovered an improvement in the quality of polyunsaturated fatty acids in Nile tilapia administered vitamin E supplementation.

CONCLUSIONS AND RECOMMENDATIONS

The results can be used to assess pumpkin seed oil nutritionally. The oil attained from the pumpkin seed may be added as a functional ingredient in Nile tilapia fingerlings diet up to 75% from the ordinary source of vegetable oil without retardation in growth. Pumpkin oil was promising for enhancing growth performance, feed utilization and without affecting on proximate composition in Nile tilapia.

NOVELTY STATEMENT

This study may be the first to evaluate different levels of pumpkin seed oil in Nile tilapia fingerlings. The use of pumpkin seed oil is expected to improve fish growth performance and feed utilization. So, we believe that the results of our study will be a new contribution in the practical field of fish nutrition.

AUTHORS CONTRIBUTIONS

All authors are contributed equally.

Conflict of interest

The authors have declared no conflict of interest.

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