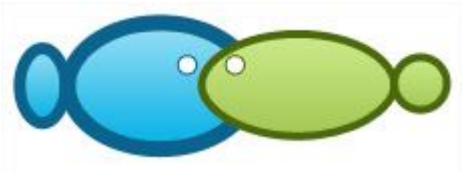

Impacts of using fresh aquatic plants as a total substitute for formulated feed on performance, health and economic efficiency of grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) fingerlings

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Abstract. The objectives of this study are to determine the effects of total replacement of formulated feed (FF) with four fresh aquatic plants (AP): (1) grass weed, *Echinochloa stagnina*; (2) coontail, *Ceratophyllum demersum*; (3) water hyacinth, *Eichhornia crassipes*; and (4) duck weed, *Spirodela polyrhiza* on growth, feed utilization, health and economic value of grass carp, *Ctenopharyngodon idella*. Fish were fed to satiation for 10 weeks. The best growth was observed in the FF-fed group. Protein, albumin, globulin, and cholesterol levels in blood serum were reduced in the AP-fed groups. Positive changes in biometric indices and midgut histology were detected in AP-fed groups. A selective feeding preference and subsequently a potential positive economic efficiency of utilizing duckweed and water hyacinth as alternative feedstuffs instead of high-priced formulated feed, has been confirmed in the present study. Feeding grass carp AP results in healthier fish, reduces production cost, increases net profit, and is more environment-friendly ecosystem. Besides, using grass carp is considered to be very useful as one of the biological solutions to overcome the over vegetation in fish ponds, waterways, rivers and freshwater lakes. However, more research works are needed to define exactly how many fishes are required to control each case.

Key Words: grass carp, aquatic plants, growth, feed utilization, blood, economic value.

Introduction. In modern aquaculture, aquaculture feed management is certainly vital because it is directly correlated to economic and environmental sustainability (White 2013). Feed is the primary cost of production, which should have balanced nutrients and sufficient energy for fish growth (Choi 2013). Fish feed represents around 60% of production cost, according to Yang et al (2003), Erondu et al (2006), and White (2013) and 30-70% of the overall operating cost according to Webster et al (2001). Bad artificial feed quality and overfeeding fish can also negatively result in considerable impacts on the environment (White 2013). Nutrients, specifically carbon, nitrogen, and phosphorous, released from fish farms into the environment and the ecosystem will definitely lead to the environmental pollution in terms of reducing water quality, eutrophication, an influx of disease-carrying fish, etc. (Amirkolaie 2011).

One of the most important ways to solve this problem is the expansion of the organic aquaculture. So, interest in organic aquaculture has been expanded, and many farmers have shifted from the traditional feeding systems to organic cultivation to produce safe seafood stuff that are environmentally friendly (Majhi et al 2006). Rana et al (2009) reported that since 2005, prices increased 20 to 40% due to the rising cost of raw ingredients for commercially manufactured or on-farm aqua feed in all countries of the world. Consequently, this obliged researchers and fish farmers were seeking

alternative sources of cheap feed ingredients to alleviate the rising feeding costs. Research on feeding techniques that promotes growth and reduces the amount of wastes in water is progressively aligned to achieve the optimum utilization of fish production (Singh et al 2005).

Grass carp, *Ctenopharyngodon idella* is basically herbivorous and stomachless fish (Nekoubin & Sudagar 2012a). It naturally grazes on certain aquatic weeds (Cui et al 1991; Ni & Wang 1999). Grass carp is directed in many types of research as a biological agent for vegetation control in lakes, channels, and ponds (Fowler & Robson 1978; Pine & Anderson 1991). It has been introduced in over 50 countries around the world for aquatic weed control and culture. In some countries, the grass carp is an indispensable part of the fish culture and serves as an essential source of protein for human consumption (Sutton et al 2012). The ability of this species to convert substantial quantities of a wide variety of aquatic plants to high-quality animal protein makes grass carp an ideal candidate for culture worldwide, especially in tropical regions (Opuszynski 1972; George 1982; Pipalova 2006). Therefore, Filizadeh et al (2004) mentioned that grass carp prefer feeding on plants, where smaller fish select softer plant tissue and youngest plants while bigger fish eat a wide variety of tough and fibrous plants. Although it has been reported that grass carp prefer filamentous algae and duckweed rather than macrophytes, there are little published data on the feeding's preferences of this species for macrophytes (Swanson & Bergersen 1988).

In Egypt, aquatic plants cause major economic and environmental problems such as increasing siltation, hindering water flow in freshwater canals, the loss of a huge amount of water via evaporation, the interference in navigation and blocking or reducing fishing effort (Khattab & EL-Gharably 1984). Grass weed (*Echinochloa stagnina*), coontail (*Ceratophillum demersum*), water hyacinth (*Eichhornia crassipes*) and duck weed (*Spirodela polyrhiza*) are aquatic plants, broadly available in the Egyptian water-bodies. Those available aquatic plants are often cheaper than other alternative plant protein sources. Therefore, the use of aquatic plants in the production of aquafeed is one of the most promising feed additives in the whole world. At the same time, the exploitation of these plants as feedstuffs also creates additional advantages by helping the ecology of water-bodies and reducing production costs of fish farms. Therefore, the primary goal of this study is to examine the effects of using fresh aquatic plants as alternative diets on the growth performance, welfare and economic value of grass carp fingerlings.

Material and Method

Fish and experimental system. This experiment was conducted at the Fish Nutrition Laboratory, Baltim Research Station, National Institute of Oceanography and Fisheries (NIOF), Egypt from October to December 2015. Grass carp fingerlings were purchased from El-Khashaa Fish Farm, Kafr El-Sheikh Governorate, Egypt. After acclimation in a concrete tank (5 × 10 × 1 m) for two weeks, thirty fish with an average weight of 10.3±0.4 g were randomly stocked into each of 15 concrete tanks (2 × 5 × 1 m) representing five treatments with three replicates per each. Experimental tanks were cleaned and supplied with fresh water and aeration. Water quality parameters including temperature, pH (Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (Jenway Ltd., Model 970-dissolved oxygen meter) were measured weekly. Ambient water temperature, dissolved oxygen and pH through the experimental period were 19.0±2.0^{o}C, 6.5±2.0 mg L^{-1}, and 7.5±0.2, respectively. Fish were fed to satiation the experimental diets twice a day every day at 09.00 and 14.00 h, for 10 weeks. The system used here is flow-through with 20% daily water exchange.

Experimental diets and feeding regime. Five experimental diets were evaluated: (1) formulated feed (25% crude protein (CP)) shortened as (T1); (2) grass weed *E. stagnina* (T2); (3) coontail *C. demersum* (T3); (4) water hyacinth *E. crassipes* (T4) and (5) duckweed *S. polyrhiza* (T5). Aquatic plants were identified and authenticated morphologically. All aquatic plants were collected from Edku Lake, El Beheira Governorate, Egypt, as pictured in Figure 1. The formulated feed was prepared in the Fish Nutrition lab. (NIOF). The proximate chemical analyses of the formulated feed (FF)

and fresh aquatic plants (on dry matter (DM) basis) are presented in Table (1). During the experiment duration, the total quantity of feed consumed by fish in each tank was determined, and the feed intake for each individual fish was calculated accordingly.

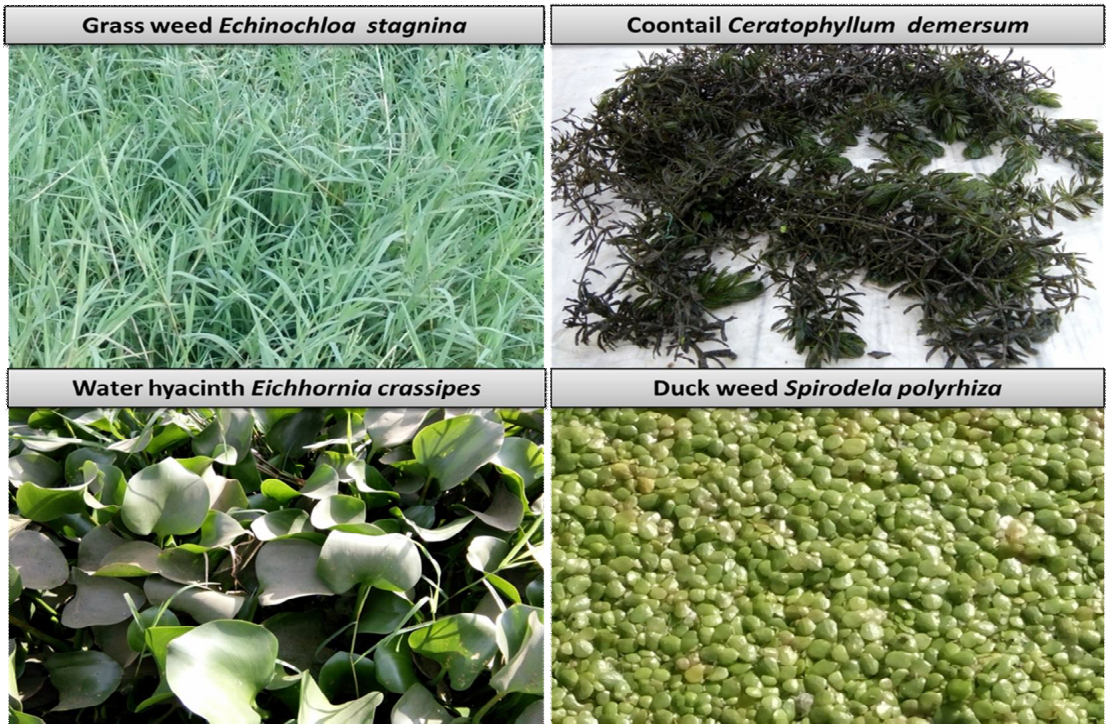

Figure 1. Aquatic plants used in this experiment: grass weed *Echinochloa stagnina* (T2); coontail *Ceratophyllum demersum* (T3); water hyacinth *Eichhornia crassipes* (T4); and duckweed *Spirodela polyrhiza* (T5).

Table 1

Proximate chemical analysis (%; on dry matter (DM) basis) of the experimental formulated feed and fresh aquatic plants used in the present experiment

Item	*Formulated feed*[1]	*Fresh aquatic plants*[*]			
	T1	*T2*	*T3*	*T4*	*T5*
Dry matter (DM)	93.5	16.8	21.3	16	19.2
Crud protein (CP)	24.9	13.1	23.4	12	14.2
Ether extract (EE)	7.0	6.8	7.3	14.5	7.4
Crude fibre (CF)	6.9	14.6	13.8	12.6	11.3
Ash	8.5	12.1	42.4	11	23.2
Nitrogen free extract (NFE)[2]	52.7	53.4	13.1	49.9	43.9
Gross energy (GE; MJ kg^{-1} DM)[3]	17.7	14.96	10.66	17.1	13.8

[1] The formulated feed (25% CP) composed of rice bran (27%), wheat bran (25%), soybean meal (20%), yellow corn (10%), corn gluten (7%), fish meal (5%), soy oil (2%), premix (3%); premix (mg kg^{-1}) contains: p-amino benzoic acid (9.48); D-biotin (0.38); inositol (379.20); niacin (37.92); Ca-pantothenate (56.88); pyridoxine-HCl (11.38); riboflavin (7.58); thiamine-HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); folic acid (0.76); cyanocobalamin (0.08); menadione (3.80), vitamin A-palmitate (17.85); a-tocopherol (18.96); calciferol (1.14); K_2PO_4 (2.011); $Ca_3(PO_4)2$ (2.736); Mg SO_4 $7H_2O$ (3.058); NaH_2PO_4 $2H_2O$ (0.795).
[2] Nitrogen free extract (NFE) = 100 - [% ash + % lipid + % protein + % fiber].
[3] GE (kJ g^{-1}) = ((protein content × 23.6) + (lipid content × 39.5) + (carbohydrate content × 17.2)).
* Tested feeds: T1 = formulated feed; T2 = grass weed *Echinochloa stagnina*; T3 = coontail *Ceratophyllum demersum*; T4 = water hyacinth *Eichhornia crassipes*; T5 = duck weed *Spirodela polyrhiza*.

Growth performance and feed utilization efficiency. At the end of the experiment, fish were harvested, counted, and weighed. Parameters of growth performance and feed utilization were determined as follows:

Weight gain (WG; g) = FW – IW

Specific growth rate (SGR; %/fish/day) = 100 * [(Ln FW) - (Ln IW)] / experimental days

Where: FW = final fish weight (g); IW = initial fish weight (g).

$$\text{Condition factor (K value)} = 100 * [\text{fish weight, g} / \text{fish length}^3, \text{cm}]$$

$$\text{Fish survival (\%)} = 100 * [\text{final number of fish} / \text{initial number of fish}]$$

$$\text{Daily Feeding Rate (\%/fish weight/day)} = 100 * [FI/FW]$$

Where: FI = total actual feed intake (g) based on fresh matter (FM) or dry matter (DM); FW = average weight of fish (g).

$$\text{Feed conversion ratio (FCR) based on DM} = \text{feed intake (g) as dry weight/weight gain (g)}$$

$$\text{Feed conversion ratio(FCR) based on FM} = \text{feed intake (g) as fresh matter/weight gain (g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{weight gain (g)/protein intake (g)}$$

$$\text{Protein productive value (PPV; \%)} = 100 * [\text{protein gain (g)/protein intake (g)}]$$

$$\text{Relative gut length (RGL; \%)} = 100 * [GL/L].$$

Where: GL = absolute gut length (cm); L = total fish length (cm).

Proximate chemical analyses of feed and fish. At the beginning of the experiment, a specimen of the tested fish (about 30 fish) was randomly collected and preserved for initial body chemical composition. At the end of this experiment, 15 fish from each treatment were sampled for determination of proximate chemical composition. Specimens of the tested feeds and fish samples were exposed to proximate chemical analyses to measure moisture, crude protein, crude lipid, crude fiber, and ash according to AOAC (2000).

Serum biochemistry. Blood samples were collected, placed in centrifuge tubes and allowed to clot at room temperature. Serum was then separated by centrifugation at 3000 (rpm) for 10 minutes. The serum was kept at -20^{o}C until analysis of total protein, albumin, globulin, urea, cholesterol, alanine transaminase (ALT), aspartate aminotransferase (AST) and amylase. Serum total protein (g dL^{-1}) was determined using the biuret test according to Henry et al (1974). Albumin content was determined according to Doumas & Biggs (1972). The globulin content (G) was estimated by subtracting the albumin content (A) from total protein content then A/G ratio was calculated. Serum cholesterol (mg dL^{-1}) was estimated by the enzymatic colorimetric methods as described by Thomas (1992). Serum triglycerides (mg dL^{-1}) were estimated according to the method of Friedewald et al (1972). Quantitative estimation of urea (mg dL^{-1}) was estimated according to the method of Henry et al (1974). Activities of aspartate aminotransferase (AST, U/l) and alanine aminotransferase (ALT, U/l) were determined by the colorimetric method according to Reitman & Frankel (1957). Amylase (U/l) was colorimetrically measured using commercial Kits purchased from Bio-diagnostic Co. (Alexandria, Egypt).

Histological examination. Mid intestine samples were carefully excited and fixed in 10% neutral buffered formalin. After dehydrating in ethanol and clearing in xylene, the tissues were embedded in paraffin wax and sectioned at 5-µm thickness layers. The sequential sections were stained with Hematoxylin and Eosin (H&E) (Bancroft & Layton 2013). Histological examination was conducted using light microscopy (Nikon E600, Tokyo, Japan).

Economic efficiency. The economic efficiency was estimated using FCR, the local price of commercial feed in Egypt, the estimated cost of collecting and drying aquatic plants, and the local price of selling grass carp according to the prices of 2017. Feeding cost, other production costs, total production costs, production costs of grass carp fed on the aquatic plants were compared with the control (as %), and the net profit or loss were calculated as follows:

Feeding cost (US$/kg fish) = FCR × feed price (US$/kg feed) abbreviated as [A]
Other production costs (US$/kg fish) = all other costs including (seeds, labor, farm rent, water pumping, etc.) abbreviated as [B]

Total production costs (US$/kg fish) = A + B; abbreviated as [C]

Net profit or losses (US$/kg fish) = price of fish (US$/kg) – total production costs [C]

Statistical analysis. Data of the investigated traits (growth performance, feed utilization, biochemical and blood serum analyses, and morphological measurements of midgut) were analyzed with one-way analysis of variance (ANOVA) using SPSS version 16 statistical package (SPSS Company Inc., Chicago, IL., USA.) to evaluate the differences between the tested treatments. The differences within each experimental treatment are assessed using Duncan's Multiple Range Test at $p \leq 0.05$.

Results

Feed analyses. The chemical composition values for the formulated feed and the fresh aquatic plants were presented in Table 1. The average content of protein in T1, T2, T3, T4, and T5 were 24.9, 13.1, 23.4, 12, and 14.2%, respectively. Furthermore, the level of ash was the highest in T3 (42.4%) compared with the other treatments.

Growth and survival. Growth performance, survival, and condition factor of grass carp fingerlings fed on the experimental diets are presented in Table 2. The results show that there were significant differences ($p \leq 0.05$) in the final body weight, weight gain, and specific growth rate among the diets. The greatest means were detected in T1 treatment followed by T5, T4, T3 respectively, while the poorest means were in T2. Survival was significantly greater in T1 (98.9%), T3 (94.4%) and T5 (93.3%) than T4 (80.00%) and T2 (66.7%). Results of condition factor indicated that grass carp fed on grass weed (T2) showed the lowest significant value (0.85) while carp fed on T1 exhibited the highest condition factors (1.07).

Table 2

Effect of using formulated feed and fresh aquatic plants on growth performance, condition factor and survival indices of grass carp, *C. idella* fingerlings. Data are means±SEM

Treatments[1]*	*Initial fish weight (g)*	*Final fish weight (g)*	*Weight gain (g)*	*SGR (%/fish/day)*	*Condition factor (K)*	*Survival (%)*
T1	10.60±0.12	22.91±0.25^{a}	12.32±0.37^{a}	1.13±0.03^{a}	1.07±0.04^{a}	98.89±1.11^{a}
T2	9.74±0.17	10.42±0.21^{e}	0.69±0.25^{e}	0.10±0.04^{e}	0.85±0.03^{b}	66.67±1.93^{c}
T3	10.60±0.03	12.97±0.23^{d}	2.37±0.24^{d}	0.30±0.03^{d}	1.00±0.05^{ab}	94.44±2.94^{a}
T4	10.92±0.08	14.81±0.10^{c}	3.88±0.10^{c}	0.45±0.01^{c}	0.91±0.06^{ab}	80.00±1.92^{b}
T5	11.18±0.22	16.46±0.37^{b}	5.27±0.53^{b}	0.57±0.06^{b}	0.91±0.05^{ab}	93.33±1.93^{a}

*Means in the same column followed by different superscript are significantly different at $p < 0.05$; [1] Tested feeds: T1 = formulated feed (25% CP); T2 = grass weed *Echinochloa stagnina*; T3 = coontail *Ceratophyllum demersum*; T4 = water hyacinth *Eichhornia crassipes*; T5 = duckweed *Spirodela polyrhiza*.

Feed utilization. Results of feed utilization are presented in Table 3. Feed utilization indices for T1 were substantially ($p \leq 0.05$) the best compared with the other treatments. The best feed intake rate was grass carp fed on T1 followed by T2, T3, T4, and T5, respectively. Feed conversion ratio (FCR based on FM and DM basis) and protein efficiency ratio (PER) differed significantly between treatments, where T1 showed the best values and T2 the poorest. There were significant differences between treatments. The same tendency was noted for protein productive value (PPV). In addition, energy utilization (EU) exhibited substantial differences between treatments, where the greatest value was achieved in T1, and the negative values were significantly ($p \leq 0.05$) observed for T3 and T2 feeds.

Table 3

Effect of using formulated feed and fresh aquatic plants on feed and nutrients utilization indices of grass carp, *C. idella* fingerlings. Data are means±SEM

Treatments[1]*	*Actual feed intake (g feed or food/fish)*		*DFR (%/fish weight)*[2]		*FCR*[3]		*Feed utilization*		*Energy utilization (%)*
	FM	*DM*	*FM*	*DM*	*FM*	*DM*	*PER*	*PPV (%)*	
T1	36.02 ± 0.40^{d}	7.51^{a}	3.16^{c}	3.05^{b}	2.93 ± 0.12^{d}	3.04^{d}	1.43 ± 0.06^{a}	18.28 ± 1.19^{a}	14.80 ± 0.81^{a}
T2	132.42 ± 2.28^{c}	0.80^{c}	19.32^{a}	0.42^{a}	256.15 ± 92.93^{a}	30.14^{a}	0.04 ± 0.02^{c}	0.02 ± 0.20^{c}	-0.79 ± 0.06^{c}
T3	144.16 ± 0.34^{bc}	28.71^{b}	17.98^{ab}	3.83^{b}	62.16 ± 6.46^{b}	12.11^{b}	0.14 ± 0.01^{b}	0.18 ± 0.16^{bc}	-1.17 ± 0.04^{d}
T4	148.56 ± 1.06^{b}	22.22^{c}	16.98^{b}	2.72^{b}	38.29 ± 1.04^{c}	5.73^{c}	0.19 ± 0.00^{b}	2.43 ± 0.06^{b}	0.04 ± 0.02^{bc}
T5	152.07 ± 3.03^{a}	7.30^{b}	17.44^{b}	3.35^{b}	29.58 ± 3.75^{c}	5.18^{c}	0.15 ± 0.02^{b}	1.47 ± 0.21^{bc}	0.46 ± 0.12^{b}

*Means in the same column followed by different superscript are significantly different at $p \leq 0.05$; [1] Tested feeds: T1 = formulated feed; T2 = grass weed *Echinochloa stagnina*; T3 = coontail *Ceratophyllum demersum*; T4 = water hyacinth *Eichhornia crassipes*; T5 = duckweed *Spirodela polyrhiza*; [2] Daily feeding rates (DFR), calculated based on actual feed and aquatic food consumption; [3] Feed conversion ratio (FCR) was calculated based on fresh matter (FM) and dry matter (DM) basis.

Feeding rate. The recorded daily feeding rate (DFR) was significantly ($p \leq 0.05$) influenced by the feed type when calculated on both FM and DM (or either FM or DM or both). On an FM basis, the plant feeds exhibited significant differences in DFR with minimum values at T4 (16.98%) and maximum values at T2 (19.32). Contrarily, when DFR were calculated on a DM basis, there were no significant ($p > 0.05$) differences between the formulated feed and aquatic plants; except grass weed (T2) which was significantly greater than the other tested feeds.

Carcass analyses. Results of the whole-body composition including moisture, CP, EE, and ash were significantly ($p \leq 0.05$) affected by the dietary treatments (Table 4). The greatest value for moisture was recorded in T3 which was significantly higher than all other feeds; moisture in T1 was significantly lower compared to the plant feeds. CP content was substantially higher in fish in T4, T1, and T2 while the lowest significant value was observed in carp fed on T3. Conversely, ether extract for T1 was significantly the highest compared to fish fed on the plant feeds. Also, values of whole body ether extract differed among the plant feeds, where T5 exhibited the highest and T3 the lowest. For ash content, grass carp fed on the aquatic plants showed higher levels, especially in T5, while the smallest level was recorded in T1 with significant differences among treatments.

Table 4

Effect of using formulated feed and fresh aquatic plants on the proximate composition (%; on wet weight basis) of the whole body of grass carp, *C. idella* fingerlings

Treatments[1]*	*Moisture*	*Crude protein*	*Ether extract*	*Ash*	*Carcass energy (Kcal./100g)*
T1	75.43 ± 0.24^{d}	13.57 ± 0.20^{a}	8.80 ± 0.04^{a}	2.58 ± 0.02^{e}	653.47 ± 7.31^{a}
T2	81.81 ± 0.19^{b}	13.51 ± 0.12^{a}	1.44 ± 0.05^{d}	3.32 ± 0.01^{b}	494.75 ± 6.66^{c}
T3	83.45 ± 0.41^{a}	12.06 ± 0.13^{b}	1.29 ± 0.08^{d}	3.19 ± 0.04^{c}	486.55 ± 4.05^{c}
T4	81.12 ± 0.14^{b}	13.77 ± 0.12^{a}	2.32 ± 0.01^{c}	2.90 ± 0.01^{d}	537.38 ± 4.45^{b}
T5	80.01 ± 0.19^{c}	12.89 ± 0.5^{ab}	3.49 ± 0.02^{b}	3.50 ± 0.02^{a}	530.87 ± 4.45^{b}

*****Means in the same column followed by different superscript are significantly different at $p \leq 0.05$; [1] Tested feeds: T1 = formulated feed (25% CP); T2 = grass weed *Echinochloa stagnina*; T3 = coontail *Ceratophyllum demersum*; T4 = water hyacinth *Eichhornia crassipes*; T5 = duckweed *Spirodela polyrhiza*.

Blood analyses. Results of the blood serum parameters are presented in Table 5. Total protein decreased in grass carp fed on the aquatic plants compared with those fed on the formulated feed; with T3 producing the lowest significant values. Albumin values decreased significantly in fish fed on the aquatic plants compared with those fed on the formulated feed (T1). Additionally, a significant increase in globulin concentration was noted for the T2 feed, while carp fed on the T3 feed exhibited the lowest globulin values. Blood urea increased significantly for treatments (T1 and T2) in comparison to T3, T4, and T5 feeds, which had similar values. Feeding grass carp on the formulated feeds significantly increased ($p \leq 0.05$) the content of cholesterol compared to the aquatic plants. Results of ALT analysis showed that fish fed on the aquatic plants exhibited significantly lower values than the control group. AST content in T5 exhibited the highest significant value. Amylase level increased significantly ($p \leq 0.05$) in fish groups fed on the aquatic plants in contrast to the T1 group, with the greatest value recorded in the T2 group.

Anatomy of the gastrointestinal tract. At the end of the experiment, gut length, relative gut length, thickness of the muscularis layer, height and width of villi, and the number of goblet cells per villi were measured in the midgut of grass carp (Figure 2; Table 6). Gut length and relative gut length significantly increased the aquatic plants fed on groups compared with fish fed on the formulated feed (T1). Regarding the thickness of the muscularis, no significant differences between T1, T4, and T5 were detected. The lowest value (4.2 µm) was measured in fish fed on T2. Similar trend was observed in the length of villi and the number of goblet cells/villi. Contrarily, the width of villi showed significantly higher ($p \leq 0.05$) values in T3 and T2. Absorption areas (µm) of T1 and T4 were significantly higher ($p \leq 0.05$) than other treatments. The number of goblet cells per villi decreased significantly in fish fed on the fresh aquatic than T1 group.

Table 5

Effect of using formulated feed and fresh aquatic plants on the serum constituent of grass carp, *C. idella* fingerlings

*Treatments1**	*Total protein (g/dl)*	*Albumin (g/dl)*	*Globulin (g/dl)*	*A/G ratio*	*Urea (mg/dl)*	*Cholesterol (mg/dl)*	*ALT (U/l)*	*AST (U/l)*	*Amylase (U/l)*
T1	2.4 ± 0.12^{a}	1.7 ± 0.12^{a}	0.7 ± 0.20^{b}	2.85 ± 0.76^{a}	19.0 ± 1.15^{a}	183.0 ± 4.36^{a}	126.0 ± 5.86^{a}	86.0 ± 6.76^{b}	148.0 ± 5.51^{d}
T2	2.2 ± 0.06^{ab}	1.3 ± 0.50^{ab}	0.9 ± 0.17^{a}	1.64 ± 0.53^{c}	18.0 ± 0.58^{a}	120.0 ± 3.46^{d}	55.0 ± 4.36^{d}	65.0 ± 5.82^{d}	586.0 ± 8.96^{a}
T3	1.7 ± 0.06^{c}	1.2 ± 0.12^{b}	0.5 ± 0.10^{c}	2.64 ± 0.62^{a}	15.0 ± 1.53^{b}	102.0 ± 5.51^{e}	72.0 ± 3.79^{c}	79.0 ± 4.49^{c}	246.0 ± 6.08^{b}
T4	2.1 ± 0.15^{ab}	1.4 ± 0.15^{ab}	0.7 ± 0.10^{b}	2.13 ± 0.47^{b}	16.0 ± 1.15^{b}	133.0 ± 4.04^{c}	76.0 ± 3.06^{c}	64.0 ± 5.22^{c}	211.0 ± 6.66^{c}
T5	2.0 ± 0.15^{bc}	1.4 ± 0.10^{ab}	0.6 ± 0.06^{bc}	2.35 ± 0.13^{ab}	16.0 ± 1.00^{b}	159.0 ± 5.51^{b}	97.0 ± 6.81^{b}	103.0 ± 5.53^{a}	214.0 ± 6.35^{c}

*Means in the same column followed by different superscript are significantly different at $p < 0.05$; [1] Tested feeds: T1 = formulated feed (25% CP); T2 = grass weed *Echinochloa stagnina*; T3 = coontail *Ceratophyllum demersum*; T4 = water hyacinth *Eichhornia crassipes*; T5 = duckweed *Spirodela polyrhiza*.

Table 6

Effect of using formulated feed and fresh aquatic plants on the morphology and fine structure of midgut of grass carp, *C. idella* fingerlings

*Treatments[1]**	*Gut length (GL) (cm)*	*Relative gut length (RGL) (%)*	*Thickness of muscularis (µm)*	*Villi length (µm)*	*Villi width (µm)*	*Absorption area (µm)*	*Goblet cells/villi*
T1	15.3 ± 1.00^{b}	138.0 ± 1^{b}	5.8±0.2a	28.3 ± 0.3^{a}	7.4 ± 0.5^{b}	209.4 ± 6.4^{a}	23.6 ± 0.6^{a}
T2	18.3 ± 0.12^{ab}	173.0 ± 6^{ab}	4.2 ± 0.3^{c}	23.5 ± 0.7^{b}	7.9 ± 0.3^{a}	185.7 ± 5.6^{b}	13.3 ± 0.4^{c}
T3	17.2 ± 0.95^{ab}	162.0 ± 7^{ab}	4.8 ± 0.2^{b}	22.0 ± 0.4^{b}	8.3 ± 0.8^{a}	182.6 ± 4.7^{b}	16.4 ± 0.5^{b}
T4	20.2 ± 0.88^{a}	185.0 ± 15^{a}	5.6 ± 0.3^{a}	27.7 ± 0.6^{a}	7.2 ± 0.4^{b}	199.4 ± 7.5^{a}	17.0 ± 0.5^{b}
T5	19.3 ± 2.59^{a}	159.0 ± 22^{ab}	5.4 ± 0.2^{a}	27.9 ± 0.3^{a}	5.3 ± 0.3^{c}	147.9 ± 4.2^{c}	18.7 ± 0.7^{b}

*Means in the same column followed by different superscript are significantly different at $p < 0.05$; [1] Tested feeds: T1 = formulated feed (25% CP); T2 = grass weed *Echinochloa stagnina*; T3 = coontail *Ceratophyllum demersum*; T4 = water hyacinth *Eichhornia crassipes*; T5 = duckweed *Spirodela polyrhiza*.

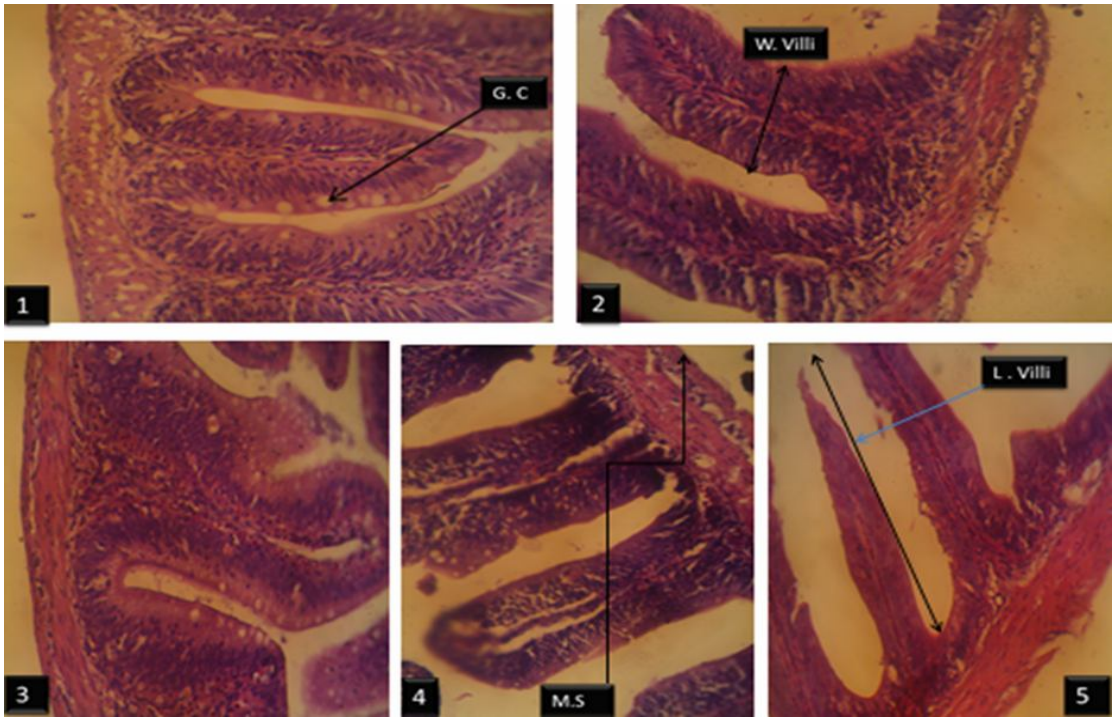

Figure 2. Photomicrograph (H&E staining, 100x magnification) of the middle portion of intestine showing muscularis, (M.S), and goblet cells (G.C) of the grass carp, tested with experimental feeds (T1, T2, T3, T4 and T5 respectively) for 10 weeks.

Economic efficency. Economic evaluation in terms of feeding cost, the total production costs, the net profit or loss and the increase or decrease in the production costs of grass carp are showed in Table 7 and Figure 3. A substantial decline in the production costs (feed and/or total) was detected in grass carp fed on the aquatic plants (except T2) compared to the control group (T1). Feeding grass carp duckweed (T5) and water hyacinths (T4) resulted in higher net profit than the other treatments while the economic loss was calculated in carp fed on the T1 and T2 feeds.

Table 7

Effect of using formulated feed and fresh aquatic plants on the economic evaluation of grass carp, *C. idella* fingerlings

Treatments[*]	*Feed cost (US$/kg fish*[1]*)*	*Other production costs (US$/kg fish)*	*Total production costs (US$/kg fish)*	*Production cost of grass carp fed AP compared with fish fed FF (%)*[2]	*Net profit or losses – (US$/kg fish)*[3]
T1	1.47[b]	0.3	1.77[b]	0	-0.37[c]
T2	1.80[a]	0.3	2.10[a]	+18.64[c]	-0.70[d]
T3	0.72[c]	0.3	1.02[c]	-42.37[b]	0.38[b]
T4	0.34[d]	0.3	0.64[d]	-63.84[a]	0.76[a]
T5	0.31[d]	0.3	0.61[d]	-65.54[a]	0.79[a]

* Tested feeds: T1 = formulated feed (25% CP); T2 = grass weed *Echinochloa stagnina*; T3 = coontail *Ceratophyllum demersum*; T4 = water hyacinth *Eichhornia crassipes*; T5 = duckweed *Spirodela polyrhiza*;
[1] Based on commercial price of formulated feed = 0.5 US$/kg; aquatic plants = 0.06 US$/kg dry weight;
[2] AP = aquatic plants; FF = formulated feed;
[3] Fish price = 1.4 US$/kg (Note: International fish price varies between 1.2 and 2.4 US$/kg, while average was around 1.4US$/kg in Egypt, according to the prices of 2017 (https://www.youm7.com/story/2017/5/1/-ننشر-متوسط-أسعار-السمك-فى-السوق-الحر-بعد-وقف-تصديره/3213428).

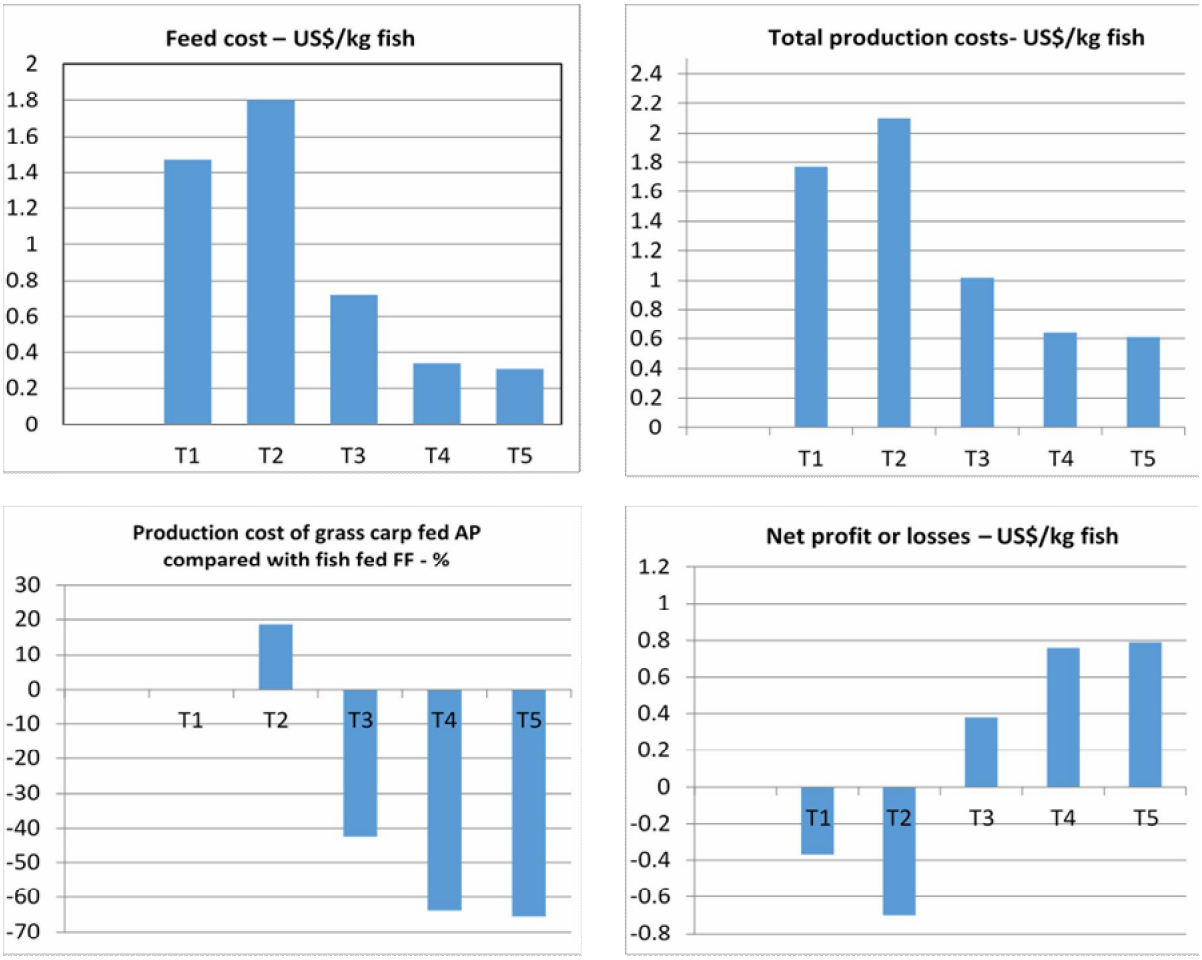

Figure 3. Effect of using fresh aquatic plants (AP)as a substitute of formulated feed (FF) on the economic evaluation of grass carp, *C. idella* fingerlings, where: T1 = formulated feed (25%CP); T2 = grass weed *Echinochloa stagnina*; T3 = coontail *Ceratophyllum demersum*; T4 = water hyacinth *Eichhornia crassipes*; T5 = duckweed *Spirodela polyrhiza*.

Discussion. In the present study, the formulated feed exhibited better growth performance than fresh aquatic plants. This result is in agreement with the findings of Amirkolaie et al (2010) and Nekoubin & Sudagar (2012a) who found that grass carp fed on the aquatic plant (*Phragmites communis*) showed poorer growth performance and survival compared with pelleted feed. Similar data on the growth and digestibility of the Indian major carp were observed by Chan et al (2002) and Safari & Boldaji (2006) when they were comparing a formulated diet to a plant diet. However, in the present study, the survival rate for grass carp fed on the T3, (coontail *Ceratophyllum demersum*) and T5, (duck weed *Spirodela polyrhiza*) feeds in the present study were high and similar to the formulated feed (control group). These data are consistent with the results of Nekoubin & Sudagar (2012b) for grass carp fed on a formulated diet containing 35% crude protein. Amirkolaie et al (2010) evaluated the effect of commercial pelleted diet and grass diet on growth parameters, mortality, and morphology of the gastro-intestinal tract of grass carp fry (1.17 g.). They found that feeding grass carp pelleted diet improved growth parameters compared to the grass diet. There was also a reduction in the mortality rate for fish fed on the pelleted diet.

The capability of grass carp to consume and utilize aquatic plants depends on the species of plant and the developmental stages of both plants and fish (Sutton et al 2012). Despite the low water temperature (less than 20^{o}C) recorded in the present study, grass carp fed on the fresh aquatic plants consumed large quantities, which ranged between 17.0 and 19.3% of body weight per day compared with 3.1% for fish fed on the formulated diet. This finding is consistent with results from other studies (Li et al 1980; Ni & Wang 1999; Filizadeh et al 2004; Nekoubin & Sudagar 2012a, b; Sutton et al

2012). Li et al (1980) explained that when grass carp were fed on the aquatic plants, the daily ration (total mass feed consumed per day/fish mass) may reach 49.9%. The daily feeding rates recorded in the present study for grass carp fed on the aquatic plants supports the scientific conclusion that grass carp is an ideal fish for biological control of submersed aquatic plants in integrated management systems (Silva et al 2014). However, preference of non-target plant species by grass carp is an extremely serious issue (Silva et al 2014) which should be taken into account to avoid the risk of over-predation of ecologically useful plants (Milstein 1992).

Variation in the number of plants consumed by grass carp in the current study may be related to the selective preference of grass carp for certain plant species. For example, the quantity of the consumed duck weed (T5 group) is considerably greater than other aquatic plants. This is in close agreement with other published researchers (Filizadeh et al 2004; Nekoubin & Sudagar 2012a, b; Sutton et al 2012). Sutton et al (2012) stated that grass carp prefer submersed plants and the soft tips of young tender plants. Furthermore, young grass carp prefer musk grass over hydrilla when both plants are present, but large fish will consume hydrilla before musk grass. Although juvenile fish feed on different species of *Cladophora*, *Spirogyra*, and other filamentous algae, the grass carp is not commonly considered as an efficient species to manage many types of algae. Moreover, if the preferred aquatic plant food of grass carp is not available, they will feed on terrestrial vegetation hanging over the surface of the water (Sutton et al 2012).

Poorer feed utilization for fish groups fed on the aquatic plants in the present study may be attributed to a lower nutritive value of some aquatic plants and/or weakened digestive efficiency than for pelleted feed. Comparing to the control feed, lower nutritive value of some of the aquatic tested plants can be noticed here, such as low CP (T4, T2, T5), low gross energy (T3, T5), high fiber content (T2, T3, T4), and high content of ash (T3, T5). This explanation is supported by the results of other researchers (Shireman et al 1983; Du et al 2005; Amirkolaie et al 2010). Despite the high feeding rates, aquatic plants pass through the digestive tract of the herbivorous fish undigested and the whole leaves are often found in the feces (Ni & Wang 1999). In this context, the intestinal evacuation time of aquatic plants for grass carp is 4 hrs. compared with 12 hrs. for a formulated diet (3 times slower) (Du et al 2009). Therefore, to enhance the digestion and the nutrient absorption of aquatic plants, the passing time of food in the digestive tract should be maximized to get sufficient energy and protein. This might be achieved by processing aquatic plants through drying, grinding, and possibly supplementing some deficient essential nutrients, such as enzymes.

Data on body composition in the current study showed greater levels of lipids and lesser levels of moisture in the fish group fed on the formulated feed compared with the other groups fed on the aquatic plants. This result is in accordance with the results collected by Amirkolaie et al (2010) and Nekoubin & Sudagar (2012b). The previous authors noticed that pelleted diet increased fat content and decreased moisture content in the body of grass carp. Also, in the current study, a lower content of ash, and higher content of carcass energy were recorded in the control diet (T1). However, our findings slightly differ from those of Mahmood et al (2018). They found that grass carp gained more ash (13.24%) in the pelleted diet than with water hyacinth supplemented diets. The discrepancy in the previous results may be attributed to the differences between the ingredients and processing technology used in both diets.

Blood chemistry parameters in fishes are influenced by many factors. Water quality, temperature, feeding habitat and physiological status can impact these values either directly or indirectly. Fish hematology is gaining a considerable concern in fish culture as an indication of fish health status (Hrubec et al 2000). In the present study, grass carp fed on T3 exhibited lower values of the majority of the evaluated blood chemistry parameters compared to the other aquatic plants. This result is consistent with the results of Keskinkan et al (2004) in which they observed that submerged aquatic plants, such as coontail, *C. demersum* can be successfully utilized for heavy metals removal in dilute concentrations in rearing water within a relatively short period of time (20 min). Therefore, this may explain why blood parameters in carp fed on T3 were lower

than for the fish fed on the other aquatic plants. In confirmation of the previous scientific opinion, Shaltout et al (2010) found that *C. demersum* had the ability to accumulate more concentrations of heavy metals than the other studied aquatic plant species.

The pattern of distribution and activity of the digestive enzymes were found to be depended on the type of diet consumed by the fish. So, the enzymatic activities of grass carp can be affected by the type of feed (Das & Tripathi 1991). The mRNA levels of trypsin and amylase in fish fed on the duck weed are greater and lipoprotein lipase expression is significantly poorer than those fed on the Chironomid larvae (He et al 2013). Similar changes were noted in the enzymatic activities of amylase in the present study. The results of the present study are in agreement with Das & Tripathi (1991). The presence of higher amylase activity in grass carp groups fed on the fresh aquatic plants reflects the type of food in fish ponds under investigation. The analyses of lipase and protease were not on the same level of importance as amylase in our study that is why we have preferred not to mention the results of these analyses in this study.

The histology of fish is important in understanding the differences in response to the nutritional properties of various kinds of feed. Generally, the histological parameters observed in the current study showed acceptable characteristics in grass carp fed on the formulated diet, such as the highest number of goblet cells/villi (23.6) and the greatest absorption area (209.4 µm). However, the midgut morphology, fine structure, gut length, and RGL showed better development in fish fed on the aquatic plants. This agrees with other published research works (Amirkolaie et al 2010; He et al 2013). The thickness of muscularis and villi' length varied among the tested groups in this study with no statistical significant differences among T1, T4, and T5, the latter two treatments showed better growth and feed utilization compared with T2 and T3. The improved growth and feed utilization for T4 and T5 may be explained by the fact that midgut is the area where the majority of nutrient absorption takes place (Olaya et al 2007), and the better gut characteristics may have played a role. Also, the increase in villi length in the T1 group could be associated with low quality of feedstuffs used to formulate the control diet. That may have caused incomplete digestion of the nutrients of the formulated feed and may have increased the absorptive surface area as shown by Aslaksen et al (2007).

Amirkolaie et al (2010) discovered that fish fed on an aquatic plant diet had a greater proportion of intestine weight to fish weight than those fed on the pelleted diet. Furthermore, He et al (2013) investigated the gut growth of young grass carp during the food transition stage from zooplankton or benthos (Chironomid larvae) to aquatic macrophytes (duck weed) for 60 days. Fish fed on the duck weed had the substantially higher gut length or gut weight than those given the Chironomid larvae. In this study, the maximum number of goblet cells was detected in grass carp fed on the T1 feed with significantly lower numbers among the plant feed groups, which were similar except for the T2 group. These cells are associated with the immune system and act through the mucus as a lubricant, containing zymogen granules, an inactive enzyme, and involved in protein digestion (Saleh & Toutou 2015). Basically, De Silva & Anderson (1995) reported that the number of goblet cells can be altered with the food habit, starvation, or feeding level. This may explain the higher growth and feed utilization recorded in the T1 group. The results of the present study agree with the findings of Toutou (2014) and Saleh & Toutou (2015) in sea bream in which the highest numbers of goblet cells were detected in fish showed higher growth performance and feed utilization.

To develop business strategies for the grass carp aquaculture industry with the self-supportive system, and economic efficiency, a feasibility study should be conducted for hatchery and on growing production scales (Kumar et al 2008). Several authors have reported that grass carp farming can be a profitable and promising enterprise (Shivakumar et al 2014; Chidambaram et al 2016) for freshwater pond polyculture systems worldwide. In the current study, despite the technical positive efficiency of formulated feed compared to fresh aquatic plants in terms of its impacts on growth, feed utilization, and health status, the higher cost of feeding is a major obstacle for feasible economic return. Feeding cost per kg of grass carp fed on FF increased 2.04, 4.32, 4.74 folds compared to T3, T4, and T5, respectively. The current findings of the present study agree with some other researchers worldwide (Shivakumar et al 2014; Chidambaram et

al 2016). In this context, the price of commercial feed ingredients continues to rise and is outpacing the market price of most species of marketable fish (Rana et al 2009), causing diminished economic efficiency for aquaculture projects in general, such as carp farms particularly (Shivakumar et al 2014) as feed accounts for about 50 percent of the production cost. Therefore, identification of cost-effective, alternative feed sources is currently of utmost importance in securing the profitability of carp culture.

Grass carp price varies between countries (Kumar et al 2008) with a maximum 1.4 US$/kg in the Egyptian hypermarkets in 2017. Sequentially, net loss was calculated for T1 group (-0.37 US$/kg fish) compared with substantial net profit for T3, T4, and T5 (0.38, 0.76, and 0.79 US$/kg fish), respectively. Values of production cost (US$/kg fish) calculated in the current study are consistent with the findings of Kumar et al (2008). However, increasing fish production capacity per hectare using economical-price and high-quality formulated feed will improve the economic efficiency of carp farms. In this context, Shivakumar et al (2014) showed that, the profit is more in intensive (well-balanced-feed-based diet) production system ((3500 kg/ha/crop) followed by semi-intensive (complementary-feed-based diet) production system (2500 kg/ha/crop) and extensive production system (1200 kg/ha/crop) without artificial feed. Therefore, the only way to reduce feed cost and improve economic value in grass carp farms, as stated in the present study, is the administration of preferable aquatic plants in the commercial diets, such as duck weed and water hyacinth. The present study showed the potential positive economic feasibility of utilizing some aquatic plants as alternative feedstuffs instead of high-priced common ones. Another viewpoint related to the indirect environmental returns is the biological control of excess aquatic plants in water resources and fish farms using grass carp. This issue is very critical to the sustainability of the aquaculture sector.

Conclusions. Generally, using aquatic plants as a substitute for formulated feed in the culture of grass carp may produce fish with better health status and less production cost, but the downside is the significant lower growth performance. The capability of grass carp to consume and control the growth of undesirable aquatic plants varies depending on plant species with a selective preference for duck weed followed by water hyacinth compared with the other plant species investigated in this study. Therefore, ecologically, grass carp could be an ideal species for integrated ecosystems to manage aquatic plants in natural water-bodies (waterways, rivers and freshwater lakes) and fish farms suffering from over vegetation. However, more research works are needed to define exactly how many fishes are required to control each case.

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