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EFFECT OF DIETARY CRUDE PROTEIN LEVEL AND STOCKING RATE OF MONOSEX ALL MALE NILE TILAPIA REARED UNDER RECIRCULATING AQUACULTURE SYSTEM

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ABSTRACT

To recognize the best dietary crude protein (CP) percentage and stocking rate (SR) of monosex Nile tilapia under recirculating aquaculture system, two CP levels (25 & 32 %) and two SR (140 & 210 / m^3) were used for 168 days. The obtained results revealed that feeding fish 32 % CP-diet at SR of 140 fish / m^3 resulted in the best final bodyweight, average daily gain, specific growth rate, feed conversion, survival, energy utilization, and return. Whereas, 32 % CP at 210 fish / m^3 was responsible for best protein productive value, body weight (with lowest inedible parts percentage), carcass protein percent (with lowest fat %), and productivity / m^3. So, it could recommend feeding Nile tilapia (under similar conditions to those of the present experiment) 32 % CP-diet and stocking rate of 140 fish / m^3.

Keywords: Nile tilapia, monosex, crude protein, stocking rate, aquaculture, recirculating system.

INTRODUCTION

Aquaculture is one of the fastest growing food production systems in the world, which plays a major role as a provider of employment and as a protein source. Aquaculture is defined as farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants, in freshwater, brackish-water and seawater environments (El-Sayed, 2006).

Nile tilapia, *Oreochromis niloticus,* is one of the most cultivated species in the world. This is due to their rusticity, fast growth, high-quality flesh and wide acceptance in the consumer market (Luz *et al.*, 2012). Tilapia culture has been growing at an outstanding rate during the past two decades. As a result, the production of farmed tilapia has witnessed a 2.6 -fold increase during the past 10 years, increasing from 1 795 235 MT in 2004 to 4 823 294 MT in 2013 (FAO, 2013).

The intensive culture of tilapia has been globally expanding, experimentally and/or commercially, in ponds, tanks, raceways, cages, recirculating and aquaponic systems. Water quality (dissolved oxygen (DO), temperature, salinity, ammonia (NH3), pH, dissolved solid metabolites, etc.), nutrition and feeding, and stocking densities are the most important factors that determine the success or failure of intensive culture of tilapia. Intensive aquaculture is simply the employment of high stocking densities of cultured species, in order to maximize the production with the minimal use of water. Such systems depend exclusively on artificial feeding and water reuse and/or exchange (El-Sayed, 2006).

Intensive systems yield high production, ranging from 100 to > 500 Mt/ha/year (Muir *et al.,* 2000). However, they require high capital and operating costs as well as high levels of technology and management tools. It is noted that there are several problems in the semi-intensive farming system, including the use of a large area of land, use a large amount of water and the limited production compared to intensive farming system. Therefore, the aims of the present study were to evaluate suitable percentage of dietary crude protein as well as stocking rate for tilapia in recirculating aquaculture system (RAS).

MATERIALS AND METHODS

The present study was carried out at an intensive fish farm depending on circulated water system. The experimental farm is located in EL-Hammoul province, Kafr EL-Sheikh governorate, Egypt. The study lasted for 21 weeks from 8 March 2014 and lasted until 23August, 2014.

Description of the experimental farm:

The experimental farm consists of 24 concrete tanks (3 x 8 x 1 m^3) which were modified to match the water recirculation system requirements. All fish tanks are located in a greenhouse with diameters 3X8 m. The tanks were arranged in two rows, each row having an irrigation channel with a drained channel in the middle. Sufficient space was made available alongside the fish tanks to build a solid waste removal tank. A separate concrete pond (3 x 30 x 1 m) was used to form the base for a trickle filter. The solid base removal tank was connected to a 2x2 m concrete tank where the water was then pumped to the top of the trickle-down filter.

The total volume of the sedimentation pond is 98 m^3 (14x7x1m) built from concrete, taking in consideration the elevations to allow complete drainage of water in the fish tanks. In addition, managing the water flows to minimize turbulence that gave the solid wastes a greater opportunity to settle to the bottom, where they can be removed periodically by pumping or siphoning outside the system. After removal of the solids, the water is further pumped using a pump of a capacity 6 m^3/minute to the biological filter in form of drops.

In order to achieve the appropriate trickle-down mechanism, an overhead tank was built to receive the pumped water, from where the water is directed through above the concrete roof, which has a large number from tubes (1 cm diameter). The distance between tubes 10 cm. This creates a steady trickle of water over the entire filter media.

The biological tank contains a plastic substrate in order to increase the surface area of the tank leading to more bacterial activity to convert the toxic ammonia NH_3 to non-toxic ammonia NH_4. The biologically treated water is then trickle down to the irrigation channel where the water is enriched with an atmospheric air to increase the oxygen content of the water coming in the fishpond.

The duration of the water recirculation in this system is about 1, 5 times every hour. The system was exposed to cleaning from feed particles and fish feces every three weeks by lowering the water column to 50 cm and the remaining water is pumped outside the system to drainage.

Water source of the experimental farm:

All water requirements of the experimental farm were ground water, which contains 12 parts per thousand salinities (ppt, ‰). The water quality parameters used in this system are given in Table 2 including water temperature, water pH, ammonia, nitrate and dissolved oxygen. Average water quality parameters and the range during the experimental period were determined every 4 weeks.

Water Management:

Two pumps (6 m^3 min-1) worked alternatively to operate the system. The water was pumped to the top of the trickle filter where it slowly flowed through the plastic media, which had been colonized by microorganisms, and then to the fish tank by gravity. The water then leaves the fish tanks to the solid waste filter by gravity.

Thirty percent of the water volume in the system is replaced daily, this removal of water being primarily used to clean the system of faces and other suspended solids. During this cleaning process the fish tanks were divided into two groups; each group of 12 ponds was drained almost completely, the fish being retained in approximately 1 m^3 water, maintaining maximum available water flow.

The tanks were then quickly refilled with the result that the fish were stressed for only a few minutes, during which, the strong flushing helps keep the fish calm. The same operation was repeated every third day. Cleaning the solid waste filter was achieved by pumping the sludge out of the system. Until now, this regime has proved the only way to extract the faces and solid particles. Advanced mechanisms such as drum filters which are used in Europe are not yet available in Egypt.

Aeration:

Aeration was carried out by using a Venturi device. Electric pumps (1.5-Kw) push water through a 5-cm-diameter, plastic pipe, to which 2.5-cm hoses have been fitted, each hose terminating in a Venturi device. Pushing the water through this device creates a stream of air bubbles that rise through the water column. Dissolved oxygen levels measured during the summer were found to be 8 ppm on leaving the trickle filter and 6 ppm after leaving the fish tanks.

Experimental fish:

A total number of 30,757 Nile tilapia monosex fingerlings with an average initial bodyweight of 8 g were used in the present study. The experimental fish were apparently healthy and free of parasites and originated from the same experimental farm (EL-Hammoul province, Kafr EL-Sheikh governorate). The fingerlings were distributed randomly into 12 concrete ponds (8 x 3 x 0.60) =14.4 m^3 representing two dietary crude protein (CP) levels (25 and 30 %), within each protein level two stocking densities (140 and 210 fish/m^3). Each treatment was tested in triplicates. The experimental fish were weighed every two weeks using a sample from each experimental pond contains 5 kg fish in order to readjust the amount of feed required for the next two weeks. The dead fish were removed immediately from the experimental pond and the mortality was recorded.

Experimental diets:

The experimental diets were purchased from the Zoo Control Industrial Company located in 6th October City. The experimental diets were in the form of pelleted floating diets with a diameter 2 mm for the first eight weeks of the experiment, thereafter the pellets diameter was 3 mm till the end of the experimental period. The experimental diet composition as well as its proximate chemical analysis are given in Table 1. The experimental fish were fed the experimental diet at a rate of 3% of the fish biomass divided into equal five portions daily, i.e. at 8 a.m., 10 a.m., 1 p.m., 3 p.m., and 6 p.m. The fish were fed the experimental diet 6 days a week. The experimental ponds were aerated after receiving the last meal till the morning (12 hours). The oxygen supported by aerator (3 horses -320 A –ITALIC made). This aerator distributed air for 60 ponds (each of 8 x 3 x 1.25 m).

Table 1: Feed ingredients percentage and proximate chemical analysis (%) of the experimental diets used in the present experiment.

Feed ingredients	Diet	
	25% CP	32% CP
Fish meal (72% CP)	10	10
Meat meal	10	20
Soy bean meal (44% CP)	16	24
Wheat meal	20	20
Yellow corn	38	20
Corn oil	3	3
Bone meal	1	1
Vitamin premix	1	1
Mineral premix	1	1
Total	100	100
Proximate chemical analysis (%)		
Dry matter (DM %)	90.65	90.14
% on DM basis :-		
Crude protein	25.12	32.2
Crude fat	5.9	7.6
Ash	7.41	9.5
Crude fiber	4.4	4.21
Nitrogen free extract	56.17	46.49
Gross energy Kcal/Kg	427.98	444.36

Measurements:

Water quality parameters were monitored every month according to Abdelhamid (1996). Whereas the chemical composition of diet and whole fish bodies was carried out according to the methods of AOAC (1990) based on the dry matter (DM) contents. Growth performance parameters and feed and nutrients utilization were calculated according to Abdelhamid (2009). The economical evaluations of the tested factors (diet costs plus experimental fish costs) were registered according to the market prices during the experimental period. All other costs are almost the same for the treatments; therefore, electricity and labor are the same for all the treatments.

Statistical analyses:

Analysis of variance for data collected was accomplished using the SAS general Linear Models Procedure (SAS, 2006). The effects of fish stocking density and protein level were statistically analyzed by factorial analysis of variance (2x2). Means were separating by least square means.

RESULTS AND DISCUSSION

Water quality:

Collected data on water temperature, dissolved oxygen, pH, ammonia, nitrate and nitrite is summarized in Table 2. Water temperatures throughout the present experiment ranged between 25.5 and 30.4°C that were closely related to the average of optimal value for tilapia. These results were in agreement with Chervinski (1982) who reported that the temperature range for the normal development, reproduction and growth of tilapia is about 20 to 35°C, depending on fish species, with an optimum range of about 25-30°C. Fluctuations of water temperature are shown, which reached its maximum values during August, however its minimum was during March. The water-dissolved oxygen (DO) throughout the present experiment ranged between 6.1 and 7.3 mg DO/l. The fluctuations of water-dissolved oxygen (DO) are shown, which showed that the maximum values of DO were obtained in May; however, the lowest values were in July. Of all the water quality parameters, dissolved oxygen is the most important and most critical parameter, requiring continuous monitoring in intensive production systems.

Nature plays a cruel joke on aquaculture; the saturation concentration of dissolved oxygen would be highest at low temperature and lowest at high temperatures (Timmons *et al*., 2005). In general, dissolved oxygen levels were within the high standards and higher than cited by Boyd (1979) for good production of tilapia (4.20 to 5.90 mg DO/l) in aquaculture ponds. The water pH values throughout the present experiment ranged between 6.2 and 7.2. The fluctuation of pH is showing, with the highest value of 7.2 in August. The results showed that the present pH values are suitable for rearing in concrete tanks. Timmons *et al*. (2005) observed that the optimum pH for the growth and health of most freshwater aquatic animals is in the range of 6.5 to 9.0.

The water un-ionized (NH_3-N) ammonia throughout the present experiment ranged between 0.01 and 0.19 mg/l. The highest values of 0.19 mg/l during August. The fluctuation of un-ionized ammonia is shown. Timmons *et al*. (2005) showed that the ammonia exists in two forms: un-ionized NH_3, and ionized NH_4^+. The relative concentration of ammonia is primarily a function of water pH, salinity, and temperature. The sum of the two ($NH_4^+ + NH_3$) is called total ammonia or simply ammonia. Un-ionized ammonia (NH_3–N) is the most toxic form of ammonia, so the toxicity of TAN

(TAN = NH_4^+–N + NH_3–N) is dependent on the percentage of the un-ionized form in the TAN concentration. An increase in pH, temperature, or salinity increases the proportion of the un-ionized form of ammonia nitrogen. In general, warm-water fish are more tolerant to ammonia toxicity than cold-water fish, and freshwater fish are more tolerant than saltwater fish. Un-ionized ammonia concentrations should be held below 0.05 mg/l and TAN concentrations below 1.0 mg/l for long-term exposure.

Table 2: Means of water quality criteria during 168-days'experimental period (March to August 2014).

Items	Temperature °C				Dissolved Oxygen (mg DO/l)				pH				Ammonia (mg NH_3-N/l)			
Months	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**
March	26.9	26.1	26.2	25.5	7.1	6.3	6.7	6.3	6.5	6.5	6.9	7.1	0.015	0.016	0.015	0.015
April	27.2	27.6	27.4	27.1	6.9	6.5	7	6.9	7	6.7	6.9	7.1	0.02	0.03	0.02	0.01
May	30.1	29.9	29.8	29.9	7.3	6.9	6.5	7	6.3	6.4	6.9	7.1	0.03	0.05	0.06	0.03
June	29.1	29.3	27.6	28.8	7.1	6.2	6.7	6.3	6.2	6.5	6.9	6.9	0.1	0.12	0.014	0.013
July	30.1	29.9	29.8	29.9	7.3	6.1	6.5	7	6.3	6.4	6.9	7.1	0.19	0.18	0.17	0.18
August	30.4	30.4	29.9	29.4	7.1	6.2	6.7	6.3	6.6	6.5	6.5	7.2	0.19	0.19	0.17	0.19

Mean weight:

Results of the present study showed that the mean weights at all rearing intervals differ significantly (P<0.01) during all experimental periods (Table 3). Averages of fish body weights for 140 and 210 fish /m^3 were found to be 22.92, 16.12 g, respectively after 4 weeks of stocking. The statistical evaluation of results indicated that live weights at this period decreased significantly (P<0.01) with increasing of the stocking density. A similar trend was also observed in fish body weights during the other growing periods. At harvest, average body weight of fish stocked at 140 fish /m^3 was significantly (P<0.01) higher than that stocked at 210 fish /m^3, which indicates that fish weights were decreased with increasing the stocking densities (average fish weights at harvest were 219.2 and 182.37 g for 140 and 210 fish/m^3, respectively). This significant reduction in fish body weights with increasing the stocking density may be due to the reduced feed intake due to the competition between the fish at higher stocking densities or to the increased oxygen uptake at higher stocking treatments. The negative correlation between fish body weight and stocking density could be also attributed to the so called "living space effect" (Swingle, 1956).

Results presented in Table 3 show that dietary protein levels released significant (P<0.01) effects on fish body weights during the completely experimental period. Regardless of stocking density, fish fed 32% CP diet showed significantly (P<0.01) heavier body weights as compared with those fed the 25% CP. These results may indicate that dietary protein level of 32% is required for fast growth of monosex tilapia cultured in intensive culture. At the end of the experiment after 168 days, the average body weights were 171 g for 25% CP and 230 g for 32% CP.

Table 3: Mean values ± SE of body weight (g/fish) of monosex tilapia reared in recirculating aquaculture system (RAS) at two stocking densities and two dietary protein levels.

Item	Tilapia Body Weight (g)					
	Duration (days)					
	30	**60**	**90**	**120**	**150**	**168**
Stocking density (fish/m^3)						
140	22.93^a ±0.82	49.31^a ±1.34	96.35^a ±2.22	135.60^a ±2.09	177.36^a ±1.96	219.20^a ±2.42
210	16.13^b ±0.82	40.84^b ±1.34	78.65^b ±2.22	112.81^b ±2.09	160.166^b ±1.96	182.37^b ±2.42
Protein level (CP %)						
25	17.79^b ±0.82	41.23^b ±1.34	78.35^b ±2.22	111.15^b ±2.09	148.15^b ±1.98	171.41^b ±2.12
32	21.26^a ±0.82	48.93^a ±1.34	96.65^a ±2.22	137.26^a ±2.09	189.37^a ±1.98	230.16^a ±2.12

Mean weigh at start 8 g / fish. Least squares mean ±pooled standard error. a, b: Means having different letter exponents among rows are significantly different (P≤0.05).

Watanabe *et al.* (1990) indicated that the final mean weights were higher for Florida red tilapia fed a diet with 28% dietary protein (average = 176.8g) than those fed 32% dietary protein (average = 166.4g), with a significant effect (P<0.05) of dietary protein level on final weights. However, Tidwell and Webster (1993) used two levels of dietary protein (25 and 32%), they found no significant effect (P≥0.05) of dietary protein on final weight of fish. El-Sayed (2006) reported the protein requirement for optimum reproductive performance of tilapia broodstock as between 30 and 40%.

Results in Table 4 show the effect of interaction between stocking density and dietary protein level on body weight during the experiential period (168 days). Higher weight gains were obtained for the fish stocked at 140 fish / m^3, as compared with those stocked at 210 fish / m^3. This has been observed in all the rearing intervals with significant (P<0.01) differences. The same trend was found with increasing of the dietary protein level from 25 to 32%. Analysis of variance of the effect of interaction between stocking density and protein level on tilapia weight gains are presented in the same Table 4. However, the results indicated significant effect (P<0.05) of interaction between stocking density and protein levels on body weight gain throw all the experimental periods.

Table 4: Mean square ± SE of body weight (g/fish) of monosex tilapia reared in RAS system (interactions of two stocking densities and two dietary protein levels) for 168 days.

Stocking density (fish/m^3)	Item Protein level (%)	Mean square Duration (days) Stocking density* Protein levels 30	60	90	120	150	168
140	25	20.69^c ±1.16	47.21^a ±1.89	87.52^b ±3.14	120.98^b ±2.96	151.53^c ±2.81	192.10^c ±3.41
	32	25.16^a ±1.16	51.41^a ±1.89	105.19^a ±3.14	150.21^a ±2.96	203.18^a ±2.81	246.29^a ±3.41
210	25	14.89^d ±1.16	35.25^b ±1.89	69.19^c ±3.14	101.30^c ±2.96	144.77^c ±2.81	150.70^d ±3.41
	32	17.36^b ±1.16	46.44^a ±1.89	88.11^b ±3.14	124.30^b ±2.96	175.56^b ±2.81	214.04^b ±3.41

Least square means ±pooled standard error. a, b, c, d: Means having different letter exponents among rows are significantly different (P≤0.05).

Average daily gain:

Results presented in Tables 5 and 6 revealed that stocking density and dietary protein level affected significantly (P<0.01) ADG in the first and second month but ADG was not significantly affected during other experimental periods tested. Regardless of dietary protein level, ADG during all experimental periods tested (30, 60, 90, 150 and 186 days after start) decreased in almost linear manner with each increase in the stocking density from 140 to 210 fish / m^3; however, this decrease of ADG values was significant (P<0.05) after the first and second month only. Irrespective of stocking density during the experimental periods, the ADG of monosex tilapia was increased as the dietary protein levels increased from 25% to 32% CP in the tested diets, but this increase of ADG values was significant (P<0.05) after the first and second month only.

Table 5: Mean values ± SE of average daily gain (g/fish/day) of monosex tilapia reared in recirculating aquaculture system at two stocking densities and two dietary protein levels for 168 days.

Item	Average daily gain (g/fish/day) Duration (days) 30	60	90	120	150	168
Stocking density (fish /m^3)						
140	0.66^a ±0.01	0.85^a ±0.03	1.71^a ±1.71	1.59^a ±0.11	1.33^a ±0.14	1.27^a ±0.07
210	0.47^b ±0.00	0.74^b ±0.03	1.29^a ±1.70	1.09^a ±0.11	1.31^a ±0.14	0.78^a ±0.07
P > F	<.0001	0.0285	0.0083	0.0159	0.9303	0.0024
Protein level (CP %)						
25	0.49^b ±0.08	0.77^b ±0.07	1.39^a ±0.08	0.97^a ±0.11	1.08^a ±0.14	0.34^a ±0.07
32	0.64^a ±0.01	0.82^a ±0.02	1.59^a ±0.07	1.71^a ±0.11	1.55^a ±0.15	1.70^a ±0.06
P > F	<.0001	0.1645	0.1224	0.0024	0.0674	<.0001

Least square means ±pooled standard error. a, b: Means having different letter exponents among rows are significantly different (P≤0.05).

These findings agree with those found by Coche (1977), who grew *O. niloticus* from 49 g to 271 g in 122 days (1.4% day). Cruz and Ridha (1991) found that, the ADG was decreased with increasing the stocking density of tilapia (average = 1.91, 1.75 and 1.77 g/ day) at 200, 250 and 300 fish / m^3, respectively after 193 culture days in marine cages. In addition, Siddiqui *et al*. (1991) found that ADG of tilapia *O. niloticus* reared for 98 days at different water exchange in outdoor concrete tanks was 1.06 g / day at 30% dietary crude protein. On the other hand, Siddiqui *et al*. (1988) observed that the ADG of tilapia *O. niloticus* increased with increasing dietary protein levels up to 40% for small size of tilapia. De-Silva *et al*. (1991) indicated that ADG of tilapia increased with increasing dietary protein from 15 to 30%. Watanabe *et al*. (1990) reported that the ADG was higher for tilapia fed the 32% protein diet (average =1.87 g / day) under different densities. Results in Table 6 show the statistical analysis and interaction between the two factors, the results indicated significant effect (P<0.05) of interaction between stocking density and protein levels on ADG throw the periods 30, 120 and 168 days.

Table 6: Effect of interaction between stocking density and protein level on average daily gain (g/fish/day).

Stocking density (fish/m^3)	Protein level (%)	Average daily gain (g/fish/day)					
		Duration (days)					
		30	60	90	120	150	168
140	25	0.61^{b} ±0.01	0.85^{a} ±0.04	1.46 ±0.11	1.21^{b} ±0.15	0.91^{a} ±0.20	0.54^{c} ±0.1
140	32	0.72^{a} ±0.01	0.86^{a} ±0.04	1.96^{a} ±0.11	1.96^{a} ±0.15	1.75^{a} ±0.20	1.99^{a} ±0.09
210	25	0.38^{d} ±0.01	0.69^{a} ±0.04	1.34^{a} ±0.11	0.72^{c} ±0.15	1.27^{a} ±0.20	0.14^{d} ±0.09
210	32	0.57^{c} ±0.01	0.80^{a} ±0.04	1.24^{a} ±0.11	1.47^{b} ±0.15	1.34^{a} ±0.20	1.42^{b} ±0.09
P > F		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Least square means ±pooled standard error. a, b, c, d: Means having different letter exponents among rows are significantly different (P≤0.05).

Specific growth rate (SGR, %/d):

Results presented in Table 7 showed that stocking density and dietary protein level had not affected significantly (P<0.01) SGR in all experimental periods tested. Regardless of dietary protein level, SGR during all experimental periods tested (30, 60, 150 and 186 days after start) decreased in almost linear manner with each increase in the stocking density from 140 to 210 fish / m^3, however this decrease of SGR values were not significant (P<0.05), except during 90 and 120 days. Irrespective of stocking density during the experimental periods, the SGR of monosex tilapia was increased as the dietary protein levels increased from 25% to 32% CP in the tested diets, but this increasing of SGR values was not significant (P≥0.05).

Results in Table 8 show the statistical analysis and interaction between the two factors, the results indicated significant effect (P<0.05) of interaction between stocking density and protein levels on SGR throw the periods 30, 120 and 168 days.

Table 7: Mean values ± SE of specific growth rate percentage (SGR%/fish/day) of monosex tilapia reared in recirculating aquaculture system for 168 days.

Item	Specific growth rate (SGR%/fish/day)					
	Duration (days)					
	30	60	90	120	150	168
Stocking density (fish /m^3)						
140	15.29^{a} ±0.09	16.99^{a} ±0.23	21.51^{a} ±0.32	20.83^{a} ±0.60	18.90^{a} ±1.28	18.03^{a} ±1.65
210	12.89^{a} ±0.09	16.05^{a} ±0.23	19.71^{b} ±0.32	18.20^{b} ±0.60	18.91^{a} ±1.28	11.58^{a} ±1.65
P > F	<.0001	0.0257	0.007	0.021	0.996	0.033
Protein level (CP %)						
25	13.12^{a} ±0.09	16.25^{a} ±0.23	20.26^{a} ±0.32	17.40^{a} ±0.60	16.94^{a} ±1.29	8.14^{a} ±1.66
32	15.06^{a} ±0.09	16.79^{a} ±0.23	20.96^{a} ±0.32	21.54^{a} ±0.60	20.87^{a} ±1.29	21.48^{a} ±1.66
P > F	<.0001	0.141	0.174	0.003	0.074	0.001

Mean weigh at start 8 g / fish. Least square means ±pooled standard error. a, b: Means having different letter exponents among rows are significantly different (P≤0.05).

Table 8: Effect of interaction between stocking density and protein level on specific growth rate (SGR%/fish/day).

Items		Specific growth rate (SGR%/fish/day)					
Stockingdensity (fish/m^3)	Protein level (%)	Duration (days)					
		30	60	90	120	150	168
140	25	14.73^{b} ±0.13	16.97^{a} ±0.32	20.53^{a} ±0.45	19.13^{a} ±0.85	16.07^{a} ±1.82	15.37^{c} ±0.62
140	32	15.8^{a} ±0.13	17.01^{a} ±0.32	22.49^{a} ±0.45	22.54^{a} ±0.85	21.73^{a} ±1.82	22.63^{a} ±0.12
210	25	11.52^{d} ±0.13	15.52^{a} ±0.32	19.99^{b} ±0.45	15.86^{b} ±0.85	17.81^{a} ±1.82	6.99^{d} ±0.30
210	32	14.27^{c} ±0.13	16.56^{a} ±0.31	19.42^{b} ±0.45	20.53^{a} ±0.85	20.01^{a} ±1.82	20.34^{b} ±0.56
P > F		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Mean weigh at start 8 g / fish. Least square means ±pooled standard error. a, b, c, d: Means having different letter exponents among rows are significantly different (P≤0.05).

Feed conversion ratio (FCR): -

The analysis of variance of the FCR values is presented in Table 9. There was a significant (P<0.01) interaction between both variables. In addition, the results of the present study indicate the differences between FCR in the treatments, which revealed the effect of increasing the dietary protein levels. The better FCR was found at the highest density with high protein levels, being 1.53 at 210 fish / m^3 and 32% CP, followed by 1.59 at 140 fish / m^3 and 32% CP, then 1.94 at 210 fish / m^3 and 25% CP and lastly 2.02 at 140 fish / m^3 and 25% CP. The results in the present study confirm those observed by Jorgensen *et al.* (1993) who found that the FCR was always significantly lower at lower stocking densities than that of fish stocked at higher densities. Cruz and Ridha (1991) indicated that there were significant effects among stocking densities on FCR.

Table 9: Mean square values ± SE of feed conversion ratio (FCR) of monosex tilapia reared in RAS system.

Stocking density (fish/m^3)	Protein level (%)	FCR Value
140	25	2.02^a ±0.02
	32	1.59^c ±0.02
210	25	1. 94^b ±0.02
	32	1.53^c ±0.02
P > F		<.000

Least square means ±pooled standard error. a, b, c: Means having different letter exponents among rows are significantly different (P≤0.05).

Survival rate (SR):

Results in Table 10 showed that survival rates were not changed significantly (P>0.05) by stocking density. The average of survival rate was 95.84% in fish stocked at 140 fish /m^3 and 95.02% in fish stocked at 210 fish /m^3. While, the survival rate increased by increasing the protein levels from 25 to 32% being 94.26 and 96.60%, respectively. The interaction effect between stocking density and CP was significant (P≤0.05) as shown from Table 11. Cruz and Ridha (1991) observed that survival rate was decreased by increasing of stocking density. It was highest (83.35%) at 200 fish / m^3 followed by 68.67% at 250 fish and 53.67% at 300 fish / m^3. The differences among the three stocking rates were significant (P<0.05). While, Tidwell and Webster (1993) reported that fish survival was not significantly affected by stocking rate or protein levels and averaged 95.1% overall for fish stocked at lower or higher stocking density in earthen pond.

Table 10: Mean values ± SE of survival rate of monosex tilapia reared in recirculating aquaculture system for 168 days.

Survival rate (SR%)	
Item	**SR%**
Stocking density (fish /m^3)	
140	95.84^a ±0.15
210	95.02^a ±0.15
P > F	0.0089
Protein level (CP %)	
25	94.26^b ±0.15
32	96.60^a ±0.15
P > F	<.0001

Least square means ±pooled standard error. a, b: Means having different letter exponents among rows are significantly different (P≤0.05).

Table 11: Effect of interaction between stocking density and protein levels on survival rate (SR%).

Stocking density (fish/m^3)	Protein level (%)	SR%
140	25	94.72^c ±0.22
	32	96.97^a ±0.22
210	25	93.81^d ±0.22
	32	96.23^b ±0.22
P > F		<.0001

Least square means ±pooled standard error. a, b, c: Means having different letter exponents among rows are significantly different (P≤0.05).

Protein efficiency ratio (PER):

Results of PER are presented in Table 12. The results showed a significant effect (P<0.01) of stocking density on PER. The same trend was observed to protein levels on PER. The best PER was showed at fish stocked 140 fish /m^3 and 25% CP. The analysis of variance of the PER values is presented in Table 12. There was a significant (P<0.01) interaction between stocking density and protein level (Table 13). In addition, the results of the present study explain the differences between PER in the treatments which revealed the effect of increasing the dietary protein level. The better PER was found at the lowest density with low protein level, being 2.80 at 140 fish / m^3 and 25% CP, followed by 2.67 at 210 fish / m^3 and 25% CP, then 2.50 at 140 fish / m^3 and 32% CP and lastly 2.35 at 210 fish / m^3 and 32% CP. The results in the present study agree with Zaki (1993) who indicate that increasing of stocking density from 10 to 40 fish per aquarium caused decrease in PER of common carp. Eid and Magouz (1995) found that PER was significantly (P<0.05) increased with increasing the stocking density from 5 to 10 g/l which disagreed with the results of the present study. Mazid *et al*. (1979) found that PER of *T. zilli* decreased linearly from 3.21 to 1.29 as dietary protein level increased from 21.7 to 53.6%.

Table 12: Mean values ± SE of protein efficiency ratio (PER) of monosex tilapia reared in recirculating aquaculture system for 168 days.

Protein efficiency ratio (PER)	
Item	**PER**
Stocking density (fish /m^3)	
140	2.65^a ±0.00
210	2.51^b ±0.00
P > F	<.0001
Protein level (CP %)	
25	2.73^a ±0.00
32	2.43^b ±0.00
P > F	<.0001

Least squares means ±pooled standard error. a, b: Means having different letter exponents among rows are significantly different (P≤0.01).

Table 13: Effect of interaction between stocking density and protein level on protein efficiency ratio (PER).

Stocking density (fish/m^3)	Protein level (%)	Protein efficiency ratio (PER) Value
140	25	2.80^a ±0.00
	32	2.50^c ±0.00
210	25	2.67^b ±0.00
	32	2.35^d±0.00
P > F		<.0001

Least squares means ±pooled standard error. a, b, c, d: Means having different letter exponents among rows are significantly different (P≤0.01).

Protein productive value (PPV%)-

Results of PPV% are presented in Table 14. The results showed no significant effect (P≥0.01) of stocking density on PPV. The same findings were observed for protein levels effect on PPV%. The best PPV% was shown with increasing the stocking rate which were at fish stocked at 210 fish /m^3 and fed 32% CP. The results in the present study differ with Eid and El-Gamal (1997) and Moustafa (1993) who reported that PPV% values decreased by every increasing of stocking density. These differences may be due to condition of the experiment, like type of the culture system, which was in recirculation system (RAS), green house, which keep the temperature in optimum range for experimental fish, and high density, which was 210 fish/m^3.

Table 14: Mean values ± SE of protein productive value (PPV%) of monosex tilapia reared in RAS system.

Protein productive value (PPV%)	
Item	**PPV%**
Stocking density (fish /m^3)	
140	38.19 ±8.07
210	47.51 ±8.07
P > F	0.563
Protein level (CP %)	
25	37.00 ±8.07
32	48.69 ±8.07
P > F	0.492

Least squares means ±pooled standard error.

Energy utilization (EU %):

Results of EU% are presented in Table 15. The results showed no significant effect (P≥0.05) of stocking density on EU%. The same findings were observed for protein levels effect on EU. The excellent EU% was shown with decreasing of stocking rate which was for fish stocked at 140 fish /m^3 and fed 25% CP. The results in the present study agree with Zaki (1993) who indicated that increasing of stocking density from 10 to 40 fish per aquarium caused decrease in EU of common carp. In addition, El-Sagheer (2001) found that all of the measurements of protein and feed utilization becomes worse with increasing fish stocking density.

Table 15: Mean values ± SE of energy utilization (EU %) of fish reared in RAS system.

Energy utilization (EU%)	
Item	**EU%**
Stocking density (fish /m^3)	
140	29.18^a ±1.40
210	23.55^a ±1.40
P > F	0.215
Protein level (CP %)	
25	27.65^a ±1.40
32	25.07^a ±1.40
P > F	0.417

Least squares means ±pooled standard error.

Abdelhamid *et al.* (2004) working on poly-culture system (Nile tilapia, *Mugillidae*, and carp) in earthen ponds (1 Feddan each)) obtained in 6 months 1.09g daily gain, 811% relative growth rate, 0.511%/d specific growth rate, 2.66 feed conversion, 1.43 protein efficiency ratio, 22.6% protein productive value, and 14.2% energy utilization, for tilapia.

Carcass traits of monosex tilapia:

Table 16 shows carcass traits of monosex tilapia as affected by stocking density and protein level. During the harvest, 10 fish were collected from every pond to do the carcass test. The length, weight, head weight, fins weight, scale weight and viscera weight were measured. The highest average of length was found for fish at 32% protein level, the lowest was found at 25% protein. The lowest averages of head percentage mean was 15.13% for the fish stocked at 140 fish/m^3 and fed 25% CP and the highest was found at 140 fish /m^3 and 32% CP. Averages of fins and scales percentage were between 17.29% for fish treatment of 210*32% and 18.69% for fish stocked at 140*25%. The averages of fins and scales percentage decreased with increasing of either stocking density or CP-level, which were 18.69 and 18.48 at 140*25% and 140*32%. Overall average of viscera weight was 10.34% for the fish in all treatments, which differ with stocking density and protein level. An average of head percentage was 15.93% for the fish in all treatments. These results are confirmed with the findings of AL-Azab (2001) who observed that head percentages decreased with each increase in the stocking density (50,100 and 150 fish/m^3) being 11.33, 10.18 and 9.64%, respectively. On the other hand, El-Sagheer (2001) reported that head percentages increased with each increase in the stocking density (5, 10 and 15 fish/m^2) for fish stocked in the earthen ponds.

Table 16. Mean values of carcass traits of monosex tilapia reared in RAS system.

Stocking density (fish/m^3)	Protein level (%)	Length (cm)	Total weigh (g)	%	Head weight(g)	%	Fins+ scales weight	%	Viscera weight	%
140	25	20.92	182.9	100	30.33	16.58	34.2	18.69	18.11	9.90
	32	19.5	143.6	100	21.73	15.13	26.55	18.48	15.5	10.79
210	25	18.79	132.8	100	21.68	16.33	23.89	17.98	14.5	10.92
	32	21.63	193.7	100	30.38	15.68	33.5	17.29	18.89	9.752

Whole body composition:

The effect of stocking density and dietary protein level on carcass CP% of monosex tilapia is presented in Table 17. The results showed that increasing of protein level from 25% to 32% increased carcass CP%. While, increasing of stocking density had no effect on carcass CP%. The results in the present study are confirmed with AL-Azab (2001) who showed that stocking density did not released any significant effects in protein content in tilapia whole bodies. While, Moustafa (1993) reported that protein contents in the whole bodies of Nile tilapia tended to decrease with each increase in the stocking density. In addition, El-Sagheer (2001) illustrated that carcass CP% in monosex tilapia decreased with each increase in the stocking density. On the other hand, Clerk *et al*. (1990) reported that the body protein content appeared to be higher in fish fed the 30% dietary protein than in fish fed the 20% and 25% dietary protein diets. Tidwell and Webster (1993) found no significant ($P \geq 0.05$) effects or interaction of stocking density and dietary protein on whole body protein.

Results of crude fat % are presented in Table 17. The results showed that crude fat % did not significantly ($P \geq 0.05$) affected by either stocking density or CP%. Yet, crude fat % decreased with increasing of either stocking density or protein level from 32% to 25% CP which were 24.25% in T1 (140 fish/m^3* 25% CP), 21.67% in T2 (140 fish/m^3* 32% CP), 21.72% in T3 (210 fish/m^3* 25% CP) and 18.80% in T4 (210 fish/m^3* 32% CP). The results in the present study disagree with many other studies; this difference may be due to the highest of stocking rate and condition of the experiment in recirculating aquaculture system (RAS). Moustafa (1993) showed that the tissue fat of Nile tilapia reared in cages increased with each increase in the size of fish as a result of decreasing stocking density. In addition, El-Sagheer (2001) reported that crude fat % in tilapia carcass decreased in almost all experimental periods ($P<0.01$) as the fish stocking density increased. De-

Silva *et al*. (1991) indicated that the carcass lipid content increased at all three protein levels (15, 20 and 30% dietary protein), the increase being decreasingly noticeable with increasing dietary protein levels.

Abdelhamid *et al.* (1999) obtained good feed intake (40.64 – 49.38 g/fish) and feed conversion ratio (1.28 – 1.77) by Nile tilapia fish during 128 days. The male-fish muscles contained 19.41% dry matter, 86.75% crude protein, 6.45% fat, and 6.73% ash. Muscular fat and ash percentages were increased by decreasing the crude protein percentages. The fish reflected good nutrients utilization (2.81 protein efficiency ratio, 59.7% protein productive value, and 20.54% energy utilization). Moreover, Abdelhamid *et al.* (2010, 2012, 2013 and 2014) found the negative relationship between crude protein and fat percentages of gilthead seabream, meager and Nile tilapia, respectively in whole fish body.

Ash %:

Results of ash % as affected with stocking density and dietary protein level are presented in Table 17. The results showed that increasing of protein level from 25% CP to 32% CP increased carcass ash %. But, increasing of stocking density had no effect on ash %. Khouraiba (1989) showed that ash contents of tilapia increased with each increase in the stocking density at all stages of the studies. Siddiqui *et al*. (1988) observed that the body ash content of tilapia was unaffected by different dietary protein levels. De-Silva *et al*. (1991) reported that the carcass ash % did not appear to follow a pattern of change in relation to the dietary diet containing different levels of protein and lipid.

Table 17. Mean values of whole body composition of monosex tilapia reared in RAS system.

Stocking density (fish/m^{3})	Protein level %	Crude protein %	Crude fat %	Ash %
140	25	52.10	24.25	12.61
	32	54.13	21.67	15.27
210	25	52.80	21.72	14.99
	32	55.03	18.80	16.34

Fish yield:

Results of Table 18 showed fish yield (Kg) per treatment and per cubic meter (m^3). Results revealed that total yield increased for fish fed 32% CP regardless the stocking density. Results of the present study differ with many studies in the same fields that may be due to the highest stocking density, which reach to 210 fish per cubic meter (m^3), and the stress which fish found it in these treatments. On the other hand, the good production from the minimal stocking rate may be due to the availability of better growth condition like good quality and suitable protein levels. AL-Azab (2001) reported that the final yield of Nile tilapia cultured in tanks increased with each increase in the stocking density from 50 to 100 or 150 m^3. In addition, El-Sagheer (2001) showed that total yield increased significantly ($P<0.01$) with each increase in the stocking density and there was no significant effect for dietary protein level on yield of monosex tilapia. Tidwell and Webster (1993) indicated that the final biomass of fish had not significantly changed by increasing protein level from 25 to 35% in diet.

Table 18: Total yield per treatments and cubic meter (m^3) of monosex tilapia reared in RAS system at two stocking density and two protein levels.

Stocking density (fish/m^{3})	Protein level %	Kg / Treatment	Kg / m^3
140	25	461	10.67
	32	604	13.98
210	25	595.5	13.78
	32	685	15.86

Abdelhamid and Ibrahim (2003a) proved that increasing the crude protein percentage of the tilapia fish wad associated with deceased fat and increased ash percentages. They found also that increasing the dietary crude protein level from 20 to 25% led to decreased the daily growth rate (from 0.89 to 0.85 g/fish), the Feddan productivity (from 3.479 to 2.993 ton); and to worst feed efficiency (from 0.288 to 0.257), feed conversion ratio (from 3.471 to 3.885), protein efficiency ratio (from 1.314 to 0.998) and economic efficiency (from 124.3 to 100.3%), but increased the feeding cost to produce one Kg bodyweight gain (from 3.47 to 4.47 LE year 2002).

Costs and returns:

The total cost was increased by increasing dietary crude protein level or stocking density (Table 19). In addition, net return was increased by increasing the dietary crude protein level, and by increasing the stocking density at 25% CP only but not at 32 % CP (Table 20).

Abdelhamid and Ibrahim (2003b) found that feeding Nile tilapia fish the 25% crude protein sinking diet for 105 days, when increased the stoking density from 20000 to 25000 fry/pond (3200m3) resulted in higher final bodyweight (154.5 vs. 141.2g) and length (19.04 vs. 18.46cm), weight (145.5 vs. 132.2g) and length (11.3 vs. 10.7cm) gains, survival rate (97.5 vs. 82.3%), total food consumption (10547 vs. 8807Kg) and food cost (13184 vs. 11009 LE year 2002), food cost to produce 1 Kg body gain (4.68 vs. 4.37 LE), specific growth rate (1.18 vs. 1.14%/d), food conversion ratio (3.74 vs. 3.50), and the protein productive value (47.2 vs. 51.8%), but resulted in lower food efficiency (0.27 vs. 0.28), daily growth rate (1.1 vs. 1.46g/fish), PER (1.06 vs. 1.14) and economic efficiency (94.9 vs. 119.2%). Increased stocking rate was responsible for increasing DM and CP contents but decreasing ash and fat contents. With increasing CP content, fat and ash contents of the fish were decreased.

Table 19: Total costs of monosex tilapia reared in RAS system at two stocking density and two protein levels.

Stocking density (fish/m3)	Protein level %	Fry cost[1]	%	Diet cost[2]	%	Variable cost [3]	%	Fixed cost	%	Total costs
140	25	2250	30.38	4529	61.16	350	4.73	276	3.73	7405
	32	2250	26.89	5491	65.63	350	4.18	276	3.30	8367
210	25	3375	35.06	5625	58.44	350	3.64	276	2.87	9626
	32	3375	33.73	6006	60.02	350	3.50	276	2.76	10007

1- (375 L.E for 1000 fingerlings average = 8 g). 2- (Ton of 25% CP 5000 L.E and 5500 L.E for 32%CP). 3- Variable costs (L.E/pond) includes, electricity (125), labor (125) and equipment's (100). 4- Fixed costs (L.E/pond) includes, depreciation -buildings and machinery (276).

Table 20: Net return of monosex tilapia reared in RAS system at two stocking density and two protein levels.

Stocking density (fish/m3)	Protein level %	Total production (Kg)	Total income (L.E)	Total cost (L.E)	Net return (L.E)
140	25	461	9220	7405	1815
	32	604	12080	8367	3713
210	25	595.5	11910	9626	2284
	32	685	13700	10007	3693

CONCLUSION

From the foregoing results, the best dietary crude protein percentage and stocking rate of monosex Nile tilapia under recirculating aquaculture system CP levels (25 & 32 %) and two SR (140 & 210 / m^3) were used for 168 days.

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تأثير مستوى بروتين العليقة ومعدل التخزين لذكور البلطى النيلى وحيد الجنس المُربى تحت نظام الاستزراع المائى بإعادة تدوير الماء.

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لدراسة المستوى المناسب لبروتين العليقة ومعدل التخزين لأسماك البلطى النيلى وحيد الجنس المستزرع تحت نظام إعادة تدوير الماء، تم إجراء تجربة لمدة ١٦٨ يوما باستخدام عليقتين ٢٥ و ٣٢ % بروتين خام والتخزين بمعدلى ١٤٠ و ٢١٠سمكة / م^{٣}. فخلُصت النتائج المُتحصّل عليها الى أن المعاملة المُغذاة على مستوى بروتين ٣٢ % وكثافة تخزين ١٤٠ سمكة / م^{٣} قد حققت أقصى وزن نهائى، وزيادة يومية فى وزن الجسم، ومعدل نمو نوعى، وتحويل غذائى، وحياتية، واستفادة من طاقة العليقة، وعائد مادى. بينما العليقة ذات ٣٢ % بروتين خام وكثافة تخزين ٢١٠ سمكة / م^{٣} قد أدت الى أفضل استفادة من بروتين العليقة، وأعلى وزن جسم مع أقل نسبة للأجزاء غير المأكولة من جسم السمكة، وأعلى نسبة بروتين مع أقل نسبة دهن فى جسم السمكة، وأقصى إنتاجية للمتر المكعب من الماء. وعليه فيُنصح بالتغذية (للبلطى النيلى وحيد الجنس تحت ظروف مماثلة لظروف التجربة) على مستوى بروتين ٣٢ % وكثافة تخزين ١٤٠ سمكة / م^{٣}.