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THE IMPACT OF DIFFERENT STOCKING DENESITIES AND DIETARY PROTEIN LEVELS ON THE PERFORMANCE OF AFRICAN CATFISH (*Clarias gariepinus*) FINGERLINGS

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Abstract: The current experiment was randomly designed as a 3×2 factorial design to investigate the effects of two fixed factor (stocking density & dietary protein level) on growth performance, feed utilization, survival rate and physiological response of African catfish *(Clarias gariepinus)*. Fishes were allotted in 18 concrete (8×3×0.5 m^3; L×W×H) tanks at three different stocking density 30, 20 and 10 catfish fingerlings / m3 and fed on two different protein diets (25 and 30%). Each treatment was applied in three replicates. Fishes were fed 2.5% of biomass body weight twice/day. Results showed that growth performance, feed utilization, survival rate and hematological parameters were significantly improved with increased dietary protein level with low stocking density. The sixth treatment (high protein level 30% and lowest stocking density; 10 fish/m^3) exhibited the highest growth performance with no mortalities. Based on the results of the current study, it could be recommended to use protein level diet of 30% or more and stocking density of 10 catfish fingerlings/ m^3 to obtain high productivity in a short time with consequent decreased cost.

Key words: African catfish; *Clarias gariepinus*; dietary protein; growth parameters; Stocking density

Introduction

Fish is one of the most important alternative sources of dietary protein required to meet the increased human population demand in Egypt together with deceased other sources of animal protein (1). Aquaculture is considered a main key for bridging the national fish demand-supply gap (2). Increasing aquaculture production is clearly needed to meet this demand in the third millennium, because capture fisheries is showing precipitous decline due to habitat destruction, over fishing and pollution (3).

The utmost aquaculture goal is the production of high quality, high quantity, and low cost fish in short possible time. This can be achieved by careful species selection, good water quality, appropriate feeding and suitable stocking density (4).That is why it is important to determine the best carrying capacity of an aquatic environment in which we can keep certain numbers of fish in a given volume of the culture media resulting in the highest fish yield without negative

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effect on growth rate depending mainly on the amount and the quality of available food (5)

African catfish (*Clarias gariepinus)* is an endemic popular aquaculture species largely distributed in most African and Asian countries (6-10). It is widely cultured in freshwater ponds because of its high growth rate with excellent quality meat, ability to accept a wide variety of feed, resistance to diseases, easiness in reproduction and its ability to tolerate adverse environmental conditions, and high stocking densities under poly-culture conditions (7, 10-13).

The most critical aspect of aquaculture is to rear the early life stages of fish (fry and fingerlings) (14, 15) because fish at these stages are very sensitive to the different factors or determinants of production. Inadequate stocking densities and poor quality feed have a major role in poor growth and low survival rate of fry and/or fingerling stages resulting consequently in lower production

African catfish are carnivorous as well as predatory; having good special appetite for high protein diet. Therefore, dietary protein levels must be adjusted to meet their requirements. Moreover, stocking densities should be optimized to ensure that the available food is utilized more efficiently with maximum space utilization (16).

The present work was conducted to evaluate the effect of two different levels of dietary protein together with varying degrees of stocking density on the growth parameters, feed efficiency, nutrient utilization, hematological parameters and survival rate of African catfish fingerlings in concrete tanks.

Materials and methods

The present work was carried out in one of the commercial fish farms located at Kafr El-Sheikh Governorate, Egypt, in co-operation with the Animal Production Department, Faculty of Agriculture, Kafrelsheikh University, during the year 2017.

Fish

A number of 4320 African catfish fingerlings (*Clarias gariepinus*) with an average initial weight of (50 ± 2 g) were obtained from a private hatchery in Kafr El-Sheikh governorate. The fish were treated with potassium permanganate solution (3ppm) and acclimatized on the new environment (experimental conditions) for two weeks before the beginning of the experiment in concrete tanks. During the adaptation period, the fish were supplied with natural feeding (trash fish).

Experimental design

The experiment was randomly designed in a 3×2 factorial design. Three stocking densities were used with two levels of dietary protein. Thus, a total of six treatments run in triplicate were allotted into18tanks. Treatments (1-3) comprised of fingerlings fed with 25% CP and stocked at 30, 20, 10 fingerlings/m^3, respectively. However, Treatments (4-6) comprised of fingerlings fed with 30%CP and stocked at 30, 20, 10 fingerlings/m^3, respectively·

Catfish fingerlings (50 ± 2 g) were randomly stocked in 18 concrete tanks each was (8×3×0.5 m^3) capacity. All tanks were supplied with drainage water from drain El-Mohet through inlet PVC pipes (2 inch in diameter). Water outlet was found to be at a rate of 15 liter per minute. The fish were fed at a level of 2.5% of live body weight for about 90 days (experimental period).

Diets

Two tested diets containing 25 and30% protein levels were formulated from the local ingredients including fish meal, soybean meal, yellow corn, wheat bran, rice bran, wheat middling, sun flower oils, vitamins and minerals mixture, and Di-calcium phosphate to obtain control diet. The premix was added to all the experimental diets. The composition of the experimental diets was showed in table (1, A). The diets were prepared by thoroughly mixing the dry ingredients at first then mixed with oil. The diets were analyzed at The Central Laboratory of Food & Feed (CLFF), Agricultural Research Center, Ministry of Agriculture**.** Chemical composition and calculated gross energy of different experimental diets are presented in Table (1, B). First experimental diet (Diet1) consist of 25% crude protein and 408.14 kcal/100g gross energy and the second experimental diet (Diet2) consist of 30% crude protein and 420.73 kcal/100g gross energy.

Feeding regime

Catfish were fed natural feeding for two weeks during the acclimatization period in the experimental tanks. After the two weeks of acclimatization, the catfish were fed the experimental diets at 2.5% from the total biomass daily for 90 days, and were applied twice a day (at 10:00 am & 14:00 pm). The feed amount was adjusted every seven days according to the new weight of the fish.

Proximate chemical analysis

Samples of the experimental diets as well as the experimental fish at the start and at the end of the experiments were obtained and then force dried, milled and deep frozen until determination the chemical analysis according to the methods described by (17).

Water quality management

Water quality parameters, such as temperature, dissolved oxygen, pH, alkalinity, hardness, ammonia, nitrate, nitrite and water salinity were estimated throughout the experiment. Water temperature was measured using thermometer. Oxygen level was measured daily at 8 o'clock by using oxygen meter (Model FE 247, EDT Instruments LTD. Dover Kent, UK). The pH was monitored using pH meter (Model Digi-sense, Cole-Parmer Instruments Co. Vernon Hills, IL. USA, Figure 1). Alkalinity was determined using test kit (Model WAT-DR. Code 4491-DR, LaMotte CO. Chestertown, Maryland, USA). Hardness was determined using test kit (Model HA-DT Cat. 20636-00, Hach Co. Loveland, Colorado, USA). Ammonia-N was estimated using test kit (Model NI, Cat. No. 22669-00, Hach Co.). Nitrite and nitrate were measured using test kits (Model NI-16 Cat. No. 20596-00 and Model NI-14 Cat.No. 14161-00, Hach Co., respectively). Determinations were carried out weekly according to APHA (18).

Determination of fish growth parameters

Growth and feed utilization were assessed by calculating average weight gain, average daily gain, specific growth rate, protein efficiency ratio, feed intake, feed conversion ratio, feed efficiency and survival rate:

Live body weight (LBW) was assessed in g for each individual group of each experimental treatment and recorded every 2 weeks (14 days).Total weight gain (TWG) (g/fish) = final body weight – initial body weight (19). Specific growth rate (SGR %/day) = [Ln final body weight- Ln initial body weight]×100/experimental period(d) (20). Average daily gain (ADG) = (W2 – W1) / t; where: W2 is the final weight, W1 initial weight and t is the time in days. Feed conversion ratio (FCR) = feed intake (g)/weight gain (g) (21). Protein efficiency ratio (PER) = weight gain (g)/protein intake (g). Protein productive value (PPV %) =100× (protein gain (g)/protein intake (g)). Survival rate (SR %) = total number of fish at the end of the experiment × 100 / total number of fish at the start of the experiment.

Hematological investigations

At the end of the experiment, fish in each group (3-4 fish) were weighed and blood samples were taken randomly from the caudal vein for blood analysis and differential leukocyte count. Anti-coagulated blood samples were prepared immediately for counting red and white blood cells, etc. Red blood cells count ($RBCs\times10^6$/mm) and white blood cells count ($WBCs\times10^3$/mm): were determined according to the method described by Stoskopf, (22). Hemoglobin concentration (Hbgm/dl) was estimated according to the method of Zinkl (23) and Packed cell volume (PCV%): was estimated by the microhaematocrite method described by Decie& Lewis (24).

Statistical Analysis

The obtained numerical data were statistically analyzed using SPSS (25) for two-way analysis of variance at 5% level of significance. When F-test was significant, least significant difference was calculated according to Duncan (26).

Results and discussion

Growth is the principal key of energy loss and/or gains in the aquatic environment; which can be measured mainly by determination of the weight gain. Stocking density is a main factor significantly affecting the growth, feed utilization (FCR) and survival rate (SR) of the catfish fingerlings. High stocking densities act as stressors, fish aggregate for the same amount of feed and some of them cann't eat resulting in growth retardation (27) and/or decrease of feed utilization (28). Moreover, in high stocking densities, there is increased oxygen demand needed either for respiration or metabolism. Therefore, it is very important to adjust the stocking density with the carrying capacity of the aquaculture system.

Growth performance and survival rate

As shown in table 2, catfish fed on 30% protein level diet showed better final weights compared to those fed g on 25% protein level diet at the different stocking densities. The final weight was significantly increased with increased protein in diet ($P \leq 0.05$). The final weight of Treatment 1 (T1) was the lowest weight (214.3± 10.12) compared with the other treatments. While, final weight of treatment six (T6) was the highest weight (284.00± 14.32) ($P \leq 0.05$) compared with the other treatments. The average weight gain (AWG) was improved especially in groups fed on high protein level. While, the first treatment (T1) showed the lowest AWG (164.30±8.24) compared with other treatments. Similarly, average daily weight gain (ADG) of treatments (4-6) feeding on 30% protein was better than treatments (1-3) feeing on 25% protein diet in all variant stocking densities; with the highest value recorded in treatment 6 (2.60±0.63) and the lowest value in treatment 1 (1.83±0.42). Besides, the highest SGR (1.71±0.41) was observed in treatment 6. The results are similar to those reported by some authors (29-33), where they reported that increased protein concentration in the diet has a positive correlation with the final weight gain expressed as AWG, ADG and SGR.

On the other hand, results of the present study showed that the decreasing stocking density, the increased final weight. Decreased stocking density significantly affected the final weight gain, the highest stocking density in treatment 1 (30 fish/m^3)(214.30±10.12) gave the worst final body weight compared with treatment 3 (10 fish/m^3) (256.30±12.54), although both treatments feed on the same protein level diet 25%. These results also could be observed in fingerlings fed on 30% protein level diet, where treatment 6 (10 fish/$m^{3)}$ showed the best result of all growth parameters compared with other groups. This means that decreasing the stocking density gave a positive effect ($P \leq 0.05$) of final weight gain. These results agreed with many authors (34-38), where they found that increased stocking density resulted in less AWG, ADG and SGR.

Survival rate of the experimental fish was recorded. It was 100% in treatments 2, 3, 5 and 6 (low stocking density), but treatment 1 and 4 (highest stocking density) lost some fingerlings in the first days of experiment. This may be attributed to increased oxygen demand in high stocking treatments which may result in fish may succumb to suffocation. Survival rate increases with increased dietary protein level, this may be attributed to that low protein diets might have not met the nutrition requirements of catfish fingerlings and thereby leading to nutrient-deficient related mortalities (39, 40).

Water quality parameters

Water quality parameters of the experimental ponds were insignificantly affected by different treatments during the experimental period (90 days). As summarized in table 3, all water parameters for all experimental ponds showed that, dissolved oxygen not least 4 mg/ litter, toxic ammonia ($NH3$) no more than 0.6 mg/ litter, pH values between 8.2 and 8.5 degree, nitrate ($NO2$) no more than 0.2 mg/ litter, alkalinity 320, hardiness 150, salinity between 2000 and 4000 ppm (source of water was brackish water) and temperature about 27ºC. Water quality parameters observed in the current study were within the normal ranges required for normal growth of African catfish (9, 33, 39, 41-45); consequently any changes in the growth parameters may be attributed either protein level diet and/or stocking density.

Feed intake and nutrient utilization

As shown in table 4; holding all factors including the different stocking density of the present study, results showed that dietary protein has a marked influence on feed utilization. Results showed that increasing the level of protein in the diet increased significantly ($P \leq 0.05$) feed conversion ratio of fish. Diet 30% protein gave the best results ($P \leq 0.05$) of feed conversion ratio compared with diet 25% protein with different stocking densities; and the sixth treatment (1.00±0.09) gave the best feed conversion ratio (FCR) compared with the third treatment (1.13±0.11) (same of stocking density)while the first treatment (1.40±0.24) was the worst treatment compare with other treatments. Besides, increasing protein level in the diets affected significantly ($P \leq 0.05$) protein efficient ratio (PER) of fish. The same trend was observed with protein productive value (PPV); these results of feed utilization may be due to the impact of different protein levels together with different stocking densities. Protein efficiency ratio (PER) and protein productive value (PPV) were better generally with low stocking densities and high protein level diets. These results are in full agreement with some authors (35, 46-50).

Body composition of African catfish as affected by the experimental diets

Data concerning body composition responses of African catfish presented in table 5 revealed that the dry matter, crude protein, ether extract contents were increased by the time from the start to the end of experimental period (90) day, but the opposite was true for the ash percentage. Increasing protein level in the diet affected crude protein of body composition significantly ($P \leq 0.05$). Fingerlings in treatment 1 (25% protein) had low crude protein (66.92±5.36) ($P \geq 0.05$) as compared with the other treatments, while the sixth treatment (30% protein) (67.39±5.47) was the best in crude protein (CP) content ($P \geq 0.05$) compared with other treatments. However, dry matter (DM) in was low in treatment 1 (28.78±1.58) as compared with the other treatments, while sixth treatment (29.21±1.75) was the best treatment compared with the different treatments. Results also revealed that increasing level of protein in diet affected positively ($P \leq 0.05$) ether extract (EE) and gross energy (GE) of fish body.

Hematological investigation

As summarized in table (6), there was a significant increase in RBCs, Hb, PCV and WBCs in all treatments; the sixth treatment (30% protein and low stocking density) showed the best hematological parameters and treatment 1(25% protein and high stocking density) showed the worst hematological results. These results suggest that the physiological response of catfish fingerlings is improved when fed high protein level diet together with existing low stocking density.

In the present study, Growth depression observed in higher stocking densities groups may be attributed to reduced amount of adequate oxygen, followed by slowed down metabolism resulting in lower growth than in lower stocking density (27, 51). The decreased FCR at high stocking density (30 fish/m^3) may confirm that high stocking density reduced feed utilization efficiency. Due to competition for feed, there is low feed intake and consequently lower energy levels necessary for metabolism which is responsible to convert nutrients into fish flesh.

Growth depression observed in lower protein level diets has been observed in various fish species under captivity; this might be due to reduction in the available energy for growth (52, 53). Fish usually obtain energy from chemical breakdown of proteins than large animals that is why high protein is highly important in fish diet (54). Lowest growth in 25% protein level diet may be due to that most of protein was used for maintain life but unavailable for growth; however 30% protein level diet resulted in higher growth rate and better feed utilization in catfish fingerlings.

High focusing to body protein is of a great importance to meet the dietary requirements for tissue building, metabolism and repair; as a result of poor growth and poor feed utilization in fish group fed low protein level in the current study (55).This means the fingerlings survived in low stocking density and fed on 30% protein level diet could use dietary protein more efficiently than fish survived in higher stocking densities and fed on 25% protein level diet.

Table 1A: Composition of the experimental diets

Raw Ingredients	Composition (%) experimental diets Diet 1 (25% Protein)	Diet 2 (30% Protein)
Fish mail 62%	10	15
Soya bean 44%	27.5	35
Yellow Corn	10	10
Rice bran	24	21.5
Wheat middling	11	10
Wheat bran	14	5
Sun flower oil	2	2
Premix [1]	1	1
Di calcium phosphate	0.5	0.5
Total	100	100

(1)Premix Composition:- Each 3 kg contains , Vit A (1200000 i.u.), Vit D (300000 i.u.), Vit E (700 mg,) Vit K3 (500 mg,) Vit B1 (500 mg), Vit B2 200mg, Vit B6 (600mg), Vit B12 (3mg), Vit C 450mg, Niacin 3000mg, Methionine3000mg, Cholin chloride 10000mg, Folic acid 300mg, Biotin 6mg, Panthonic acid 670mg, Magnesiam salphate 3000mg, Copper sulphate 3000mg, Iron sulphate 10000mg, Zinc sulphate , 1800mg, Cobalt sulphate 300mg, Carrier upto 3000mg.

Table 1B: Proximate analysis of the experimental diets

Composition (%)	Diet 1 (25% CP)	Diet 2 (30% CP)
Dry matter	90.30	90.90
Organic matter	78.30	80.50
Crude Protein	25.45	30.40
Ether extract	5.40	5.00
Crude Fiber	6.60	6.40
Ash	12.00	10.40
Nitrogen free extract	50.55	47.80
Calculated energy value:		
*GE (kcal/100g)	408.14	420.73
**DE(kcal/g)	306.11	315.55

*GE (gross energy) was calculated according to NRC (1993) by factors of 5.65, 9.45 and 4.22 kcal per gram of protein, Lipid and carbohydrate, respectively. **DE (digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy.

Table 2: Growth performance parameters of African catfish fed different experimental diets

Treatment	Protein Levels %	Stocking density fish/ m^3	Initial BW(g)	Final BW(g)	AWG (g)	ADG (g)	SGR (%)	SR (%)
1	25	30	50.0±2.58	214.30±10.12^{c}	164.30±8.24^{c}	1.83±0.42^{c}	1.62±0.32^{ab}	99.80±5.54
2		20	50.0±2.44	227.00±11.24^{c}	177.00±9.54^{c}	1.97±0.51^{c}	1.40±0.38^{c}	100.0±0.00
3		10	50.0±2.64	256.30±12.54^{ab}	206.30±10.25^{ab}	2.29±0.61^{ab}	1.57±0.30^{b}	100.0±0.00
4	30	30	50.0±2.87	233.60±11.87^{bc}	183.60±8.98^{bc}	2.04±0.54^{bc}	1.45±0.24^{c}	99.90±4.87
5		20	50.0±2.67	247.00±12.54^{b}	197.00±9.68^{b}	2.19±0.55^{b}	1.52±0.29^{bc}	100.0±0.00
6		10	50.0±2.64	284.00±14.32^{a}	234.00±11.23^{a}	2.60±0.63^{a}	1.71±0.41^{a}	100.0±0.00

A,b and c mean the column bearing different letters differ significantly at 0.05 level

Table 3: Water parameters during the experimental period

Parameters	T1	T2	T3	T4	T5	T6	Standard no.
Temperature	27.5º	27.8º	28.1º	28.3º	27.7º	28.5º	20 : 32
Ph	8.2	8.4	8.3	8.5	8.2	8.3	6.5 – 8
Dissolved O_2	6	6	6	6	6	6	>4
NO_2	Nil	Nil	Nil	Nil	Nil	Nil	< 0.2
NH_3 (mg /l)	0.6 :0.9	0.6 :0.9	0.6 :0.9	0.6 :0.9	0.6 :0.9	0.6 :0.9	< 0.6
Alkalinity	320	320	320	320	320	320	<500
Hardiness	150	150	150	150	150	150	<500
Salinity ppm	2000 :4000	2000 :4000	2000 :4000	2000 :4000	2000 :4000	2000 :4000	0 – 5000

Table 4: Average feed intake, feed conversion ratio, protein efficiency ratio (%), feed efficiency and protein productive value of African catfish fed different experimental diets

Treatment	Protein Levels %	Stock density fish/ m^3	Feed in-take g/fish	Average weight gain (g)	FCR	PER	PPV
1	25	30	230.56±11.24	164.30±8.24^{c}	1.40±0.24^{c}	2.80±0.35^{c}	19.09±2.15^{b}
2		20	231.25±11.35	177.00±9.54^{c}	1.31±0.18^{bc}	3.01±0.57^{b}	20.56±2.22^{b}
3		10	233.33±11.57	206.30±10.25^{ab}	1.13±0.11^{a}	3.47±0.64^{a}	23.79±2.11^{a}
4	30	30	230.56±10.98	183.60±8.98^{bc}	1.26±0.17^{b}	2.62±0.38	17.96±1.98^{c}
5		20	231.25±10.89	197.00±9.68^{b}	1.17±0.12^{ab}	2.80±0.37^{c}	19.20±2.08^{b}
6		10	233.33±11.47	234.0±11.23^{a}	1.00±0.09^{a}	3.30±0.55^{ab}	22.56±2.34^{a}

A,b and c mean the column bearing different letters differ significantly at 0.05 level

Table 5: Body composition of African catfish as affected by the experimental diets (% on dry matter basis)

Treat. No	% On Dry matter basis				
	DM	CP	EE	Ash	GE** (Kcal/100g)
At the start of the experiment					
	25.00±1.15	62.80±4.56	14.10±1.22	20.95±0.38	497.14 ±11.5
At the end of the experiment					
T1	28.78±1.58	66.92±5.36	17.08±1.54	13.90±0.15	548.34±15.24
T2	29.12±1.64	67.13±4.87	17.02±1.36	13.91±0.21	548.31± 14.28
T3	29.11±1.52	67.36±5.24	16.94±1.54	13.74±0.32	548.94±13.47
T4	29.09±1.66	67.33±5.11	16.77±1.74	13.94±0.22	547.16±13.65
T5	29.19±1.87	67.36±5.22	16.77±1.64	13.87±0.19	547.50±13.78
T6	29.21±1.75	67.39±5.47	16.79±1.77	13.78±0.24	548.03±14.55

A,b and c mean the column bearing different letters differ significantly at 0.05 level.
**Gross energy was calculated according to NRC (1993) by using factors of 5.65, 9.45 and 4.22 Kcal per 1 gram of protein, lipid and carbohydrate, respectively

Table 6: Effect of protein levels and stocking denesity on the haematological parameters in catfish fingerlings

			Haematological parameters			
Treatment	Protein Levels %	Stocking density(fish / m^3)	RBCs (x10/mm^3)	WBCs (x10^3/mm^3)	Hb (g/100ml)	PCV (%)
T1	**25**	30	2.48±0.05[d]	75.86±1.12[b]	7.62±0.41[c]	21.00±0.5[a]
T2		20	2.58±0.2[b]	73.45±0.81[d]	7.63±0.15[c]	22.50±0.5[a]
T3		10	2.68±0.14[b]	74.23±1.3[c]	7.64±0.33[c]	22.00±0.5[a]
T4	**30**	30	2.63±0.4[c]	76.07±2.1[b]	7.70±0.41[a]	23.50±1.0[a]
T5		20	2.72±0.2[a]	72.51±0.5[e]	7.79±0.5[ab]	23.50±1.0[a]
T6		10	2.83±0.08[a]	67.23±0.59[f]	7.82±0.28[a]	24.00±0.19[a]

RBCs= Red Blood cells; WBCs= White Blood cells; Hb = Hemoglobin; PCV = Packed Cell Volume

Conclusion

Both Stocking density and Protein level in fish diet have a significant effect on growth rate, feed utilization, physiological responses and survival rate *Clarias gariepinus* fingerlings in concrete tanks. Based on the results of the current study, it could be recommended to use protein level diet of 30% or more in combination with stocking density of 10 fish/ m^3 to obtain higher production in a short time.

Conflict of interest

The authors declare that they have no conflict of interest.

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