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Effect of Dietary Inclusion of Sieving Wastes of the Egyptian Clover Seeds Instead of Soybean Meal for Tilapia

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ABSTRACT

An indoor feeding experiment for 57 days was conducted on Nile tilapia to evaluate the effects of dietary inclusion of graded levels (0, 50, 75, and 100%) of sieving wastes meal of Egyptian clover seeds instead of soybean meal based on crude protein content. A total of 8 glass aquaria (each of 70 × 35 × 40 cm, filled with 65 L dechlorinated tap water, and stocked with 12 fingerlings 17.3 g as average initial body weight and 9.5cm as average total length) were used. The obtained results recommended the best replacement level of 50% which significantly realized the best feed composition, highest feed consumption, final body weight, and best body composition of Nile tilapia, to be reflected in many benefits for both the producers and the consumers.

Keywords: Clover seeds, sieving wastes, tilapia performance.

INTRODUCTION

Nowadays aquaculture in Egypt supplies ca. 80% of human fish consumption. Egypt occupies the tenth rank all over the world concerning aquaculture production (FAO, 2016). Nevertheless, the Aqua-feeds are shorter than the demands, besides their expensive costs; therefore, there is a need to recycle and reuse all possible and available field and industrial by-products that have a nutritional importance for fish (Koeleman, 2018a & b). Since fish are the most feed converter than all terrestrial animal species (Shehab, 2018). However, adult tilapia is herbivores, omnivores, or detritovores (El-Sayed, 1991). Thus, many authors evaluated fish diets containing various agro-industrial wastes (El-Sayed, 1999) as fish meal, soybean meal, linseed meal, corn gluten, tomato waste, clover leaves, duck weed, poultry by-product meal, and bone and meat meal (Eid *et al*., 1995), sugar beet pulp (Magouz, 1996), lupine seed meal (Hassanen, 1998), corn gluten meal (El-Ebiary *et al*., 2001), black seed and roquette seed meals (Abd Elmonem *et al*., 2002), sunflower meal (El-Saidy and Gaber, 2002 a & c), dried dropping dates (Srour *et al*., 2002), sugarcane bagasse compost (Hafez *et al.,* 2003), sesame hulls by-product (Abd Elmonem *et al*., 2004), fennel seed meal, dried marjoram leaves, and dried basil leaves (El-Dakar *et al*., 2004 a, b, & c, respectively), leaf protein concentrate from water hyacinth (El-Saidy *et al*., 2004), olive cake (Khalafalla and Salem, 2006), and silkworm meal (Salem *et al*., 2008) etc…. Therefore, the present research aimed to study the possibility of feeding Nile tilapia diets containing graded levels of sieving wastes meal of Egyptian clover seeds instead of soybean meal.

MATERIALS AND METHODS

Mono-sex Nile tilapia fingerlings (17.3g as average initial body weight and 9.5cm as average total length) were purchased from a private hatchery at Tolombat 7, Kafr El-Sheikh governorate. After transportation to Sakha Aquaculture Research Unit, Central lab. of Aquaculture Research, Abbasa, Abou Hammad, Sharqia governorate, Agricultural Research Center, Cairo; fish were adapted on the experimental lab. conditions for two weeks in a plastic tank of 1000 L capacity. Then distributed into eight glass aquaria, each volume of 70 × 35 × 40 cm stocked with 12 fingerlings, two aquaria / treatment. Aquaria were provided with 65 L dechlorinated tap water, electric air compressors, air stones and heaters with thermostats. Aquaria water was partially replaced with syphoning five times weekly and completely once a week. Rearing water criteria were periodically measured. Natural light was available 10 h daily. Four iso-nitrogenous (25.36 – 25.92% crude protein) diets were formulated to contain the graded levels of sieving wastes meal of the Egyptian clover seeds instead of dietary soybean meal on basis of crude protein content. Table 1 illustrates formulation of the experimental diets. Diets 1, 2, 3, and 4 contained 25.92, 25.64, 25.36 and 25.36% calculated crude protein (CP), respectively.

Table 1. Formulation of the experimental diets (Kg)

Ingredients	Diets (replacement rate) No.			
	1 (0%, control)	2 (50%)	3 (75%)	4 (100%)
Soybean meal (44%,crude protein)	320	160	100	-
Ground wastes of sieving the Egyptian clover seeds	-	200	300	400
Fish meal	40	40	40	40
Gluten (62%, crude protein)	80	80	80	80
Rice bran (12.5%, crude protein)	120	120	120	120
Corn, fine ground (8%, crude protein)	400	360	320	320
Plant oil	20	20	20	20
Broilers' concentrate	20	20	20	20
Total	1000	1000	1000	1000

All dietary ingredients were purchased from the local market; where, the gluten (62% crude protein) was purchased from Badamas Feed Factory belonging to Dakahlia Company for Poultry and rice bran (4000, 12.5% crude protein) from the Rice Blower at Al-Gamal village, Mansoura. Corn (imported from Argentine) contained 8% crude protein was purchased from Dakahlia Company for Poultry at Badamas, then was ground on 3 mm sieve at Al-Ezz factory of feeds. Broiler concentrate (Holman) was purchased from Dakahlia Company for Poultry at Badamas. It consists of mineral salts and vitamins as given in Table 2. Diets were manually offered five days a week at daily

feeding rate of 3% of the actual body weight of fish, at two meals daily 9 am and 12 mid-day. Fish were weighed bi-weekly to readjust feed quantity. Diets and whole fish body were chemically analyzed using AOAC (2000) procedures. Growth performance [body weight, total length, specific growth rate (SGR), relative growth rate (RGR), condition factor (Froese, 2006), and mortality rate] and feed utilization (feed intake and feed conversion ratio) were measured or calculated. All numerical data collected were statistically analyzed using SAS (2006) and Duncan (1955).

Table 2. Specifications and ingredients of Holman broiler concentrate 45% crude protein (Alpha Feed International, Al Obour – Industrial Zone) Reg. No. 8813

Specifications	Ingredients
Crude Protein 45%	Corn Gluten 60%
Crude Fiber 1.36%	Limestone
Crude Fat 1.38%	Mono Calcium Phosphate (2002)
Calcium 6.32%	Soya bean 48%
Phosphorus available 2.33%	L – Lysine Hydrochloride
Methionine 4%	Salt
Methionine + Cystine 4.69%	Vit.Mix. + Min. Mix. (4578)
Lysine 3.89%	D – L – Methionine
Sodium 0.80%	Choline Chloride (1309)
Metabolizable Energy 2590 Kcal/kg	

RESULTS AND DISCUSSION

Some water quality criteria

The rearing water criteria periodically measured from 19/11/2017 to 16/1/2018 did not differ among treatments or throughout the experimental period. Since water temperature ranged between 28.20 and 28.65ºC, pH 7.85-8.70, dissolved oxygen (DO) 9.10-9.85 mg/L, total ammonia (T. amm.) 0.70-0.85 mg/L, and salinity (Sal.) 1.20-1.55‰ (Table 3). These conditions are suitable for rearing Nile tilapia (Abd El- Hakim *et al*., 2002).

Table 3. Mean values of some quality criteria of the fish rearing water throughout the experimental period

Date	Temp., ºC	pH	DO, mg/L	T. amm, mg/L	Sal., ‰
19/11/2017	28.65	8.30	9.65	0.85	1.30
3/12/2017	28.20	8.35	9.10	0.76	1.40
19/12/2017	28.55	7.85	9.35	0.73	1.20
2/1/2018	28.20	8.00	9.60	0.79	1.30
16/1/2018	28.15	8.70	9.85	0.70	1.55

Proximate analysis of the experimental diets

The gradual levels of the replacement (sieving wastes meal of the Egyptian clover seeds instead of soybean meal in the fish diets) significantly and gradually increased each of the dietary contents of dry matter, crude protein and carbohydrates; but significantly and gradually reduced the dietary contents of both ether extract and crude fibers (Table 4). These results are in partial agreement with those obtained by Abdelhamid and Saleh (2015) and Abdelhamid *et al*. (2016). It was proved that the increase of dietary CP increases significantly the body weight during certain physiological phases, and significantly and positively affects the RGR, as well as significantly influences each of feed intake and dry matter content of fish body (Abdelhamid *et al*., 2001 and Khalil *et al*., 2001).

Feed intake of the experimental fish

Feed intake by the experimental fish significantly differed by the dietary replacement levels. The highest feed intake (P≤0.01) was calculated for the fish group fed at 50% replacement comparing with the other replacement levels (Table 5). These currently findings are confirmed by those reported by Abo-Donia *et al*. (2004), Abdelhamid and Saleh (2015) and Abdelhamid *et al*. (2016) with some terrestrial animals.

Table 4. Mean values of the chemical analysis for the experimental diets, % on dry matter basis

Rep. %	Moisture	CP	EE	CF	Carb.	Ash
0	29.14^{A}	25.15^{D}	13.16^{A}	12.92^{A}	26.78^{bB}	5.77^{C}
50	26.82^{aB}	26.60^{C}	11.52^{B}	9.21^{B}	28.60^{aB}	6.44^{bB}
75	25.78^{bB}	27.40^{B}	10.65^{C}	7.72^{C}	29.39^{aA}	6.78^{aB}
100	24.84^{Bc}	28.09^{A}	9.88^{D}	5.78^{D}	30.06^{aA}	7.12^{aB}

Rep.: replacement, CP: crude protein, EE: ether extract, CF: crude fibers, Carb.: carbohydrates. a-c: Mean in the same column superscripted with different small letters significantly differ (P≤0.05). A-D: Mean in the same column superscripted with different capita letters significantly differ (P≤0.01).

Table 5. Mean and standard errors of feed intake throughout the experimental period (g/fish / 57 days)

Replacement levels, %	Mean	Standard errors (±)
0	23.20^{aB}	0.20
50	25.35^{aA}	0.05
75	23.85^{a}	0.55
100	23.45^{aB}	0.25

a: Means in the same column superscripted with the same small letter do not significantly differ (P>0.05). A-B: Means in the same column superscripted with different capita letters significantly differ (P≤0.01).

Final live body weight of the experimental fish

Concerning the body weight of the experimental fish, there were no significant (P>0.05) differences among dietary treatments at the start of the experiment; yet, there were significant (P≤0.01) variations among treatments at the end of the experiment. Where, the final body weight of Nile tilapia fed dietary 50% sieving wastes meal of Egyptian clover seeds instead of soybean meal was significantly (P≤0.01) heavier than those of the other treatment groups (Table 6). This improvement in the final body weight of that treatment may be due to the significant increase in feed consumption of those group's fish (Table 5), whereas the lower final body weight of fish fed the two highest replacement levels (75 and 100%) may be related to the significant high content of carbohydrates and crude fibers and significant low fat contents of their diets (Table 4). These results are in line with those documented by Abo-Donia *et al*. (2004), Abdelhamid and Saleh (2015) and Abdelhamid *et al*. (2016) although the variation among species of the experimental animals. In the same direction, replacement of 25% freshwater crab meal or 50% mixer (1/1) of duck weed meal with freshwater crab meal instead of fish meal in Nile tilapia diet led to the best final body weight, body weight gain, daily body weight gain, feed conversion rate, and economic efficiency, as well as the highest CP and the lowest EE of fish body and flesh (Abdelhamid *et al*., 2009a & b; 2011 and 2012b). In an another research field for feed evaluation of unconventional carbohydrate resources (one of agro-industrial by-products, i.e corn wastes of "Karata" manufacture) instead of corn in tilapia diets, the 100% replacement rate significantly succeeded; since, it was responsible for the best final body weight, body weight gain, daily body weight gain, RGR, SGR, feed intake, feed utilization, and fish body CP content (Abdelhamid *et al*., 2011). Moreover, in the field of recycling

the agro-industrial by-products in fish feeding, Abdelhamid *et al*. (2015) proved that the distillers dried grains (DDGS) could be used in tilapia diets at 20% replacement level instead of fish meal to obtain the best growth performance; whereas, 100% replacement level led to the best economic efficiency. More recently, Khadr (2018) found that 30% replacement rate of the DDGS did not negatively affect fish performance and health, but even improved the economic efficiency.

Table 6. Mean and standard errors of final body weight (g/fish) of the experimental fish

Replacement level, %	Mean	Standard errors (±)
0	33.05^{aB}	2.05
50	45.50^{A}	2.50
75	26.20^{aB}	1.60
100	27.50^{aB}	2.70

a: Mean in the same column superscripted with the same small letter do not significantly differ (P>0.05). A-B: Mean in the same column superscripted with different capita letters significantly differ (P≤0.01).

Growth rates of the experimental fish

Table 7 illustrates the response of the experimental fish to the dietary treatments tested in form of their growth rates. Throughout the experimental period, both of RGR and SGR negatively affected by all replacement levels, thus reflected lower values than the control (0% replacement level). In an evaluation for plant protein sources, Abdelhamid *et al*. (2004a) reported that mallow (*Malva parviflora* L.) plants meal could replace 10% of the dietary protein as an alternative protein source for Nile tilapia (*Oreochromis niloticus*) fingerlings without negative effects on fish growth performance, fish muscular area, and economic efficiency. In another trial, Abdelhamid *et al*. (2004b) found that it was possible to replace 10% soybean meal protein by sesame meal protein in Nile tilapia fingerlings diet without any negative influences on growth performance, feed and nutrients utilization, and fish body composition. Moreover, it could partially (25-50%) and successfully replaces soybean meal protein by black seeds (*Nigella sativa*) meal protein in Nile tilapia diet, which realized the best growth rates and economic efficiency when black seeds meal is available (Abdelhamid *et al*., 2005b). Water hyacinth protein could also partially (10-20%) replace soybean meal protein realizing the best growth rates and economic efficiency (Abdelhamid *et al*., 2006). Yet, Abdelhamid *et al*. (2010a & b) confirmed the opposite results; since the dietary inclusion of water hyacinth (particularly from polluted resources) negatively affected fish weight, their SGR, survival, feed conversion, and fish body composition. Consequently, they recommended to does not use water hyacinth from polluted water resources in fish nutrition, but when it is necessary and in case of feed shortage, it could be used only from uncontaminated resources and a maximum limit not exceed 30% of the dietary soybean meal protein. Although many people in Kafr El-Sheikh Governorate (as the highest governorate allover Egypt in producing fish) talk about sewage using in fish nutrition; yet, different freshwater fish species (Nile tilapia, silver carp, common carp, and African catfish) reflected low values of all tested measurements (growth, feed utilization, and fish body composition, dressing, and boneless meat) by their feeding with sewage comparing with those fed commercial-artificial diet. Thus, Abdelhamid *et al*. (2014) recommended do not feeding fish with treated sewage.

Table 7. Mean of RGR and SGR of the tested fish throughout the experimental period

Replacement levels, %	RGR, %	SGR, %/d
0	90.99	1.24
50	53.50	0.63
75	58.25	0.60
100	60.35	1.00

Final total length and condition factor of the experimental fish

The calculated condition factor (CF) of the experimental fish at the start of the experiment was 2.018%, since the initial body weight and total length were 17.3 g and 9.5 cm, respectively. Values of final total length and CF of the experimental fish (Table 8) revealed no significant (P>0.05) differences among treatments; yet, fish fed the 50% replacement diet gave superior CF (2.33%) than all other treatments. This superiority of CF with that diet may be due to the significant highest final body weight of fish in that treatment (Table 6) than with all other treatments. Sieving wastes of Egyptian clover (berseem) seeds were rarely used in animal nutrition (Abo-Donia *et al*., 2004; Abdelhamid and Saleh, 2015 and Abdelhamid *et al*., 2016), but there is no available data about its use in fish nutrition. Generally, in the field of using novel (unconventional) feeds, dried live yeast as a source of mono-cellular protein was used with/or without LactoSac® (as a commercial probiotic) in Nile tilapia diets. Its mixture (at a level of 20g/Kg diet) was significantly improved the weight, length, CF, and feed utilization of fish (Abdelhamid *et al*., 2000).

Table 8. Mean of the final total length and condition factor of the experimental fish (each value is the mean of 8 fishes)

Replacement levels, %	Final total length, cm	Condition factor, %
0	13.69	1.29
50	12.50	2.33
75	12.75	1.26
100	12.50	1.41

Feed conversion ratio of the experimental fish

Although the significant elevation of the feed intake by fish in the treatment offered the 50%-replacement diet; yet, it reflected the best feed conversion ratio (FCR) (0.90), since it gave the significant highest final body weight than all other treatments. The values of FCR were 1.47, 0.90, 2.68, and 2.30 for the diets included 0, 50, 75, and 100% replacement levels, respectively. Feed evaluation process continued, Abdelhamid *et al*. (2005a) successfully replaced till 50% of soybean meal protein by linseed meal protein in Nile tilapia diets and obtained improved growth, FCR, nutrients utilization, fish body protein content, and economic efficiency.

Chemical analysis of the whole fish body at the end of the experiment

Table 9 presents mean values of the chemical composition of the whole body of Nile tilapia at the end of the experimental period. There is a gradual and significant (P≤0.01) decrease in moisture percentage, i.e. increase in dry matter content in fish body by increasing the replacement level. This led to significant (P≤0.01) increase in CP and ash contents by the increase of replacement percentage. The opposite was true, concerning EE that significantly (P≤0.01) decreased with increasing the replacement rate. In this respect, Hassanen *et al.* (1995) reported that increasing levels

of some fermented waste foods (tomato pulp silage) in diets reduced lipid content, but increased ash content in whole fish body as that found in the present work. Also, Kheir and Sweilum (1997) stated that increasing dietary CP content led to increase fish body CP and decrease its ash content. Since the negative relationship between CP and EE is a fact (El-Saidy and Gaber, 1997, 2002b & 2005; Hassanen, 1998; Abd Elmonem *et al.,* 2002; Azab *et al.,* 2002; Gaber, 2002a & b & 2006; Zaki and El-Ebiary, 2003; El-Dakar *et al*., 2004b; Gaber and Hanafy, 2004; Abdelhamid *et al*., 2006, 2009b, 2011, 2012b, & 2015; Diab *et al.,* 2006; Hanafy, 2006; Soltan *et al*., 2006; Saad, 2007 & 2010; Ali, 2008; Salem *et al*., 2008 and Farrag *et al*., 2013). Although other researchers reported positive correlation between fish body CP and EE (Kheir and Sweilum, 1997; Mabrouk *et al*., 2000; Soltan *et al*., 2001; El-Saidy and Gaber, 2002a & b; El-Sayed, 2003; Shalaby *et al*., 2003; El-Dakar *et al*., 2004a & b; El-Saidy and Gaber, 2004; Nour *et al*., 2004; Ayaad and Hassouna, 2005a & b; Gaber, 2005; Eweedah *et al*., 2006 and Soltan *et al*., 2008); but others (Mohamed and Hanafy, 2002; Soltan, 2002; Soltan *et al*., 2002 and El-Dakar, 2004) found no effect on fish chemical composition due to dietary treatments.

However, carbohydrates did not significantly influence by the dietary treatments. The improvement in chemical composition of the whole fish body (concerning CP content) may be attributed to the increase of dietary CP content with increasing the replacement level (Table 4). By evaluating medicinal plants in fish nutrition, Abdelhamid and Soliman (2012a &c) obtained significant improvement in Nile tilapia utilization of energy and protein of the diets included fenugreek seeds or Cresson seeds at 1-2% levels, thus the growth rates and fish body protein increased. Also, Abdelhamid and Soliman (2012d) recommended the dietary inclusion of 2% dried guava leaves or camphor trees for its improving effects on growth performance, feed utilization, and fish body composition. Additionally, Abdelhamid and Soliman (2013) also recommended the addition of 1-2% of the common carp diets either of *Mentha arvensis* leaves meal, *Camellia sinesis* leaves meal, *Marticaria chmomilla* meal, *Origanum majorana* leaves meal, *Zingiber officinale* meal, or *Cinnamum zeylanicum* meal (according to its availability and prices to preserve fish production economy), because its beneficial roles in improving growth performance, feed and nutrients utilization, and fish body composition. On the other hand, Magouz (1996) found that dietary inclusion of sugar beet pulp did not influence Nile tilapia body composition.

Table 9. Mean of chemical analysis of whole body of the experimental fish at the end of the experiment (% on fresh weight basis)

Rep., %	Moisture	CP	EE	Ash	Carb.
0	75.23^{A}	17.10^{C}	3.52^{A}	1.66^{bC}	2.48
50	74.54^{B}	18.02^{cB}	3.01^{aB}	2.13^{aC}	2.27
75	73.63^{C}	18.81^{ab}	2.43^{aB}	2.72^{B}	2.39
100	72.95^{D}	19.70^{aA}	1.96^{C}	3.17^{A}	2.21

Rep.: replacement, Carb.: carbohydrates. a-c: Mean in the same column superscripted with different small letters significantly differ (P≤0.05). A-D: Mean in the same column superscripted with different capita letters significantly differ (P≤0.01).

CONCLUSION

From the foregoing results, it could conclude and recommend the best replacement level of 50% (sieving wastes meal of Egyptian clover seeds instead of soybean meal based on crude protein) which significantly realized the best feed composition, highest feed consumption, final body weight, and best body composition of Nile tilapia, that may be reflected in many benefits for both the producers and the consumers.

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تأثير محتوى العليقة من مخلفات غربلة بذور البرسيم المصرى كبديل لكسب فول الصويا للبلطى

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أُجريت تجربة تغذية معملية لمدة 57 يوما على أسماك البلطى النيلى لتقييم تأثير احتواء العلائق على مستويات متدرجة (صفر، 25، 50، 75، 100%) من مسحوق مُخلفات غربلة (عُصافة) بذور البرسيم المصرى بدلا من كسب فول الصويا على أساس المحتوى البروتينى. استخدمت 10 أحواض زجاجية كل منها بأبعاد 70 × 35 ×40 سم، مُلئت بـ 65 لتر ماء صنبور منزوع الكلور، وخُزن بكل منها 12 اصباعية بمتوسط وزن أولى 17.3 جم وطول كلى 9.5 سم. وأوصت النتائج المُتحصل عليها بمستوى استبدال 50%، والذى حقق معنويا أفضل تركيب للعليقة، وأعلى استهلاك للعلف، وأقصى وزن نهائى للأسماك، وأفضل تركيب جسم للأسماك، مما ينعكس بدون شك بالعديد من الفوائد على كل من المنتجين والمستهلكين.