Effect of different feeding times on growth performance and blood parameters of African catfish (*Clarias gariepinus*) under different stocking densities.

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ABSTRACT:

The current study was designed to evaluate the effect of feeding time and different stocking densities on water quality parameters, growth performance, feed utilization, chemical composition, and blood biochemistry of African catfish (*Clarias gariepinus*). One hundred and eighty catfish with an average initial weight of 196.8 ± 0.47 g were randomly distributed in 18 concrete ponds in (6 treatments). The experiment was based on the design of a 3×2 factorial with three different feeding times (8 am, 12 pm and 6 pm), two levels of stocking density (1kg/m^3 and 2kg/m^3). The experimental fish were fed on a commercial diet (36% crude protein), at a feeding rate of 3% of body weight. Water quality parameters, growth performance, feed utilization, chemical composition, and blood biochemical in all groups were calculated at the end of the experiment after 60 days. The results indicated that water quality, growth performance, feed utilization, chemical composition and blood parameters were improved significantly in groups raised under density of (1kg/m^3) compared to those raised under density of (2kg/m^3) at the three feeding times. As for the three feeding times, there were statistically significant differences in (8 am and 6 pm) groups about the 12 pm group. The current study recommends using the stocking density (1kg/m^3) in African catfish culture which improves water quality parameters, growth performance, feed utilization, chemical composition, and blood parameters with feeding time (8 am and 6 pm) about 12 pm.

Keywords: Feeding time; Stocking density; African catfish; Growth performance**.**

INTRODUCTION

The world's population are about 7 billion people, and its population is expected to reach more than 9.1 billion people by 2050, and therefore this increase requires a difficult challenge to balance this number with its requirement of animal protein considering the decline in natural fisheries that suffer from a shortage food where the world production of fish reached about 179 million tons in the year 2018. The most common fish species are carp, catfish, and salmon (FAO (2014) and the most widely farmed species is the African catfish (*Clarias gariepinus*) (FAO 2014). Aquaculture sector is responsible for more than 89% of the increase in total fish production and is expected to expand further in Africa (an increase of up to 48%) driven by the placement of additional farming capacity in recent years. Among the most important types of fresh water that are widely distributed in aquaculture are carp and pangas catfish. African catfish aquaculture is an important aquaculture species that is farmed in different parts of the world. The largest producing country is Nigeria followed by the Netherlands, Brazil, Hungary, Kenya, Syrian Arab Republic, Egypt, South Africa, Cameroon, and Mali (FAO (2016b). Total African catfish production officially reported by FAO was 1,245.3 tons during 2019 (FAO (2020). (GAFRD, 2019) The total production of African catfish from natural fisheries (lakes + Nile River) was about 39,507 tons, and from fish farming 8454 tons. (GAFRD, 2019) African catfish belongs to the Clariidae family and is a native fish species in African countries.

African catfish tolerate a variety of feeding habits (Anyanwu et al., 2012), and they tolerate difficult environmental conditions and are adapted to living in them (Nwani et al., 2015) and tolerate low levels of oxygen due to the presence of a respiratory organ (the labyrinth organ) that allows It inhales oxygen from the atmosphere, and it is a benthic fish that feeds on phytoplankton and zooplankton (Bruton, 1979). There is no doubt that stocking density is one of the most important factors that determine fish farming and meet the requirements of the increase in animal protein with the increase in population. Stocking density is defined as the number of fish stored at the beginning of the culture period (Ruane, N.M, et.2002). Storage density affects survival rates, and research continues to find the best storage density and survival rate (Ellis, T.et, 2002). The current study was carried out to compare the effect of different stocking densities and feeding times on growth performance, feed utilization and blood

parameters of African catfish *Clarias gariepinus* reared in concrete ponds for 60 days.

MATERIALS AND METHODS

Fish rearing and management

This experiment was conducted in the experimental fish unit of fish, Department of Fish Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. 180 African catfish (*Clarias gariepinus*) with an average initial weight of 196.8 ± 0.47 g were randomly distributed in 18 concrete ponds in Six treatments (3 replicates for each treatment). Each pond was filled with water to a level of 50 cm and this level was maintained during the duration of the experiment, each pond connected with tube for aeration (24h per day). The experiment was based on a 3 × 2 factorial design with three feeding times per day (8 am, 12 pm and 6 pm) at a rate of 3% of their wet body weight and two levels of stocking density ($1kg/m^3$) and ($2kg/m^3$) for 60 days on diet 36% (Table 1). Fish in each pond were weighed every 2 weeks, and feed amount was adjusted accordingly.

Water Quality Parameters

Water temperature was measured in each pond daily using a mercury thermometer of 0 to 100°C range. Weakly dissolved oxygen was measured directly using an oxygen thermometer apparatus (XSI model 58, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Ammonia (NH4 –N mg/L) was measured using Ammonia MR Reagent Hi 93715-01. Nitrate (NO3 –N mg/L) was measured using Nitrate reagent Hi 93728-01. Nitrite (NO2 –N mg/L) was measured using Nitrite reagent Hi 93708-01. Hydrogen ion (pH) was measured using pH reagent Hi 937110-01.

Growth performance and Feed Utilization

All fish were weighed separately to the nearest 0.1 g at the start of the experiment and every 2 weeks through the duration of the experiment. Fish mortality was recorded every 2 weeks at the time of weighing. Growth performance and feed utilization efficiency were calculated as follows:

Weight gain % (WG %) = 100 [(final fish weight (g) – initial fish weight (g))/ initial fish weight].

Specific growth rate (SGR; %/day) = 100 [(Ln final fish weight – Ln initial fish weight/ experimental time (days)].

Feed conversion ratio (FCR) = Feed intake (g) / weight gain (g).

Daily gain = Weight gain, g /period in days.

Survival rate (SR) %= Final number of fish /Initial number of fish × 100.

Diet and carcass composition

Twelve fish samples were dried in an oven at 110 °C to constant weight and kept at 20 °C for further analysis. A standard method was used for the chemical composition (moisture, ash, fibre, fat, and crude protein) of the whole fish body and nutritional profile of test diets (AOAC, 2007). Moisture content was measured by oven drying at 110 °C to constant weight, while moisture content was measured using the kjeldahl method. Crude fat was also determined by the Soxhlet method and ash extracted by burning in a muffle furnace at 550°C for 6 hours.

Blood Sampling

At the end of the experiment, fish were not fed for 24 hrs. prior to blood sampling. Three fish per pond were anesthetized using clove oil (0.1 mL /L). The blood samples were obtained from caudal vein with a 450 angle according to (Feldman et al., 2000) and were collected in sterilized tubes. The syringe and microtube used to draw blood were one rinsed with 10% EDTA plasma and another plain for serum. The separation of blood serum was completed by centrifugation for 20 minutes at 3000 rpm.

Hematological analysis

The erythrocytes and leukocytes were counted according to the method described by Stockpot (1993) using hemocytometer and Natt- Herrik solution.

Hemoglobin concentration was determined according to Stockpot (1993) using the cyanomet hemoglobin method Drabkin's solution. According to (Dacie and Lewis (1991) the micro hematocrit method was used for estimation of the Packed Cell Volume (PCV) %.

Differential leukocytic count (DLC) determination, the white blood cells were counted among one hundred of blood smear according to (Stockpot (1993). The absolute DLC was calculated according to Thrall (2004) according to the following formula:

Absolute DLC = no. of each white cell x no. of total leukocytic count/100

Phagocytic activity

Leukocyte phagocytic function followed the method of (Cai et al. (2004) using the blood smears stained by Giemsa/May-Grunwald (Rosenfeld 1947). Phagocytic activity (PA=number of phagocytic cells with engulfed bacteria/number of phagocytic cells × 100. Phagocytic index (PI, i.e., number of engulfed bacteria per cell) = number of engulfed bacteria/numbers of phagocytic cells.

Serum biochemical analysis

Serum biochemical were determined calorimetrically according to the manufacturers of instructions using readymade chemicals (kits):

Serum total proteins (REF:310 001 Spectrum co. Egypt (Cannon et al., 1974).

Albumins (CAT. No. AB 10 10 Biodiagnostic co. Egypt.) (Doumas B.T et al., (1971).

Globulins content was calculated mathematically.

Aspartate aminotransferase (AST), CAT. No. AS 10 61 (45) Biodiagnostic co. Egypt. (Reitman, A. and Frankel, S. (1957).

Alanine aminotransferase (ALT) CAT. No. AL 10 31 (45) Biodiagnostic co. Egypt. (Reitman, A. and Frankel, S. (1957).

The serum lysozyme activity was assayed by ELISA micro-well technique using fish lysozyme ELISA kit (CAT. No. SL0050FI Sunlong Biotech co. China.) at the wavelength 450 nm using the micro plate ELISA reader following the manufacturer's instructions.

Immunoglobulin M (IgM) was measured by ELISA using a commercial kit (CAT. No. SL0048FI Sunlong Biotech co. China.) following the manufacturer's instructions

T3, FT4 and GH hormones were measured by Fluorescence Immunoassay rapid quantitative test using a commercial kit and FIA meter (Finecare FIA meter plus, Guangzhou Wondfo Biotech co., China)

Statistical analysis

The data were statistically analyzed by SAS (2002) according to the following model:

$$Yijk = \mu + Di + Mj + DMij + eijk$$

Where, μ is the overall mean, D is the constant effect of feeding times (i = 1 ... 3), M is the constant effect of stocking density (j = 1 ... 2), DMij is the interactive effect of feeding times and storage density, and random error eijkis. Differences between treatments were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS

Water quality

Water quality parameters were monitored and maintained at the same levels in all experimental groups were 26.9±0.5ºC, Dissolved Oxygen 5.62±0.2mg/L, pH 7.06±0.15 and ammonia 1.42±0.61mg/L) so, there was no significant difference recorded in any water quality parameter among the groups during the experimental period except for ammonia levels in (2kg/m^3) stocked groups which might reached to 1.91mg/L.

Survival rate

There are no mortalities recorded in all experimental groups.

Growth performance and feed utilization

High stocking density (2kg/m^3) groups showed significant differences in FW, WG and DWG with the lowest level recorded for the 12 pm fed group (Table 2). Also, it is noticeable that was no significant effect for interaction on feed utilization parameters.

Similarly, the feed efficiency ratio decreased in all the 2k stock density groups than the low stocking density (1k groups) with the lowest level observed in the 12 pm fed group. While the feed conversion ratio was positively related to the high stocking density recording the highest level in (12*2k group) indicating the influence of the time of feeding and the negative impact of the high stock density on catfish growth performance parameters and feed utilization (Table 3). Also, it is noticeable that was no significant effect for interaction on feed utilization parameters.

Chemical Composition

The Chemical Composition of catfish fed on diets 36% with various levels of stocking density and feeding time for 60 days is illustrated in (Table 4). The crud protein (CP) and Ash content significantly increased in the lower stock density groups (6*1k, 8*1k and12*1k group) when compared with that of the higher stock density groups. While dry Matter (DM) showed no significant differences.

Blood parameters

Data presented in (Table 5) showed that there was significant increase in RBCs counts, HB% and PCV% in fish reared in the lower

stock density with a positive impact of the feeding time toward the 8 am and 6 pm fed groups over the 12 pm fed group when compared with those levels of fish reared at the higher stock density.

Similarly, WBCs, heterophils, lymphocytes, monocytes, esinophils and basophils (Table 5) recorded declined counts in 2k stock density reared fish with the lowest levels recorded in the 12 pm fed group comparing with that of 1k stock density reared fish.

Immune response

Lysozyme activity, Phagocytic activity, Immunoglobulin M(IgM), Total proteins, Albumin and Globulins are presented in (Table 6 and table 7). Significantly increased in the fish reared in 1k stocking density groups with the higher levels observed in 8 am and 6 pm fed groups. While the higher stock density 2k groups recorded dropped levels of Lysozyme activity, Phagocytic activity, Immunoglobulin M(IgM), Total proteins, Albumin and Globulins indicate immunosuppression specially at 12 pm fed fish group.

Blood biochemical

Blood biochemical of African catfish fed on diets 36% with various levels of stocking density and feeding time for 60 days are illustrated in (Table 8). It was observed that high stock density (2k) fish groups showed an increase in AST, ALT enzymes activity, Urea, and creatinine concentration levels with the highest level in 12 pm fed group (12*2k group) over the 1k stock density groups, while the cholesterol and triglycerides showed decreased concentrations in the higher stock density.

Glucose, antioxidant, and digestive enzymes

Data in (Table 9) explained that Glucose levels recorded significant increase in 2k stock density groups when compared with the 1k stock density groups with the highest level in (12*2k group).

Digestive enzymes: Lipase and Amylase enzyme activity showed significant decrease in the 2k high stock density groups specially 12*2k group when compared with 1k low stock density fish groups. Antioxidant enzymes activity superoxide dismutase (SOD) and catalase (CAT) enzymes activity recorded significant increase in low stock density groups (1k groups) with the higher levels observed for the 6*1k and 8*1k groups when compared with the decreased activities of these enzymes in the 2k reared groups.

Malondialdehyde (MDA) enzyme activity showed a significant increase in the 2k groups with the highest level recorded in the 12 pm fed group compared with its activities in the 1k groups.

Triiodothyronine (T3), Free thyroxine (FT4) and Growth hormone (GH)

T3, T4 and GH recorded lowest levels in the high stock density specially in 12*2k group when compared with the 1k groups (Table 10).

DISCUSSION

Water quality

Water quality is a key factor which affect growth and feed utilization of fish (Ellis et al., 2002). High stocking density causes higher stress in Nile tilapia, consists of higher oxygen consumption, increased ammonia level, lower DO, and higher CO2 levels (Gomes et al., 2003; Jia et al., 2016 and Wu et al., 2018. Fish under stressful conditions excrete more ammonia (Randall and Tsui, 2002), but because all treatment does not reach higher level of stocking density, there weren't effect of water quality on growth.

Growth performance, feed utilization and survival rate

In agreement with Mostafa et al. (2001) and ANI et al., (2013), different feeding times influenced the growth performance of African catfish. Growth performance increased significantly in fish groups fed at 8 am or 6 pm while the lowest level recorded for the 12 pm fed group; this is compatible with Okpako, (2010) who recorded that the best time to feed the African catfish is morning or evening.

Kerdchuen and Legendre (1991) recorded the higher growth rate in Catfish, (*Heterobranchus longi- filis*) that fed at night when compared with those fed during the daytime at the same feeding rate. Determining the best time of the day to feed catfish will maximize growth performance and discourage waste minimizing deterioration of water quality arise from the feeds decomposition due to feeding fish at inappropriate time. Therefore, decreasing fish mortality rate due to bad pond water quality so the overall fish production will be improved (Norm, 2000). Environmental factors, feeding time and water quality have a significant effect on the feed intake and growth of the fish as they can induce all sort of stress (NRC, 2009). (Narejo et

al.,2010 and Zeng et al.,2010) reported that lower fish stocking densities are consistent with better survival rates due to the more space and food provided. also, the lower competition therefore determining the optimal stock densities provide maximum growth performance, survival rates, production, and profitability.

In a similar study Chakraborty et al., 2010) reported that stocking density considered as key parameter in fish culture and production with direct influence on fish growth and survival rates. Stocking density influence on fish health and growth has been recorded in several species; European sea bass, lake sturgeon, Atlantic salmon, catfish, piabanha, Senegalese sole (Fajfer, 1999; Hosfeld et al.,2009; Tolussi et al.,2010 and Salas-Leiton et al., 2010).

Chemical Composition

Fish fed at different times influenced converting the food consumed into muscle and energy influencing the muscle formation (Anne Gélineau et al., 2002). However, Morteza Najafi et al., (2009) observed no significant difference in the growth performance and muscles rates of juvenile beluga sturgeon larvae fed at different times. Feeding time caused differences in fish growth (Sundararaj et al., 1982). Feeding fish at some times of day lead to lean body growth but fattening at other times. Increased frequency (or feeding at both times of the day influences both processes. Light and temperature cycles have direct influences on the fish behavioral and physiological daily activities and because food conversion efficiency varies with the feeding time, so timing the daily feed meal provides greatest growth and fattening at a lowest cost (Banning, 1973; Noeske and Meier, 1983). Many fish species have a daily preferred feeding time which may be changed seasonally (Davis and Bardach, 1964) and the feeding time that provides the maximum growth performance also, may be changed seasonally (Noeske and Spieler, 1984). Channel catfish has been offered food ad libitum with demand feeders have a daily preferred feeding time of 2000 h (Randolph and Clemens, 1976).

Blood Parameters

The evaluation of hematological and blood biochemical parameters levels is useful in stress response assessment (Braun et al., 2010), assessing the fish physiological condition and wellbeing (Tavares-Dias and Moraes, 2007; Duston et al., 2003) and used as useful pointers for the disturbances in fish physiology (Tavares-Dias and Morares 2007).

Hb evaluation is a useful indicator for the erythropoietic status and the oxygen carrying capacity of the fish blood (Baker et al., 2000). Fish hematology provides good information about stress level and fish response. According to Hastuti and Subandiyono, (2015) decrease in the number of erythrocytes, hemoglobin, haematocrit, and leukocrit of African catfish (*Clarias gariepinus)* reared at high stocking density indicating that the fish was under stress. Blood hemoglobin contents are influenced by many factors, such as water quality and oxygen availability so when fish reared at optimum stocking density (Kurniawan, 2019).

Erythrocyte levels dropped at the higher stock densities due to the increased metabolism and fecal waste leading to higher ammonia content compared with that at low stock densities (Addini et al., 2020). Erythrocytic counts influenced by the distribution density that meaning higher stock density caused the higher ammonia level in the fish culture. Also, the blood cells performance of African catfish influenced by the culture water quality, especially the ammonia count (Ni et al., 2014). Hematocrit level is related to the erythrocytes number as it is the ratio between blood plasma and the red blood cells. The increase in the erythrocytes number will be followed by an increased hematocrit percentage (Fitria et al.,2019). Increased hematocrit level in fish blood related to the red blood cells higher number formed by the fish hematopoiesis tissue, because the red blood cells number being positively proportional to the hematocrit value (Fadil et al., 2011). Leucocytes Fish reared at high stock densities exhibited suppressed immune responses; decreased WBCS, lysozyme activity, IgM and phagocytosis (Dawood et al., 2019). The lower total leukocytes may be influenced by the production of anti-stress substances by the fish body (Mohapatra el al., 2014). The decrease in total leukocytes indicated a decrease in stress level due to fish being able to adapt to its environment. The total leukocyte level can be influenced by water quality, stress, and pathogens in the rearing media (Fitria et al., 2019). Lymphocytes numbers are known to show variability according to the physiological condition of the fish (Klontz, 1972). Decreased lymphocyte numbers were observed under stressed conditions –hypoxia, cortisol induced or during handling and transport, but

neutrophil numbers tend to increase under stressful condition (Ellsaesser and Clem 1987).

ALT, AST, total protein, and albumin serve as diagnostic aids to a disturbed metabolic process (Baker et al., 2000). The decrease in ALT, AST and ALP levels indicates transamination while their increased levels indicate a distortion in the liver activities or damage liver or kidney tissues. Blood glucose levels dropped with the lower stocking density. According to Ajani et al., (2015) the blood glucose level is positively proportional to the stocking density increase, increased when the fish were under stress. These agree with (Van de Nieuwegiessen et al.,2009) who concluded that *Clarias gariepinus* responded to stress by elevating their blood glucose levels. At increased stocking densities stress related hormones adrenaline and noradrenaline are associated with increased secretion of cortisol leading to glucose contents fluctuate to control energy consumption and use (Putra et al.,2020). lipase and amylase are the major digestive enzymes, have the main roles in digestion and absorption of the feed. The increased activity of lipase and amylase resulted in increased overall body metabolism (Dawood et al., 2014 and Liu et al., 2018). Liu et al., (2018) and Bolasina et al., (2006) observed decreased digestive enzyme activities in Nile tilapia and Japanese flounder of intensive stocking conditions. Decreased activity of digestive enzymes consisted with dropped growth performance and feed efficiency. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) are considered as antioxidant enzymatic defenses that managing the cellular response to oxidative stress under physiological stress conditions (David et al., 2008). Dawood et al., (2019) observed decreased SOD, CAT activities as well as increased MDA activity in blood of tilapia reared in intensive conditions suggesting suppressed antioxidant response. In agreement with our results dropped antioxidant enzyme (SOD and CAT) recorded by (Braun et al., 2010) and higher (MDA) levels observed in fish reared under intensive conditions (Andrade et al., 2015). Immunoglobulins heterodimeric glycoproteins have a crucial role in natural antigens recognition and present in fish skin, gut mucus, gill, bile and systemically found in the fish plasma (Magnadottir, 2010). The lysozyme activity could damage the polysaccharide walls of the pathogenic bacteria via breakdown its glycosidic bonds providing stronger innate- immune response resulting in enhanced fish defense against such stressors (Ellis et al., 2002). The lysozyme activity related to the leucocytic counts which produce the lysozymes lead to enhanced phagocytosis and complement system (Cecchini et al., 2000). Phagocytosis is one of the most significant fish cellular immune system components (Zhang et al., 2008) that guarantee that fish can efficiently avoid attacks of a pathogen; recognizing the pathogen and constrain its spread and progress (Harikrishnan et al.,2011), dropped phagocytosis, suggesting weak immune response and tolerance against higher or intensive stocking density. Li, (2012) reported that the high stocking density had a negative impact on fish growth and altered the serum thyroid hormones through crowding stress and decline the circulating peripheral thyroid hormones levels. In agreement with (Li, 2012) high stocking density, decreased growth rate and plasma T4 concentration level in rainbow trout, brook charr and Amur sturgeon. Pickering et al., (1991) recorded increased plasma ACTH and cortisol levels and a significant decrease in the circulating growth hormone concentration in rainbow trout as a response to the handling acute stress.

CONCLUSION

The fish were stressed when the stocking density was not in optimal level and fish were healthy with the optimal stocking density. High stocking density can impair the catfish welfare, depress the growth, the digestive enzyme activity, immune response and oxidative status. Morning or evening time of the day could be considered the preferable time of the day to feed catfish over the afternoon time. The use of stocking density ($1kg/m^3$) in aquaculture in African catfish culture improves water quality parameters, fish growth performance, feed consumption, chemical composition, and blood parameters at different feeding times with most preferable times at 8 am and 6 pm over the 12 pm feeding time.

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Table 1: Feed formula (%) and proximate chemical composition of the experimental diet.

Ingredients	Experimental diet
Fish meal (72% CP)	64
Rice flour	14.5
Wheat flour	10
Rice brane	2.5
Vitamin Premix[a]	2.0
Mineral Premix[b]	2.0
Soybean oil	5
Total	100
Proximate chemical analysis (%)	
Moisture (%)	3.9
Crude protein (%)	36.9
Crude lipid (%)	18.3
Ash (%)	18.0
NFE[c] (%)	26.8
GE[d] (KJ/g)	17.5

[a]Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; vitamin C (coated), 0.9 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; α-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

[b] Mineral premix (per kg of premix): CaHPO4.2H2O, 727.2 g; MgCO3.7H2O, 127.5 g; KCl 50.0 g; NaCl, 60.0 g; FeC6H5O7.3H2O, 25.0 g; ZnCO3, 5.5 g; MnCl2.4H2O, 2.5 g; CuCl2, 0.785 g; CoCl3.6H2O, 0.477 g; CaIO3.6H2O, 0.295 g; CrCl3.6H2O, 0.128 g; AlCl3.6H2O, 0.54 g; Na2SeO3, 0.3 g.

[c] NFE, nitrogen free extract = 100 - (CP + CF + EE + Ash %).

[b] GE, gross energy (Jobling, 1983).

Table 2: Growth performance of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	IW (g)	FW (g)	WG (g)	DWG(g/fish/day)
Feeding time				
6	197.16	521.66^{a}	324.49^{a}	2.89^{a}
8	196.33	503.33^{ab}	307.16^{ab}	2.73^{a}
12	199.49	480.33^{b}	280.83^{a}	2.51^{b}
Standard error	±0.96	±2.93	±2.98	±0.02
P-value	0.0434	0.0094	0.026	0.0262
Stocking density				
1k	197.55	534.99^{a}	337.44^{a}	3.01^{a}
2k	197.66	468.55^{b}	270.88^{b}	2.41^{b}
Standard error	±0.78	±2.39	±2.43	±0.02
P-value	0.3858	0.0094	0.0061	0.0061
Interaction between feeding time and stocking density				
6*1k	199	566.33	367.33	3.28
8*1k	195.33	529	333.66	2.97
12*1k	198.33	509.66	311.33	2.78
6*2k	195.33	477	281.66	2.51
8*2k	197	477.66	280.66	2.5
12*2k	200.66	451	250.33	2.23
Standard error	±1.36	±4.14	±4.22	±0.03
P-value	0.2499	<.0001	<.0001	<.0001

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

Table 3: Feed utilization of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	SGR	FI	FCR	PI	FER	PER
Feeding time						
6	0.37	612.76^{b}	1.89^{b}	183.83	0.52	1.75
8	0.36	673.56^{a}	2.19^{a}	202.08	0.48	1.52
12	0.33	604.19^{c}	2.17^{a}	181.26	0.46	1.55
Standard error	±0.003	±8.19	±.033	±2.45	±.008	±.025
P-value	0.1251	<.0001	0.0013	<.0001	0.0014	0.0013
Stocking density						
1k	0.38^{a}	662.15^{a}	1.96^{b}	198.65^{a}	0.51^{a}	1.7^{a}
2k	0.33^{b}	598.20^{b}	2.21^{a}	179.46^{b}	0.45^{b}	1.51^{b}
Standard error	±.002	±6.68	±.027	±2.008	±.006	±.021
P-value	0.0104	0.0888	0.0043	0.0889	0.004	0.0037
Interaction between feeding time and stocking density						
6*1k	0.4	667.2	1.81	200.16	0.55	1.83
8*1k	0.38	708.4	2.12	212.53	0.47	1.57
12*1k	0.36	610.86	1.96	183.26	0.51	1.7
6*2k	0.34	558.33	1.98	167.5	0.5	1.68
8*2k	0.34	638.73	2.27	191.63	0.44	1.46
12*2k	0.31	597.53	2.38	179.26	0.42	1.39
Standard error	±.004	±11.58	±.046	±3.47	±.011	±.036
P-value	<.0001	0.0001	<.0001	0.0001	<.0001	<.0001

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

Table 4: Chemical composition of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	DM	CP	EE	ASH
Feeding time				
6	24.72	60.24	15.45^{ab}	24.33^{a}
8	25.22	60.72	15.05^{b}	24.22^{ab}
12	25.02	56.29	16.18^{a}	23.54^{b}
Standard error	±0.44	±0.27	±0.29	±0.24
P-value	0.733	0.425	0.05	0.081
Stocking density				
1k	24.99	60.06^{a}	15.63	24.55^{a}
2k	24.98	58.11^{b}	15.49	23.51^{b}
Standard error	±0.36	±0.22	±0.23	±0.19
P-value	0.994	0.042	0.673	0.003
Interaction between feeding time and stocking density				
6*1k	24.67	61.25	14.74	24.07
8*1k	25.04	61.69	14.61	24.03
12*1k	25.25	57.23	17.55	25.55
6*2k	24.77	59.23	16.16	24.6
8*2k	25.4	59.76	15.5	24.4
12*2k	24.79	55.35	14.81	21.53
Standard error	±0.63	±0.38	±0.41	±0.34
P-value	0.804	0	0.001	0

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

Table 5: Blood parameters of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	RBCs (x10^6 /mm^3)	Hb (g/100ml)	PCV %	MCV	MCH	MCHC
Feeding time						
6	4.95±1.16^{a}	15.38±3.32^{a}	48.25±11.88^{a}	97.33±1.50	31.22±1.63	32.07±1.60
8	4.82±1.08^{a}	14.90±3.00^{a}	46.50±10.66^{a}	96.21±1.21	30.99±0.87	32.22±1.02
12	3.57±0.80^{b}	11.46±2.22^{b}	35.00±8.36^{b}	97.62±1.35	32.20±1.08	33.00±1.51
P-value	0.0032	0.0077	0.0066	0.3307	0.3282	0.4280
stocking density						
1k	5.29±0.89^{a}	16.22±2.49^{a}	51.67±8.55^{a}	97.73±1.21	30.75±0.71	31.47±0.71^{b}
2k	3.62±0.60^{b}	11.61±1.84^{b}	34.84±5.88^{b}	96.37±1.27	32.20±1.29	33.40±1.09^{a}
P-value	0.0002	0.0006	0.0003	0.1183	0.0652	0.0164
Interaction between feeding time and stocking density						
6*1k	5.95±0.08	18.18±0.22	58.50±0.70	98.24±0.10	30.54±0.77	31.09±0.75
8*1k	5.70±0.11	17.25±0.54	55.00±1.41	96.49±0.56	30.27±0.35	31.37±0.19
12*1k	4.21±0.57	13.21±1.60	41.50±6.36	98.47±1.55	31.43±0.55	31.92±1.07
6*2k	5.95±0.08	12.57±1.15	38.00±1.41	96.43±1.85	31.88±2.36	33.05±1.81
8*2k	3.95±0.65	12.55±2.17	38.00±7.07	95.92±1.94	31.71±0.24	33.07±0.42
12*2k	2.94±0.07	9.71±0.05	28.50±0.70	96.77±0.31	32.98±0.86	34.08±0.99
P-value	0.3955	0.5122	0.4605	0.7617	0.9916	0.9493

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

Table 6: Immune responses of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	lysozyme (µg/ml)	Phagocytic activity%	Phagocyticindex%	IgM (µg/ml)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Feeding time							
6	4.71±1.66	18.86±7.27	18.86±7.27	2.96±1.05	6.35±0.67^{a}	3.24±0.12^{a}	3.11±0.64^{a}
8	4.32±0.99	17.54±6.58	17.54±6.58	2.67±0.89	5.97±0.41^{a}	3.10±0.15^{a}	2.87±0.27^{ab}
12	3.31±0.61	12.19±4.26	12.19±4.26	2.21±0.63	5.18±0.78^{b}	2.71±0.64^{b}	2.46±0.29^{b}
P-value	0.1039	0.1321	0.1321	0.1531	0.0049	0.0043	0.0418
Stocking density							
1k	4.92±1.21^{a}	20.37±6.64^{a}	20.37±6.64^{a}	3.25±0.74^{a}	6.32±0.57^{a}	3.24±0.10^{a}	3.08±0.53^{a}
2k	3.30±0.48^{b}	12.01±1.62^{b}	12.01±1.62^{b}	1.97±0.18^{b}	5.34±0.64^{b}	2.78±0.50^{b}	2.55±0.24^{b}
P-value	0.0116	0.0131	0.0131	0.0030	0.0016	0.0012	0.0172
Interaction between feeding time and stocking density							
6*1k	5.83±1.78	24.12±6.88	24.12±6.88	3.85±0.24	6.93±0.11	3.30±0.14	3.64±0.03
8*1k	5.14±0.16	22.89±3.88	22.89±3.88	3.29±0.85	6.25±0.22	3.19±0.13	3.06±0.10
12*1k	3.82±0.22	14.12±6.27	14.12±6.27	2.62±0.66	5.80±0.54	3.26±0.05	2.54±0.49
6*2k	3.60±0.28	13.61±1.01	13.61±1.01	2.06±0.10	5.78±0.22	3.18±0.14	2.60±0.37
8*2k	3.50±0.54	12.19±0.77	12.19±0.77	2.04±0.07	5.69±0.41	3.01±0.15	2.69±0.28
12*2k	2.80±0.23	10.25±0.45	10.25±0.45	1.80±0.23	4.56±0.11	2.17±0.20	2.40±0.10
P-value	0.5822	0.4634	0.4634	0.3887	0.3112	0.0042	0.1341

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

Table 7: TLC and DLC of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	WBcs (x10³/mm³)	Heterophil (x10³/mm³)	Lymphocyte (x10³/mm³)	Monocyte (x10³/mm³)	Esinophils (x10³/mm³)	Basophils (x10³/mm³)
Feeding time						
6	37.67±8.70^{a}	4.51±0.77	29.90±7.76^{a}	2.71±1.20	0.34±0.27	0.21±0.24
8	33.78±6.74^{ab}	4.92±0.95	25.80±5.55^{ab}	2.60±0.61	0.26±0.18	0.18±0.21
12	27.87±5.52^{b}	3.77±0.91	21.64±4.26^{b}	1.75±0.58	0.35±0.38	0.35±0.16
P-value	0.0654	0.3465	0.0225	0.1600	0.8931	0.6139
Stocking density						
1k	38.11±6.97^{a}	4.39±0.80	30.37±5.71^{a}	2.84±0.93^{a}	0.24±0.19	0.25±0.21
2k	28.11±4.63^{b}	4.41±1.14	21.20±3.12^{b}	1.87±0.55^{b}	0.38±0.32	0.23±0.22
P-value	0.0101	0.9724	0.0018	0.0434	0.4285	0.8965
Interaction between feeding time and stocking density						
6*1k	44.39±6.00	4.20±0.25	36.16±4.58	3.59±1.11	0.20±0.28	0.24±0.34
8*1k	38.81±5.37	5.08±1.25	30.37±2.84	2.75±0.92	0.39±0.05	0.21±0.30
12*1k	31.12±0.75	3.89±0.31	24.58±0.15	2.18±0.48	0.15±0.21	0.31±0.007
6*2k	30.95±3.24	4.83±1.15	23.64±1.83	1.83±0.24	0.48±0.26	0.16±0.23
8*2k	28.76±2.57	4.77±1.03	21.24±1.09	2.45±0.41	0.13±0.019	0.15±0.21
12*2k	24.62±6.98	3.64±1.53	18.71±4.46	1.33±0.22	0.54±0.48	0.39±0.27
P-value	0.6041	0.7813	0.3554	0.3519	0.2974	0.8919

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

Table 8: Blood biochemical of catfish of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	AST (U/L)	ALT (U/L)	Urea (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
feeding time						
6	20.15±1.34^{a}	19.14±2.11	1.94±0.13	0.35±0.06	97.49±2.33	107.40±10.51
8	20.91±1.53^{b}	20.13±1.01	2.02±0.11	0.38±0.04	97.14±6.40	111.46±14.20
12	22.85±2.89^{b}	21.37±2.06	2.04±0.13	0.43±0.11	94.40±6.57	111.13±9.98
P-value	0.0142	0.2372	0.5909	0.3176	0.5110	0.9016
stocking density						
1k	19.82±0.76^{b}	19.26±1.74	1.96±0.13	0.33±0.04^{b}	99.88±3.14^{a}	107.80±7.55
2k	22.79±2.18^{a}	21.17±1.61	2.04±0.10	0.44±0.07^{a}	92.80±4.28^{b}	112.20±13.67
P-value	0.0013	0.0918	0.3388	0.0251	0.0201	0.6027
Interaction between feeding time and stocking density						
6*1k	19.20±1.21	18.17±3.10	1.86±0.15	0.30±0.02	99.32±0.92	103.32±3.74
8*1k	19.91±0.08	19.68±1.41	2.04±0.06	0.35±0.03	100.50±6.84	109.96±0.40
12*1k	20.35±0.29	19.94±0.11	1.99±0.20	0.36±0.06	99.82±0.36	110.10±14.49
6*2k	21.11±0.61	20.11±0.05	2.04±0.08	0.42±0.03	95.66±1.40	111.48±15.84
8*2k	21.91±1.75	20.59±0.55	2.01±0.19	0.42±0.05	93.77±5.54	112.95±24.42
12*2k	25.36±0.08	22.82±2.11	2.09±0.09	0.50±0.13	88.96±3.56	112.16±9.16
P-value	0.0877	0.7162	0.5786	0.7020	0.4714	0.9460

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

Table 9: Glucose, antioxidant, and digestive enzymes of catfish of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	Glucose (mg/dl)	Lipase (U/L)	Amylase (U/L)	SOD (U/ml)	CAT (U / L)	MDA (nmol / ml)
feeding time						
6	25.93±6.60^{b}	61.96±10.23^{a}	32.34±7.57	12.18±2.23^{a}	14.41±4.23	10.94±1.72
8	27.73±6.95^{b}	55.86±7.18^{a}	29.43±7.95	11.00±2.57^{ab}	13.35±3.90	10.97±1.46
12	32.60±7.30^{a}	45.91±12.11^{b}	25.93±6.60	9.82±1.68^{b}	12.37±2.11	12.51±2.02
P-value	0.0051	0.0074	0.1678	0.0214	0.6366	0.0986
stocking density						
1k	22.88±3.17^{b}	62.51±6.94^{a}	34.91±74.96^{a}	12.74±1.54^{a}	15.54±3.43^{a}	10.13±1.13^{b}
2k	34.66±3.63^{a}	46.64±9.36^{b}	23.55±3.53^{b}	9.25±1.05^{b}	11.22±1.08^{b}	12.82±1.09^{a}
P-value	<.0001	0.0010	0.0030	0.0004	0.0422	0.0029
Interaction between feeding time and stocking density						
6*1k	20.34±2.37	69.73±2.37	38.84±1.69	14.05±0.70	17.51±3.58	9.54±0.19
8*1k	21.94±2.26	62.06±0.83	35.02±6.89	13.18±0.85	15.20±5.44	9.77±0.81
12*1k	26.34±0.90	55.76±6.26	30.90±3.19	10.99±0.93	13.90±1.72	11.07±1.72
6*2k	31.51±0.38	54.21±8.26	25.85±0.72	10.29±0.38	11.30±1.48	12.33±0.97
8*2k	33.52±2.30	49.67±0.73	23.85±4.24	8.82±0.23	11.50±1.48	12.15±0.13
12*2k	38.84±1.65	36.07±3.64	20.98±4.71	8.64±1.41	10.85±0.98	13.95±0.99
P-value	0.8711	0.5631	0.8712	0.2979	0.7330	0.9278

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

Table 10: T3, FT4 and GH of catfish of African catfish fed on diets 36% with different levels of stocking density and feeding time for 60 days.

Item	T3 (ng/dl)	FT4 (pmol/L)	GH (ng/ml)
6	117.89±5.01	13.48±1.26	0.22±0.07
8	117.86±7.86	13.58±0.72	0.20±0.09
12	116.70±7.78	13.29±1.27	0.15±0.07
P-value	0.9936	0.9380	0.1729
stocking density			
1k	118.40±8.17	13.92±1.06	0.24±0.07[a]
2k	116.58±4.63	12.98±0.76	0.14±0.05[b]
P-value	0.8732	0.2087	0.0148
Interaction between feeding time and stocking density			
6*1k	118.84±3.54	14.02±1.88	0.27±0.02
8*1k	118.80±13.50	13.87±0.25	0.25±0.10
12*1k	117.55±11.74	13.87±1.41	0.19±0.03
6*2k	116.94±7.91	12.94±0.04	0.16±0.00
8*2k	116.93±1.48	13.28±1.05	0.16±0.06
12*2k	115.85±6.36	12.70±1.19	0.09±0.00
P-value	0.9890	0.9297	0.9788

Means in the same column of each parameter having different letters are significantly differ ($P < 0.05$).

تأثير أوقات التغذية المختلفة على أداء النمو ومقاييس الدم للقرموط الأفريقى تحت معدلات التسكين المختلفة.

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الملخص العربى:

صممت الدراسة الحالية لتقييم تأثير أوقات التغذية وكثافات التخزين المختلفة على معايير جودة المياه، وأداء النمو، واستخدام العلف، والتركيب الكيميائي، والتركيب الكيمائى لدم سمك القرموط الأفريقى. تم توزيع 180 سمكة قرموط بمتوسط وزن ابتدائي 196.6 ± 0.47 جم بشكل عشوائي في 18 حوض خرساني. اعتمدت التجربة على التصميم الإحصائى 3 × 2 بثلاث أوقات تغذية مختلفة (8 صباحًا، 12 مساءً، 6 مساءً)، ومستويين من كثافة التخزين (1كجم / م^3) و (2كجم م^3). تم تغذية أسماك التجربة على أعلاف تجارية (36٪ بروتين) بمعدل تغذية 3٪ من وزن الجسم لجميع المجموعات. تم قياس معايير جودة المياه، مقاييس أداء النمو، الإستفادة من الغذاء، التركيب الكيميائي، معايير الدم في جميع المجموعات في نهاية التجربة بعد 60 يوم. أشارت النتائج إلى أن جودة المياه وأداء النمو والإستفاده من الغذاء والتركيب الكيميائي ومعايير الدم تحسنت معنويا في مجموعات كثافة التخزين (1كجم/ م^3) مقارنة بالمجموعات (2كجم/ م^3)في أوقات التغذية الثلاثة. أما بالنسبة لأوقات التغذية الثلاثة، فقد كانت هناك فروق ذات دلالة إحصائية في مجموعات (8 صباحا و6 مساء) مقارنة بمجموعة 12 مساءا. لذلك فإن استخدام كثافة التخزين (1كجم/ م^3) في استزراع القرموط الأفريقي يحسن من مقاييس جودة المياه، وأداء النمو للأسماك، والإستفادة الغذائية، والتركيب الكيميائي للأسماك ومعايير الدم وذلك مع أوقات التغذية (8 صباحًا و6 مساءً) بشكل أفضل من وقت التغذية 12مساءً.

الكلمات الاسترشادية : وقت التغذية، معدل التسكين، القرموط الأفريقي, أداء النمو.