

Evaluation of use effective microorganisms (EM) with different feeding strategies on growth performance, body chemical composition and economic efficiency of monosex Nile tilapia *Oreochromis niloticus* juveniles

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Abstract – A factorial trial was conducted to detect the effect of different feeding strategies of supplementation of effective microorganisms (EM) liquid on the growth performance, feed utilization, body chemical composition and economic efficiency of monosex Nile tilapia (*Oreochromis niloticus*) juveniles. Three experimental treatments were formulated a basal diet without any addition of EM (control; A), a diet supplemented with 2% EM (B), and a diet supplemented with 4% EM (C). All treatments were offered to fish through two different strategies of feeding the meal: 2/3 in the morning and 1/3 in the afternoon or 1/3 in the morning and 2/3 in the afternoon. Each treatment was replicated three times. Juveniles Nile tilapia with an average initial body weight of 3.85 ± 0.22 g (\pm SE) were randomly stocked at a rate of 90 juveniles per 1.5 m³ tank. Fish growth performance and feed utilization significantly ($P \leq 0.05$) increased with increasing EM supplementation and were positively affected by different feeding strategies. Moreover, the economic evaluation showed that there were more benefits by when using the 4% EM diet and feeding 2/3 of daily meal in the morning.

Keywords: Nile tilapia / effective microorganism / feeding strategies / growth performance

1 Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most commonly farmed fish in African and worldwide. Currently, tilapia farms provide more than 75% of global tilapia production as a result of the species' ability to adapt in a wide range of environmental conditions, resistance to stress and diseases and ease of rearing under different production systems, such as monoculture or polyculture systems (FAO, 2016). Nile tilapia can feed on a wide variety of dietary sources, including phytoplankton and zooplankton, and its growth rates are excellent. In addition to its tasty flesh, Nile tilapia is rich in essential amino acids, vitamins and minerals and it conveniently priced for most people (FAO, 2016). The increased demand for animal protein and increasing challenges

of fish farming in Egypt, such as high feed prices and fresh water shortages, has led to the use of new materials that increase the ability of the fish to obtain take more benefits from feed ingredient, maintaining water quality and enhancing feed utilization. The incorporation of probiotics into fish diets can decrease the usage of antibiotics and other synthetic drugs in feed (Fuller, 1989). Therefore, the supplementation of probiotics in fish diets has become ubiquitous in aquaculture. The use of probiotics results in reduced feed costs, which plays an important role in assessing the practicality of fish diets (Abareethan and Amsath, 2015). Additionally, probiotics used to stimulate aquatic animal performance. Previous research has shown that probiotics can enhance the feed utilization of aquatic animals through: improved feed efficiency, higher growth performance, the prevention of intestinal disorders, enhancing immunity and stress tolerance and the pre-digestion of anti-nutritional factors present in ingredients (Suzer et al., 2008; Soltan and El-Laithy, 2008; Abdelhamid et al., 2014).

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Probiotics include many blends of beneficial microbial such as effective microorganisms (EM) which contains Lactic acid bacteria (LAB), yeast, photosynthetic bacteria, actinomycetes and other organisms, the liquid of EM has a pH 3.5 (Guim et al., 2002). The microorganisms in EM liquid culture are beneficial and highly efficient; they are not harmful, not pathogenic, not genetically modified and not chemically synthesized. They include well-known natural microorganisms which promote the process of beneficial anti-oxidative fermentation, accelerate the decomposition of organic matter, and promote the balance of microbial flora (Kriangsak, 2001; Kolk and Smink, 2005). EM is a beneficial and safety product. It's used as a probiotic in livestock and fishes without any adversely effects on fish, human and environmental condition (Abdel-Aziz et al., 2020). EM improves growth rates, reproductive performance, water quality and the overall flavor and quality of fish muscles and it control diseases in aquaculture whereas it is a good alternative to antibiotics (Omar et al., 2017; EMRO, 2010; Abdel-Aziz et al., 2020; Gao et al., 2021).

On the other hand, the feeding management include the suitable amount of daily meal and optimum feeding time with regard the highest fish growth by least cost diet are very important for fish production. The feeding regime has become diverse, but the thumb rule of feeding stock at optimum level should be very economical to savings in feed cost and economic justification (Webster et al., 1992). The amount of daily feed intake, presentation the predetermined ration is the key factors of feed management strategies, affecting the growth and feed conversion (Goddard, 1995; Yones et al., 2019a). Several study have reported that fish fed on the morning are better than evening, where (Ani et al., 2013) reported that fish fed at the morning had the best growth on African catfish than that fed at the afternoon or at the evening. Also, the effect of feeding time with energy level on rainbow trout diet were found that fish tended to eat more during the morning than in the evening, also the group fed high energy level in the morning with low energy in the evening ingested more energy during the morning meal than in the evening (Gélineau et al., 2002). For example, trout has a peak of feeding activity at dawn and trout fed at dawn has higher WG than those fed at midnight (Boujard et al., 1995; Sanchez-Vazquez and Tabata, 1998). Consequently, optimal time to offering feeds lead to increasing feed utilization, decreasing uneaten diets, improving digestion and absorption, reducing effluents and excretion of ammonia in fish ponds, hence optimal water quality with maximum growth rate (Yones et al., 2019a).

Economic efficiency refers to the situation in which each resource is optimally allocated to provide best service for each individual or entity while minimizing costs as much as we can. Or the term refers to the optimal use of resources in order to maximize the production of goods and services. Economic efficiency includes both technical or productive efficiency as well as price efficiency. Thus, economic efficiency is the result of multiplying productive efficiency by price efficiency (Azevedo et al., 2015). The current study was undertaken to evaluate the adding of different levels of EM in fish diets by use two feeding systems on growth performance, feed utilization, body chemical composition and economic evaluation.

2 Materials and methods

2.1 Study area

This study was conducted in fish rearing laboratory in Fayoum research station (beside Qaroun lake, 29°30'N, 30°40'E) National Institute of Oceanography and Fisheries (NIOF) El-Fayoum Governorate, Egypt. Monosex Nile tilapia *Oreochromis niloticus* juveniles were obtained from a private fish farm in Louqanda region- in the south coast of Qarun Lake. They were transferred in plastic bags. Fish were acclimated in laboratory conditions for two weeks before beginning the experimental. Total numbers of 90 juveniles per tank, were randomly distributed into 18 rectangular fibreglass tanks of 1.5 m³ water capacity. All tanks were provided with continuous aeration. The juveniles had an initial average weight (W_1) as (3.85 ± 0.22) g.

2.2 Trial design and distribution of fish in tanks

A factorial trial was conducted to evaluate the influence of different daily quantities of effective microorganisms (EM) diet supplementation on growth, feed efficiency, body chemical composition and economic efficiency of monosex Nile tilapia. Three levels of EM supplementation (0%, 2%, and 4%) were tested in diets containing an average of 35% crude protein (CP). Every level of supplementation was tested with two feeding systems; 2/3 of meal fed in the morning and 1/3 of meal fed in the afternoon and 1/3 of meal was fed in the morning and 2/3 of meal in the afternoon. The treatments were referred to as A1, A2, B1, B2, C1, and C2 as shown in Figure 1. Juveniles' fish were stocked at 90 fish per tank. The feeding times were 8 am (in the morning), 4 pm (in the afternoon) and the feeding rate was 5% of fish body weight. The fish were weighed every two weeks to adjust the quantity of feeds. Fish were reared in fresh water and this trial was conducted for a period of 51 days.

2.3 Diet formulation and preparation

The EM liquid culture was obtained by the Ministry of Agriculture, Egypt as a commercial product. This EM (liquid culture) accelerates the natural decomposition of organic matter using 3 well-known groups of microorganisms including LAB, yeasts and phototrophic or photosynthetic bacteria from green seaweed and soil

All diets were prepared and formulated by using a pelleting machine with a diameter of 1 mm then it were air dried at 30°C, the used EM was added to pellets by spraying immediately before feeding at three levels as 0%, 2% and 4% in the experimental diets. The proximate chemical analyses of the diets are shown in Table 1.

2.4 The system of running water in the experimental tanks

The system consisted of two water pumps connected with two sandy filters unit and forced the water into two storage large tanks with a total volume of 10000 L per tank.

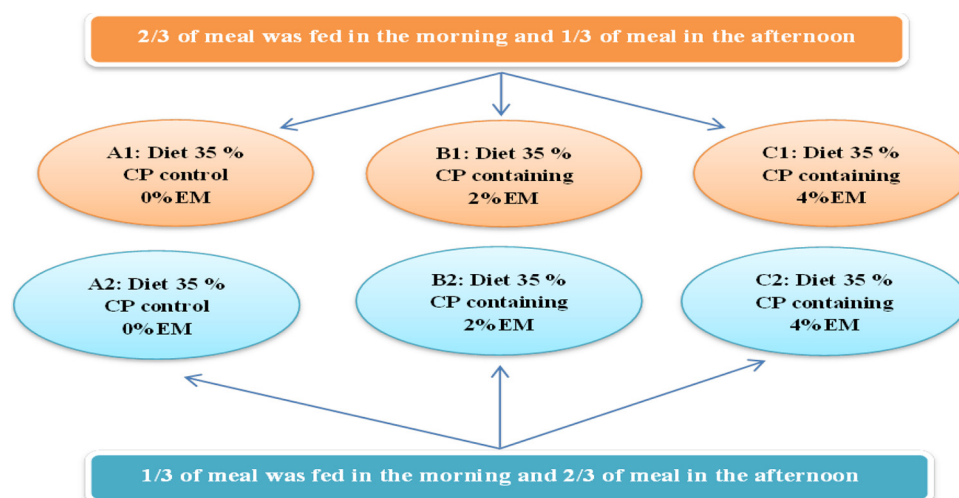


Fig. 1. Trial design.

Table 1. Formulation and proximate composition of experimental diets.

Ingredients (g·100 g ⁻¹)	Diet A (0% EM)	Diet B (2% EM)	Diet C (4% EM)
Fish meal (72% CP)	20	20	20
Extruded full fat Soybean meal (37% CP)	40	40	40
Yellow corn	20	19	18
Wheat bran	12	11	10
EM*	0.0	2.0	4.0
Fish oil	4.5	4.5	4.5
Starch	1.0	1.0	1.0
Vit. & Min. & premix	2.5	2.5	2.5
Total	100	100	100
<i>chemical analysis % on Dry matter basis</i>			
Moisture (M)	6.93	8.09	9.41
Dry matter (DM)	93.07	91.91	90.59
Crude protein (CP)	36.22	35.72	35.80
Ether extract (EE)	15.13	18.70	18.10
Total carbohydrate	40.32	37.28	37.88
Ash	8.33	8.30	8.22
Gross energy (GE, kJ·g ⁻¹)**	21.60	22.35	22.25

Notice: Chemical analysis was determined according to [A.O.A.C. \(2010\)](#) and total carbohydrate was calculated by difference.

*EM effective microorganism was produced by ministry of Egyptian environment under supervision Effective microorganism research organize in japan. EM includes lactic acid bacteria, yeast, photosynthetic bacteria, actinomyces and other organisms at the same amount as it was analyzed by [Guim et al. \(2002\)](#) who found lactic acid bacteria by 1×10^5 CFU·ml⁻¹, saccharomyces was 2×10^6 CFU·ml⁻¹ and few amount of photosynthetic bacteria and actenomyces.

**Calculated according to [NRC \(2011\)](#).

2.5 The system of aeration in the experimental tanks

An air central blower was used to pump the air in a network of plastic pipes. These pipes transport the air to the experimental tanks and the air diffused through all experimental units.

2.6 Water quality

Temperature and pH, were measured daily at 1pm by centigrade thermometer and Orion digital pH meter model 201,

respectively, while dissolved oxygen (DO) was measured every week, by oxygen meter (Cole Parmer model 5946). These measurements were presented in [Table 2](#).

2.7 Measurements of growth performance

These measurements were calculated according to the following equations:

Weight gain (WG, g)=final weight (W_2) – initial weight (W_1), average daily gain (ADG, g·day⁻¹)=weight gain, g per experimental period, day, Relative growth rate

Table 2. Mean (\pm SE) of water quality parameters.

Parameters	Mean (\pm SE)
Temperature, C	26.56 \pm 0.52
pH	7.94 \pm 0.12
DO, mg·l ⁻¹	7.25 \pm 0.13

(RGR, %) = $[(W_2 - W_1) / W_1] \times 100$, specific growth rate (SGR, % day⁻¹) = $[(\ln W_2 - \ln W_1) / t] \times 100$ whereas ln: is the natural log. and t: is the time in days, Condition factor (CF, g·cm⁻³) = $(W_2 / L_2^3) \times 100$ whereas L₂: is the final standard length of fish in cm, survival rate (SR, %) = (Number of fish at end/ Number of fish at start) \times 100, Hepatosomatic index (HSI, %) = (liver weight/body weight) \times 100 and Viscerosomatic index (VSI, %) = (weight of viscera and associated fat tissue/body weight) \times 100.

2.8 Measurements of feed efficiency

These measurements were calculated according to the following equations:

Feed intake (FI, g·fish⁻¹) = feed intake during the trial period/ the final number of fish for this trial, feed conversion ratio (FCR) = feed intake, g/ weight gain, g, protein efficiency ratio (PER) = Weight gain, g/ Protein intake, g, energy efficiency ratio (EER) = (Weight gain, g/ energy intake (kJ).

2.9 Chemical analysis of feeds and whole-body fish

The conversional chemical analysis of diet and whole body fish samples were carried out as described by A.O.A.C (2010) and Gross energy (GE) was estimated for formulated diets the factors 23.62, 39.5 and 17.56 kJ g⁻¹ for CP, EE and carbohydrates respectively were used (NRC, 2011).

2.10 Measurements of economic efficiency

Determinants of economic efficiency are used to estimating economic valuation criteria according to Azevedo et al. (2015). These include fixed costs, variable costs, total costs, quantity of fish production, and the price of productive unit, total revenue and net income.

Fixed costs: the costs paid by the producer whatever the production done or not, Variable costs: the costs paid by the producer only in case of production done only. Here it includes cost of Feed, employment, energy, fry, additives, chemicals and others. Total Costs: This is the sum of fixed costs and variable costs. Price of the unit produces Fish: The average price of the unit produce fish in the study samples was about 22 L·E·g⁻¹ = (1.3 \$). Total Revenue: it is the product of multiplication of the quantity of fish production multiplying unit price. Net profit: it is total revenue minus total costs. Where total revenue expresses the quantity of production multiplied by the unit price produced.

2.11 Current evaluation criteria

Value added according to Macfadyen et al. (2011)

Calculated by subtracting (total revenue – variable costs). This ratio is considered one of the criteria of economic efficiency for the use of variable assets and the ability of the production unit to pay its monetary and non-monetary obligations to the production process. The higher ratio, reflect more economic efficiency of the productive unit in the use of its resources.

Operating Ratio (%) according to Aswathy et al. (2015)

Calculated by dividing (total costs/total revenue). This ratio is one of the criteria used to detect economic efficiency by use the fixed and variable assets and the ability of the production unit to pay its monetary and non-monetary liabilities to the productive currency. The lower ratio, reflect more economic efficiency of the productive unit in the use of its resources.

Return on Costs according to Aswathy et al. (2015)

Calculate by dividing (total revenue/costs). It is the reverse standard for Standard operating ratio. This criterion shows the extent to which the production unit covers its production costs and an economic surplus.

2.12 Statistical analysis

The analysis of variance (two-way ANOVA) and LSD of Duncan Waller were used to compare treatment means. Data were analysed using stratigraphic package software (SPSS, 2007) SPSS Inc. Released 2007. SPSS for Windows Version 16.0. Significant.

3 Results

3.1 Water quality

The water quality parameters were within the acceptable range for rearing juvenile Nile tilapia as shown in Table 2.

3.2 Growth performance and feed efficiency

3.2.1 Effect of dietary supplementation of effective microorganisms (EM) on growth parameters of juveniles Nile tilapia

Growth parameters were not significantly different between treatments (Tab. 3) However, final weight, weight gain, average daily gain, relative growth rate, specific growth rate, daily growth coefficient and survival rate (SR) increased with increasing levels of EM in the diets. Fish fed on diet C, which contained 4% EM had the highest performance in terms of growth parameters as illustrated in Figure 2. Fish fed diet B (2% EM) showed lower performance for these growth parameters than those fed control diet (0% EM) had the lowest performance of the three diets. However, the fish fed diet B (2% EM) had the highest condition factor (CF), hepatosomatic index (HIS) and viscerosomatic (VSI) values. No significant differences were found in the feed utilization parameters among the treatments; while fish fed diet C (4% EM) had the lowest feed conversion ratio (FCR) and the highest protein efficiency ratio (PER), energy efficiency ratio (EER) compared with other diets.

Table 3. Effect of EM on growth performance of tilapia juveniles.

Parameters [†]	Treatments			LSD*
	A (0% EM)	B (2% EM)	C (4% EM)	
W ₁ , g	3.85	3.85	3.85	–
L ₂ , cm	10.55 ± 0.19	10.35 ± 0.14	10.50 ± 0.17	0.560
W ₂ , g	20.97 ± 1.05	21.37 ± 0.42	22.60 ± 0.39	2.210
WG, g	17.12 ± 1.05	17.52 ± 0.42	18.75 ± 0.39	2.210
ADG, g day ⁻¹	0.33 ± 0.02	0.34 ± 0.01	0.36 ± 0.01	0.040
RGR, %	444.80 ± 27.03	455.26 ± 11.10	487.01 ± 10.11	57.560
SGR, % day ⁻¹	3.32 ± 0.10	3.36 ± 0.04	3.50 ± 0.03	0.210
CF, %	1.72 ± 0.05	1.92 ± 0.06	1.91 ± 0.13	0.280
SR, %	93.29 ± 2.97	96.38 ± 2.91	96.94 ± 2.69	9.170
HSI, %	4.56 ± 0.43	5.13 ± 0.55	4.53 ± 0.54	1.630
VSI, %	9.65 ± 1.09	9.83 ± 0.12	9.66 ± 1.25	3.080
FI, g fish ⁻¹	20.06 ± 0.93	19.70 ± 0.66	20.14 ± 0.61	2.400
FCR	1.17 ± 0.03	1.12 ± 0.05	1.08 ± 0.01	0.110
PER	2.35 ± 0.05	2.50 ± 0.11	2.60 ± 0.03	0.238
EER	0.039 ± 0.001	0.040 ± 0.001	0.041 ± 0.001	0.007

Values are means ± S.E. of triplicate groups of three tank.

*Least significant difference.

[†]Weight gain (WG), average daily gain (ADG), Relative growth rate (RGR), specific growth rate (SGR), condition factor (CF), survival rate (SR), Hepatosomatic index (HSI), Viscerosomatic index (VSI), Feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and energy efficiency ratio (EER).

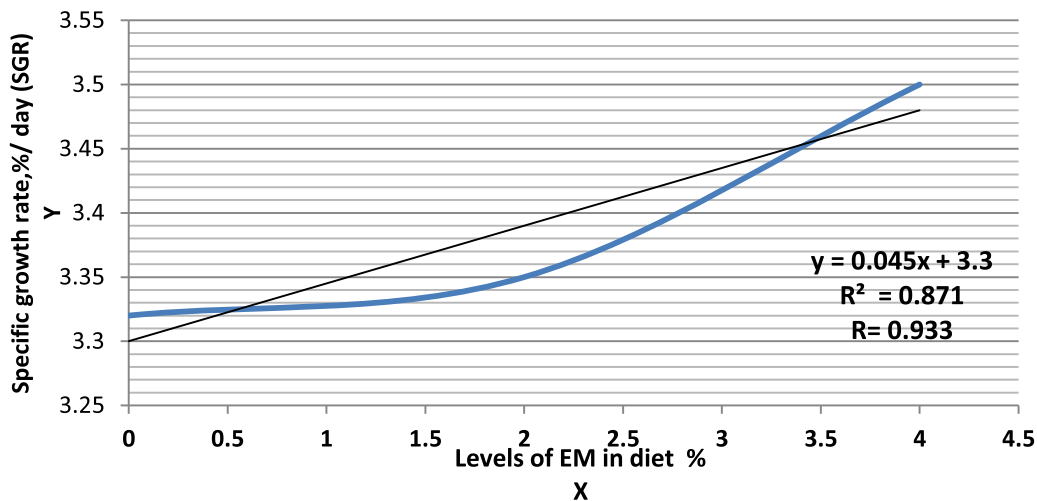


Fig. 2. The relationship between SGR with EM level in diet. Linear regression: $Y = a + bX$ whereas Y : is the dependent variable, a : is regression constant, b : is regression coefficient and X : is independent variable. Correlation coefficient R and Determination Coefficient R^2 .

3.2.2 Effect of feeding regime on the growth parameters of juveniles Nile tilapia

Different feeding regimes caused significant differences in growth performance parameters. As shown in Table 4 and Figure 4, fish fed 2/3 of their meal in the morning showed the highest growth indices and feed intake (FI, g fish⁻¹). The others parameters such as CF, SR, HSI, VSI, FCR, PER and EER were not significantly different between the two feeding regime.

3.2.3 Interaction between supplemented dietary EM and feeding regime on growth and feed efficiency of juveniles Nile tilapia

Results of the effects of the interaction between dietary EM supplementation and feeding regime on growth parameters are shown in Table 5. There were no significant differences between treatments A1, B1, C1 and C2 in W₂, WG, average daily gain (ADG), relative growth rate (RGR) and specific growth rate (SGR) but these treatments were significantly

Table 4. Effect of feeding regime on growth performance of tilapia juveniles.

Parameters [†]	Treatments		LSD*
	2/3 of the meal at the morning	2/3 of the meal at the afternoon	
W ₁ , g	3.85	3.85	–
L ₂ , cm	10.57 ± 0.15	10.43 ± 0.13	0.360
W ₂ , g	22.41 ^a ± 0.27	20.88 ^b ± 0.75	1.320
WG, g	18.56 ^a ± 0.27	17.03 ^b ± 0.67	1.320
ADG, g day ⁻¹	0.364 ^a ± 0.01	0.333 ^b ± 0.01	0.031
RGR, %	482.20 ^a ± 7.21	442.50 ^b ± 17.54	34.330
SGR, % day ⁻¹	3.45 ^a ± 0.02	3.31 ^b ± 0.06	0.120
CF, %	1.87 ± 0.09	1.84 ± 0.06	0.200
SR, %	94.44 ± 2.47	96.84 ± 2.07	5.840
HSI, %	4.39 ± 0.24	5.22 ± 0.48	0.980
VSI, %	10.12 ± 0.66	9.3 ± 0.78	1.850
FI, g fish ⁻¹	20.80 ^a ± 0.47	19.14 ^b ± 0.45	1.170
FCR	1.11 ± 0.01	1.14 ± 0.04	0.081
PER	2.51 ± 0.02	2.46 ± 0.94	0.181
EER	0.0405 ± 0.001	0.040 ± 0.001	0.003

Values are means ± S.E. of triplicate groups of three tank. (a and b) Average in the same row having different superscripts significantly different at ($P \leq 0.05$).

*Least significant difference.

[†]Weight gain (WG), average daily gain (ADG), Relative growth rate (RGR), specific growth rate (SGR), condition factor (CF), survival rate (SR), Hepatosomatic index (HSI), Viscerosomatic index (VSI), Feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and energy efficiency ratio (EER).

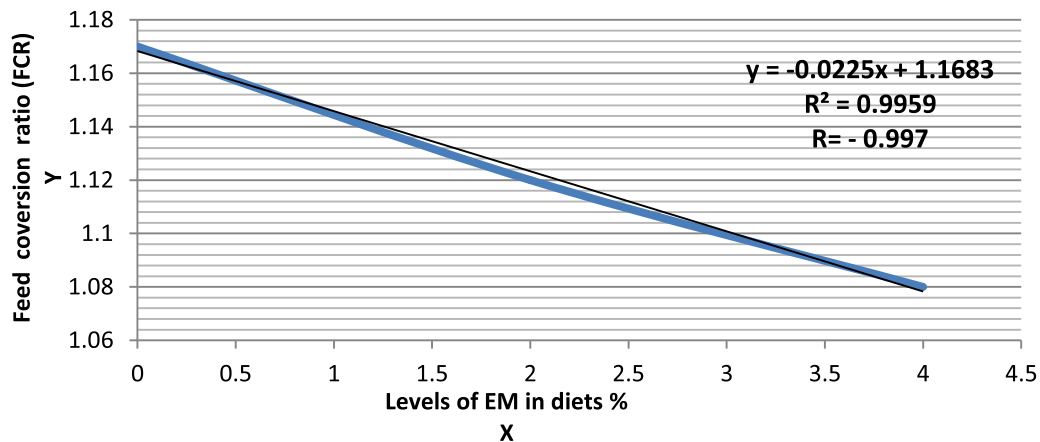


Fig. 3. The relationship between FCR with EM level in diet. Linear regression: $Y = a + bX$ whereas Y : is the dependent variable, a : is regression constant, b : is regression coefficient and X : is independent variable. Correlation coefficient R and Determination Coefficient R^2 .

different from treatments B2 and A2. However treatments, A1, C1, C2 and B1 showed higher values for these parameters than the B2 and A2 treatments. Statistical analyses did not show any significant differences between all the treatments in L₂, CF, SR, HSI and VSI. However, the C2 treatment had the highest L₂ followed by A1, B1, C1, A2 and B2, respectively. The highest CF was obtained for C1 followed by B2, B1, A1 and C2, while the lowest CF was obtained for the A2 treatment. The C2 treatment had the highest SR (99.44%) and the lowest SR was found for the A1 treatment. Measurements of feed efficiency did not significantly differ among treatments but, the best FCR was recorded for the C2 and C1 treatments followed

by the B2, B1 and A1 while the A2 treatment had the highest FCR value.

3.3 Body chemical composition

3.3.1 Effect of dietary EM supplements on the body chemical composition of juveniles Nile tilapia

The effect of EM supplemented feed on the whole body chemical composition and gross energy of Nile tilapia juveniles at the start and end of the study is presented in Table 6. Statistical analysis showed significant differences among treatments in body content of dry matter (DM),

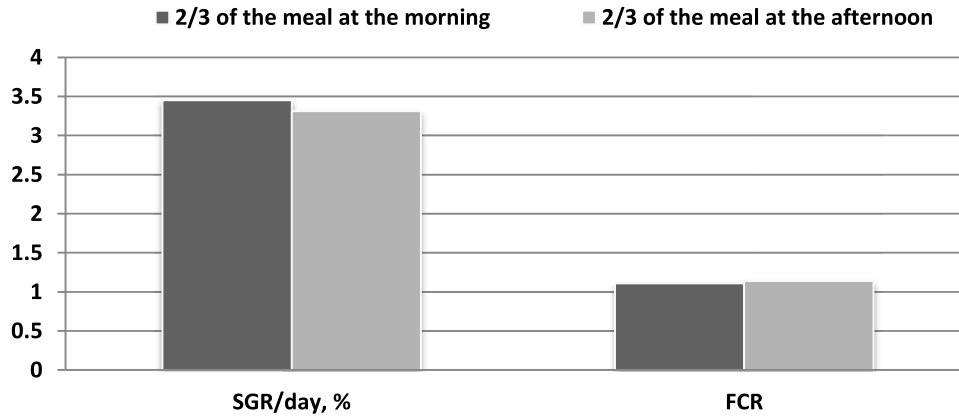


Fig. 4. SGR, FCR with different of feeding strategies.

Table 5. Effect of interaction between EM and the feeding regime on growth performance of tilapia juveniles.

Parameters [†]	Treatments [‡]						LSD [*]
	A 1	A2	B1	B2	C1	C2	
W ₁ , g	3.85	3.85	3.85	3.85	3.85	3.85	–
L ₂ , cm	10.75 ± 0.40	10.35 ± 0.01	10.57 ± 0.13	10.13 ± 0.03	10.37 ± 0.31	10.81 ± 0.02	0.740
W ₂ , g	22.69 ^a ± 0.01	19.25 ^b ± 0.84	21.93 ^a ± 0.21	20.83 ^{ab} ± 0.66	22.62 ^a ± 0.87	22.58 ^a ± 0.40	2.050
WG, g	18.84 ^a ± 0.01	15.40 ^b ± 0.84	18.08 ^a ± 0.21	16.97 ^{ab} ± 0.66	18.77 ^a ± 0.87	18.73 ^a ± 0.40	2.050
ADG, g day ⁻¹	0.370 ^a ± 0.001	0.302 ^b ± 0.016	0.354 ^a ± 0.004	0.332 ^{ab} ± 0.010	0.367 ^a ± 0.020	0.367 ^a ± 0.010	0.040
RGR, %	489.48 ^a ± 0.10	400.13 ^b ± 21.50	469.16 ^a ± 5.45	440.91 ^{ab} ± 17.30	487.53 ^a ± 22.60	486.49 ^a ± 10.10	53.280
SGR, %day ⁻¹	3.50 ^a ± 0.00	3.15 ^b ± 0.08	3.41 ^a ± 0.02	3.31 ^{ab} ± 0.0600	3.50 ^a ± 0.07	3.50 ^a ± 0.03	0.190
CF, %	1.82 ± 0.08	1.73 ± 0.07	1.85 ± 0.05	2 ± 0.08	2.04 ± 0.26	1.78 ± 0.04	0.430
SR, %	89.99 ± 4.44	96.60 ± 3.40	98.88 ± 1.11	93.88 ± 6.11	94.44 ± 5.56	99.44 ± 0.56	14.210
HSI, %	3.96 ± 0.04	5.18 ± 0.62	4.38 ± 0.05	6.25 ± 0.20	4.83 ± 0.70	4.23 ± 1.04	2.000
VSI, %	11.54 ± 0.10	7.75 ± 0.05	9.83 ± 0.05	9.83 ± 0.29	8.98 ± 1.81	10.33 ± 2.29	4.140
FI, g fish ⁻¹	21.49 ± 0.74	18.64 ± 0.78	20.22 ± 0.60	19.17 ± 1.32	20.65 ± 1.23	19.62 ± 0.43	3.160
FCR	1.14 ± 0.04	1.21 ± 0.01	1.17 ± 0.02	1.13 ± 0.12	1.09 ± 0.01	1.06 ± 0.02	0.188
PER	2.48 ± 0.03	2.28 ± 0.03	2.5 ± 0.05	2.49 ± 0.27	2.54 ± 0.04	2.62 ± 0.04	0.415
EER	0.041 ± 0.001	0.038 ± 0.001	0.040 ± 0.001	0.040 ± 0.004	0.041 ± 0.001	0.042 ± 0.001	0.006

Values are means ± S.E. of triplicate groups of three tanks. (a and b) Average in the same row having different superscripts significantly different at ($P \leq 0.05$).

*Least significant difference.

[†]Weight gain (WG), average daily gain (ADG), Relative growth rate (RGR), specific growth rate (SGR), condition factor (CF), survival rate (SR), Hepatosomatic index (HSI), Viscerosomatic index (VSI) Feed intake g/ fish (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and energy efficiency ratio (EER) [‡], A1: fish fed a control diet with 2/3 of amount daily meal in the morning, A2: fish fed a control diet with 2/3 of amount daily meal in the afternoon, B1 fish fed dietary 2% EM supplemented with 2/3 of amount daily meal in the morning, B2: fish fed dietary 2% EM supplemented with 2/3 of amount daily meal in the afternoon, C1: fish fed dietary 4% EM supplemented with 2/3 of amount daily meal in the morning and C2: fish fed dietary 4% EM supplemented with 2/3 of amount daily meal in the afternoon.

crude protein (CP), ether extract (EE) and gross energy (GE), while the ash content did not differ. The fish fed a diet containing 2% EM (B) had the highest DM (28.31%) followed by the control (A) and fish fed 4% EM (C), respectively. The CP content was higher for fish fed diets containing EM (B: 55.90 and C: 55.19%) than the control. Fish fed the control diet (A) had the highest EE (33.33%). Therefore, fish fed the control had the highest GE (29.11 kJ g⁻¹) followed by fish fed the B and C. Ash content was highest for the fish fed the C diet.

3.3.2 Effect of feeding regime on the body chemical composition of juveniles Nile tilapia

The effect of the feeding regime on the body chemical composition of Nile tilapia juveniles at the start and end of the study period presented in Table 6. These findings showed that, DM, CP, EE, ash content and GE did not differ significantly between fish fed 2/3 of their meal in the morning and those fed 2/3 of their meal in the afternoon. Therefore, feeding regime did not affect the body chemical composition of the fish.

Table 6. Effect of EM Supplemented on body chemical composition of tilapia juveniles.

Items & Treatments	Dry matter (DM, %)	Crude protein (CP, %)	Ether extract (EE, %)	Ash, %	Gross energy (GE, KJ/g)
Start	22.20±2.00	69.30±0.50	8.47±0.40	21.83±0.04	19.72±0.2
<i>Effect of EM on body chemical composition regardless feeding regime</i>					
A (0% EM)	27.94 ^{ab} ±0.30	54.55 ^b ±0.35	33.33 ^a ±0.42	12.10±0.19	26.11 ^a ±0.10
B (2% EM)	28.31 ^a ±0.07	55.90 ^a ±0.45	31.86 ^b ±0.21	12.23±0.36	25.84 ^{ab} ±0.08
C (4% EM)	27.15 ^b ±0.33	55.19 ^{ab} ±0.20	31.83 ^b ±0.31	12.94±0.22	25.67 ^b ±0.09
LSD*	0.85	1.34	1.06	0.85	0.302
<i>Effect of feeding regime on body chemical composition regardless EM</i>					
2/3 of the meal at the morning	27.25±0.31	55.31±0.36	32.09±0.34	12.59±0.26	25.80±0.09
2/3 of the meal at the afternoon	27.85±0.28	55.12±0.35	32.59±0.43	12.28±0.25	25.90±0.11
LSD*	0.76	0.93	0.99	0.66	0.26
<i>Effect of interaction between EM and feeding regime on body chemical composition</i>					
(A1)	27.70±0.62	54.88±0.71	32.73 ^{ab} ±0.60	12.39 ^{ab} ±0.11	25.95 ^{ab} ±0.07
(A2)	28.18±0.25	54.23±0.21	33.94 ^a ±0.02	11.82 ^b ±0.23	26.28 ^a ±0.06
(B1)	28.44±0.00	55.92±0.84	32.02 ^b ±0.47	12.05 ^{ab} ±0.36	25.92 ^b ±0.01
(B2)	28.18±0.00	55.88±0.75	31.70 ^b ±0.00	12.42 ^{ab} ±0.75	25.77 ^{bc} ±0.17
(C1)	27.13±0.42	55.13±0.49	31.53 ^b ±0.64	13.33 ^a ±0.15	25.54 ^c ±0.14
(C2)	27.19±0.70	55.25±0.05	32.14 ^b ±0.05	12.60 ^{ab} ±0.10	25.81 ^{bc} ±0.03
LSD*	1.490	2.020	1.410	1.260	0.350

Values are means±S.E. of triplicate groups of three tank, (a, b, c.) Average in the same column having different superscripts significantly different at ($P \leq 0.05$).

*Least significant difference.

A1: fish fed a control diet with 2/3 of amount daily meal in the morning, A2: fish fed a control diet with 2/3 of amount daily meal in the afternoon, B1 fish fed dietary 2% EM supplemented with 2/3 of amount daily meal in the morning, B2: fish fed dietary 2% EM supplemented with 2/3 of amount daily meal in the afternoon, C1: fish fed dietary 4% EM supplemented with 2/3 of amount daily meal in the morning and C2: fish fed dietary 4% EM supplemented with 2/3 of amount daily meal in the afternoon.

3.4 Economic efficiency

The results for economic efficiency are presented in [Table 7](#). Fish fed a diet containing 4% EM were most expensive in terms of total cost (29.15 L.E, \$1.82), variable cost (21.15 L.E, \$1.32) and production (1.973 kg) followed by treatments B and A. these fish also had the highest net profit (14.24 L.E, =0.89 \$), total revenue (43.24 L.E, = \$2.7) and value added (22.27 L.E, = \$1.39), followed by treatments B, and A. Additionally, fish fed a diet containing 4% EM had the best operating ratio and return on costs whereas the economic efficiency increased with the low operating ratio. Hence, the addition of EM in the tilapia diet improved the growth rate, SR and it was economically efficient compared with the control. [Table 7](#) also shows that fish fed 2/3 of their daily meal in the morning consumed more feed than those fed 2/3 of their daily meal in the afternoon. This reflected on the operating ratio and return on costs. However, the parameters of economic efficiency such as production (1.900 kg), net profit (12.93 LE, = \$0.81), value added (20.95 L.E, = \$1.31) and total revenue (41.92 L.E, = \$2.62) were highest for fish fed 2/3 of their meal in the morning. The interaction between EM and feeding regime had an effect on the total cost and production for treatments A1, A2, B1, B2, C1 and C2, respectively. Net profit and value added were the highest for treatment C2 (15.14 and 23.15, respectively), while these parameters were lowest for treatment B2 which their fish fed diet containing 2% EM and 2/3 of the meal fed in the afternoon. The highest total

revenue was for treatment C2 (44.46) followed by treatments B1 (42.94), C1 (42.39), A1 (40.45), B2 (38.49) and treatment A2 had the lowest total revenue (38.12). Similarly, treatment C2 had the highest operating ratio and return on costs (0.65 and 1.52 respectively) of all the treatments.

4 Discussion

The water temperature, pH and dissolved oxygen during the trial were 26.56 °C, 7.94 and 7.25 mg·l⁻¹, respectively, which are similar to the optimum ranges for tilapia ([Geater and Tansakul, 1988](#); [Bergheim, 2007](#)).

In the present trial it was evident that growth performance and feed utilization increased when using high levels of EM and these findings are comparable with previous findings for tilapia by ([Opiy et al., 2019](#)). These authors have reported that, probiotic supplemented diets result in higher growth in Nile tilapia than diets without probiotic supplementation and using 0.1% probiotics in fry tilapia diet improved the growth performance and mitigated the stress factor ([Lara-Flores et al., 2003](#)). Additionally the use of probiotics increased the growth rate of trout ([Gomez-Gil et al., 2000](#)).

In similar manner to this study, [El-Dahhar et al. \(2015\)](#) tested four levels of EM (0%, 2%, 4% and 6%) by supplementing it in sea bream diets, and they detected that the highest WG, ADG, and SGR values were obtained by using 4% EM and these values were significantly better than those of

Table 7. Effect of EM and feeding regime on economic efficiency of tilapia juveniles.

Items & Treatments	Total cost (L.E)	Variable cost (L.E)	Production (kg)	Net profit (L.E)	Total Revenue (L.E)	Value added (L.E)	Operatin Ratio	Return On Costs
<i>Effect of EM on economic efficiency regardless feeding regime</i>								
A (0% EM)	27.26	19.24	1.786	11.85	39.28	20.04	0.69	1.44
B (2% EM)	28.25	20.25	1.857	12.46	40.71	20.46	0.69	1.44
C (4% EM)	29.15	21.15	1.973	14.24	43.42	22.27	0.67	1.49
<i>Effect of feeding regime on economic efficiency regardless EM</i>								
2/3 of the meal at the morning	28.99	20.97	1.900	12.93	41.92	20.95	0.69	1.45
2/3 of the meal at the afternoon	27.45	19.45	1.840	12.90	40.35	20.90	0.68	1.47
<i>Effect of interaction between EM and feeding regime on economic efficiency</i>								
(A1)	28.63	20.58	1.839	11.48	40.45	19.87	0.71	1.41
(A2)	25.90	17.90	1.733	12.22	38.12	20.22	0.68	1.46
(B1)	29.36	21.36	1.952	13.57	42.94	21.57	0.68	1.46
(B2)	27.15	19.15	1.763	11.34	38.49	19.34	0.70	1.42
(C1)	28.99	20.99	1.925	13.40	42.39	21.40	0.68	1.46
(C2)	29.31	21.31	2.020	15.14	44.46	23.15	0.65	1.52

L.E is the leaver Egyptian.

A1: fish fed a control diet with 2/3 of amount daily meal in the morning, A2: fish fed a control diet with 2/3 of amount daily meal in the afternoon, B1 fish fed dietary 2% EM supplemented with 2/3 of amount daily meal in the morning, B2: fish fed dietary 2% EM supplemented with 2/3 of amount daily meal in the afternoon, C1: fish fed dietary 4% EM supplemented with 2/3 of amount daily meal in the morning and C2: fish fed dietary 4% EM supplemented with 2/3 of amount daily meal in the afternoon.

the control diet. These results are consistent with the results shown in Table 3. Another study also found similar results for carp; the SGR for carp fed a diet containing 2%, 4% and 6% EM gradually increased to 16% compared with carp fed the control diet (Huang et al., 1999; Niang, 2013).

In the present study, the CF increased with dietary supplementation of EM compared with the control diet. These results are in agreement with the results of Omar et al. (2017) for fish fed with EM or EM with copper.

The data of the present trial showed that fish fed a diet supplemented with 4% EM had the highest SR, followed by fish fed a diet with 2% EM and the control diet. Similar findings were obtained with a higher rate of survival when using 4% EM in fish feed than the control diet (El-Dahhar et al., 2015). These findings could be due to the beneficial effects of EM and their ability to improve the immune systems of fish (Xu et al., 2021; Michalska et al., 2021). Additionally, in the present study the juveniles that were fed 2% and 4% EM demonstrated higher growth and survival than the control. A similar trend was exhibited by Abdel-Aziz et al. (2020) and Xu et al. (2021) who reported that low or high doses of EM can promote tilapia growth, and they suggested that dietary EM supplementation had positive effects on tilapia culture. Likewise, the results of Lara-Flores et al. (2003) proved that probiotics in fish diets decreased the effect of stress factors and enhanced fish performance. Previous studies indicated that dietary probiotic supplementation had a positive effect on both fish health and SR (Opiyo et al., 2019).

In line with these observations, the supplementation of EM increases the appetite of fish, enhancing feed intake and breaking down indigestible feed (Lara-Flores et al., 2003). In the present study, the feed utilization parameters did not differ significantly among treatments. However, EM had a small effect on FCR, as shown in Figure 3. Moreover, some studies have confirmed that FCR doesn't differ significantly with

dietary probiotic supplementation, but feed utilization parameters were higher in fish fed diets containing EM than the control diet (Mohit et al., 2007). The same finding was observed for Nile tilapia fed a diet containing probiotics (El-Haroun et al., 2006; Opiyo et al., 2019). In contrast, shrimp fed EM supplemented feed showed the lowest FCR values (Huang et al., 1999). The best PER and EER were achieved for fish fed diets containing 4% and 2% EM, which in agreement with the results reported by Lara-Flores et al. (2003) and El-Haroun et al. (2006) for Nile tilapia fed on a probiotic diet compared with those fed a control diet without probiotic. Similarly, the highest PER was obtained for larvae fed 2% EM followed by those fed 4% EM (El-Dahhar et al., 2015).

Moreover, Saccharomyces which are yeasts found in EM, are a good source of protein, minerals and B-complex (Gao et al., 2021). Therefore, they could improve the PER. An increase in the PER has also been detected through the addition of probiotics to fish diets (Pond et al., 2006). consequently, it is evident that EM plays a substantial role in the enhancing of growth, fish health, stress resistance, and the production of digestive enzymes, vitamins, and fatty acids. Also EM improves the gut flora and creates a balance for the micro flora in the digestive system of fish.

4.1 Effect of feeding regime on growth parameters of juveniles Nile tilapia

In general, fish farmers in Egypt offer Nile tilapia equal amounts of feed twice a day in the morning and afternoon. However, our results showed that Nile tilapia are more active and have a larger appetite in the morning than the afternoon (Tab. 4; Fig. 4). In the current study, fish fed 2/3 of their daily meal in the morning ingested more feed and hence more energy and more protein, which reflected in their growth parameters in comparison with fish, fed 2/3 of their daily meal in the afternoon.

Previous studies have also found that feeding time has a substantial effect on the growth of Nile tilapia (El-Husseiny et al., 2004), channel catfish (Noeske-Hallin et al., 1985) and goldfish (Spieler and Noeske, 1984). Therefore, the largest proportion of the daily meal should be provided for fish at their optimum feeding time to suit each of fish type.

Several studies have confirmed that fish fed in the morning had a higher growth rate than those fed in the afternoon or evening. For example, rabbit fish fed in the morning or before noon had higher growth parameters than those fed in the afternoon at 1.00 p.m. and 4.00 p.m., also it was found that the water temperature degree is lower in the morning than in the afternoon encouraging increased feed intake (Abdel-Aziz et al., 2016). In similar context, rainbow trout fed a low energy diet at 9.00 am had higher growth rate and feed efficiency than those fed at 19.00 pm (Bolliet et al., 2000). Furthermore, it was observed that, the WG of Korean rockfish was the highest when fish were fed one meal per day at 8.00 am (Mizanur and Bai, 2014). In contrast, pompano fish fed in the evening had a higher growth rate than those fed in the morning, and fish activity decreased later in the day, when energy consumption decreased, causing the highest WG (Heilman and Spieler, 1999). Similarly, greenback flounder fed at a 2% feeding rate in the evening had a higher growth rate and lower urea excretion than fish fed at a 3% feeding rate in the morning (Verbeeten et al., 1999). Moreover, a previous study reported that Nile tilapia preferred feeding in the afternoon than feeding in the morning (Van der Meer et al., 1997). Another study reported that, the optimum feeding time for tilapia growth is once daily at 19.00 pm, and there is more digestion efficiency in the afternoon (El-Husseiny et al., 2004).

The CF, SR, HSI and VSI didn't differ significantly between these two treatments, with SR ranging from 94% to 97%. This range is higher than the value of 80% recorded by Sumi et al. (2011). The increase in SR in the present study could be the result of the good condition of the fish and the management rearing system applied in our experiment. The feed utilization parameters (FCR, PER and EER) did not differ significantly between the two strategies of feeding. However, these parameters were fairly better with 2/3 of the meal amount fed in the morning than the other treatment. The FI, g/fish, was significantly high for fish fed 2/3 of their meal in the morning. These results are in agreement with the results obtained for catfish, in which feeding time does not affect the FCR (Jarboe and Grant, 1996). Furthermore, some studies found that feeding in the afternoon led to a decrease in feed intake (Wu et al., 2004). Additionally, in another study, FI decreased gradually from the morning until mid-day, and the lowest value of FCR was obtained for fish fed in the morning (Abdel-Aziz et al., 2016). Similarly, another study found that, digestion efficiency improved for fish fed in the morning compared with those fed at other times (Gélineau et al., 2002).

4.2 Effect of interaction between dietary EM supplemented and feeding regime on growth and feed efficiency of Nile tilapia juveniles

Treatment A2 had the lowest values for growth performance parameters. This is because the A2 fish were fed 2/3 of their meal in afternoon and their diet was fed without EM supplementation. However, the fish

supplemented with EM and offered 2/3 of meal in the afternoon (B2, C2), had an enhanced ability for feed utilization, and this reflected in their growth performance. Therefore, adding EM to the feed of Nile tilapia removes the negative effect of the improper of feeding time. Moreover, EM includes a high population of LAB (*Lactobacillus* and *pediococcus*), which is considered the primary group of probiotics used in fish nutrition to boost growth, feed efficiency and SR as reported by Ringø and Gatesoupe (1998). EM not only includes LAB, but it also contains a large population of yeast (*saccharomyces*), which plays a substantial role in the improvement of animal performance, and is supported by more recent works that confirm the enhancement of fish growth by feeding a diet containing yeast (Diab et al., 2006; Opiyo et al., 2019). It has been found that using live yeast in the diet of sea bass increases the activity of digestive enzymes (Tovar et al., 2002). Accordingly, several works have highlighted the effect of yeast on fish growth. The addition of yeast to the diets of juveniles sea bream improved their growth performance (Totou, 2014). Adding *Lactobacillus* and yeast (*Saccharomyces cerevisiae*) to carp diet improved growth (Dhanaraj et al., 2010). Also, *S. cerevisiae* in the diets of Nile tilapia fry resulted in good growth rates (Lara-Flores et al., 2003).

The EM liquid culture also contained photosynthetic bacteria that are able to separate the hydrogen in ammonia in hydrogen sulfide and in hydrocarbons, as it deoxidizes carbon gases and synthesizes sugar. Therefore photosynthetic bacteria play an important role in EM activity. The SR did not differ significantly among treatments, but C2 recorded the highest SR (99.44%) and A1 recorded the lowest SR (89.99%). The present results reflect the role of LAB and yeast in fish health for increasing immune system efficiency and improving fish survival. These results agree with the results of Fernandez et al. (2011). The fish that were fed the same amount of feed at different times of the day showed differences in growth parameters (Boujard et al., 1995). Although the A1 diet did not contain EM, it was fed mostly in the morning, and it had a higher SGR value than the while, the A2 diet, which was mostly fed in the afternoon. These results confirm that the best time to offer feed is in the morning, whereas the fish can most benefit from the contents of the diet. Therefore, it is preferable to offer a large proportion of the daily meal at the optimal feeding time. In general, the effects of EM and feeding regime depend on several factors, feeding behavior, which will differ between herbivorous and carnivorous fish. The interaction between EM supplementation and feeding regime EM supplementation and feeding regime did not appear to result insignificant differences in FI, FCR, PER and EER. However, the lowest FCR value was recorded for the C2 treatment, while, the A2 had the highest FCR value. Several studies have reported that feed efficiency is increased by improving the health of the digest system, than increasing of digestive enzymes activities, which takes place when fish are fed diets containing EM (Nahashon et al., 1994). Our results support many recent findings on Roha (*Labeo rohita*) fingerlings fed a diet containing a mix of three microorganisms LAB *Bacillus subtilis* and *S. cerevisia* (Mohapatra et al., 2012). Likewise, recent studies by El-Zayat (2014), Hussein et al. (2016) and Yones et al. (2019b) confirmed that, lactocel-con in tilapia diets enhances and improves the feed efficiency of fish.

Additionally, sea bream fingerlings fed a diet containing probiotics had the highest FCR, PER, and EER compared with those fed a control diet (Totou, 2014).

4.3 Body chemical composition

4.3.1 Effect of dietary EM supplementation on body chemical composition of Nile tilapia juveniles

In the present study (Tab. 6) body CP content was higher for fish fed an EM supplemented diet. This reflects the nutrient efficiency transfer from feed to fish muscles or organs, and the nutritional value of fish and its meat quality. Where, EM in the fish diet increased the CP content with decreasing in EE content. A similar result in another study emphasized that the CP content of the fish body was significantly elevated and the EE was decreased for fish fed probiotics (Opiyo et al., 2019). Moreover, the CP content is higher in fish fed EM than fish at the start of the study (Niang, 2013). In the same trend, another study agreed with our finding of dietary EM supplementation of 2% and 4% leading to higher CP content than the CP at control (El-Dahhar et al., 2015). Additionally, other studies have reported an increased CP content and decreased EE for fish fed a diet containing Biogen® (Soltan et al., 2016). This manifested the big role of EM bacteria to stimulate protease enzyme and inducing higher availability of feed protein which was more beneficial to fish (Gao et al., 2021). Furthermore, *Saccharomyces* in EM increase digestive enzymes activity, such as trypsin, hence fish benefit more from CP in their diet which is reflected by increased CP in their body content (Lara-Flores et al., 2003). On the other hand, some research has shown that, dietary probiotic supplementation did not affect the body composition of fish (Lara-Flores et al., 2003). Also, El-Haroun et al. (2006) showed that the fish carcass content of CP did not differ when fish were fed a probiotic-supplemented diet, but they reported that fish fed the control had the highest EE content while the ash content was not significantly affected. A similar result was obtained for ash content when feeding a dietary EM supplementation, with no differences were observed between fish fed EM or not (El-Dahhar et al., 2015) and this is in agreement with our results.

4.3.2 Effect of feeding regime on body chemical composition of juveniles Nile tilapia

Our results (Tab. 6) are in agreement with those of Verbeeten et al. (1999) they found that fish carcass content of DM, CP, ash and GE were not affect by feeding time. Furthermore, in another study feeding time did not cause significant differences in fish body composition (Robinson et al., 1995). Also, our results were in partial agreement with the results of Abdel-Aziz et al. (2016) who found, both EE and GE contents in whole body of rabbit fish did not differ with feeding time, but they found that the body content of DM, CP and ash were significantly affected by feeding time. These authors added that the body content of CP increased in fish fed in the morning. The same finding was relatively coinciding with our results, which cleared that body CP of fish fed 2/3 of their meal in the morning was higher than those fed most of their diet in the afternoon. Conversely, other research confirmed that muscles EE was higher in trout fed in the

afternoon than those fed in the morning (Boccignone et al., 1993). While, body CP significantly increased in fish fed once a day in the evening, and body content of EE increased with those fed once daily in the morning (El-Husseiny et al., 2004). Most of findings indicate that, feeding time don't affect the fish body composition, but changes in fish body composition when fish are fed at different times per day may be due to fish type, diet ingredients and environment condition such as water temperature, whereas warm water fish tend to deposit EE with decreased water temperature (Lara-Flores et al., 2003).

4.3.3 The interaction between dietary EM supplemented and feeding regime on the body chemical composition of juveniles Nile tilapia

The body content of EE and GE (Tab. 6) were differed significantly among treatments. The control diet that was mostly fed in the afternoon had the highest EE and GE content. These results are in agreement with increased in CP and reduction in the EE contents of tilapia fed a diet containing the probiotic *Bacillus subtilis* compared with tilapia fed a control diet (Opiya et al., 2019). Similarly, another study reported the enhancement of CP content and decrease in EE content in *Onchorhynchus mykiss* fed probiotic supplemented diets (Bagheri et al., 2008). The ash content varied among the treatments, the highest ash content obtained for C1 and the lowest for A2, while the other treatments did not significantly differ in ash content (Tab. 6). In another study, dietary probiotic supplementation had insignificant effect on CP, EE or ash content (Hassaan et al., 2018).

4.4 Economic efficiency

Our results of economic efficiency agreed with previous results for tilapia, the best indices of economic performance were achieved for fish fed a diet containing prebiotics, probiotics and symbiotic (Azevedo et al., 2015). Moreover, Table 7 showed the fish fed 2/3 of their daily meal in the morning consumed more feed than those fed 2/3 of daily meal in the afternoon, resulting in improved production, net profit, value added and total revenue for fish fed 2/3 of their meal in the morning. This reflects the optimum feeding time with an adequate amount of feed. Whereas, the amount of daily feed intake, frequency, feeding time and a predetermined ratio are important factors for the feed management strategies, which influence growth, feed utilization and economic efficiency (Goddard, 1995). Additionally, optimal feeding regimes can lead to savings in feed costs (Davies et al., 2006).

The highest total cost and variable cost were obtained for treatments B1 (29.36, 21.36 L.E=\$1.84, \$1.34), C2 (29.31, 21.31=\$1.83, \$1.33) followed by C1 (28.99, 20.99 L, E=\$1.81, \$1.32), which were fed a diet containing EM and this finding implies that these treatments had the highest feed intake or survival rate. While the lowest variable cost was achieved for A2, B2 (17.90, 19.15 L.E=\$1.12, \$1.20), where fish were offered 2/3 of their daily meal in the afternoon but, A2 was not supplemented with EM while B2 contained 2% EM. The variable cost of A1 was (20.58 L.E=\$1.29). The other indicators for fish fed 2/3 of their daily meal in the morning with an EM supplemented diet or a diet without EM

such as C1, B1 and A1, were better for production (kg), net profit, and the total revenue than fish fed 2/3 of their daily meal in the afternoon. However, the best economic parameters were for C2 (4% EM) which had the highest fish production (2.02 kg), net profit (15.14 L.E=\$0.92), total revenue (44.46 L.E=\$2.78) and added value (23.15 LE=\$1.43). Furthermore, this treatment had the best operating ratio, and return on costs. EM supplemented feed had more of an effect than feeding regime on the economic efficiency of juvenile Nile tilapia.

5 Conclusion

Juveniles Nile tilapia is benefit most when their feed is offered in the morning than those in the afternoon. Nile tilapia fed 2/3 of their daily meal in the morning performed better than those fed the same amount in the afternoon. So it is necessary to feed higher quantities of feed in the morning than in the afternoon. The use of 4% EM in the diet improved the growth and feed utilization of fish fed 2/3 of their daily meal in afternoon. Crude protein and fat content of fish body are significantly affected by the addition of EM in the fish diet, but the feeding regime did not have any effects on body chemical composition. The indicators of economic efficiency were improved with dietary EM supplementation regardless the feeding regime, and fish fed a diet containing 4% EM with 2/3 of their daily meal in the afternoon had the highest net profit and added value. So, this study recommends that, using 4% EM as feed additives in fish feed in general, especially when feeding fish at inappropriate times of them.

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References

- Abareethan M, Amsath A. 2015. Characterization and evaluation of probiotic fish feed. *Int J Pure Appl Zool* 3: 148–153.
- Abdel-Aziz M, Bessat M, Fadel A, Elblehi S. 2020. Responses of dietary supplementation of probiotic effective microorganisms (EMs) in *Oreochromis niloticus* on growth, hematological, intestinal histopathological, and antiparasitic activities. *Aquaculture Int* 1–17.
- Abdel-Aziz MF, Mohammed RA, Abou-Zied RM, Allam SM. 2016. Effect of feeding frequency and feeding time on growth performance, feed utilization efficiency and body chemical composition on Rabbitfish *Siganus rivulatus* fry and juvenile under laboratory condition. *Egypt J Aquat Biol Fish* 20: 35–52.
- Abdelhamid MA, Refaey AMM, Seden AME, Zenhom OA. 2014. Effect of different sources and levels of some dietary biological additives on: IV – immunity and hematology of Nile tilapia *Oreochromis niloticus*. *Egypt J Aquat Biol Fish* 18: 49–60.
- Ani AO, Okpako BA, Ugwuowo LC. 2013. Effect of feeding time on the performance of juvenile African catfish *Clarias gariepinus*. *Online J Ani Feed Res* 3: 143–148.
- A.O.A.C. (Official methods of analysis of AOAC International agricultural chemicals). 2010. Contaminants drugs/edited by William Horwitz. Gaithersburg (Maryland): Volume I, AOAC International, 1997.
- Aswathy N, Narayanakumar R, Philipose KK, Imelda J. 2015. Cost-benefit analysis and input requirements for mariculture of finfishes in India. *Winter School Technol Adv Maricul Prod Enhan Sustain* 301–304.
- Azevedo RVD, Fosse Filho JC, Cardoso LD, Mattos DDC, Vidal Júnior MV, Andrade DRD. 2015. Economic evaluation of prebiotics, probiotics and symbiotics in juvenile Nile tilapia. *Rev Ciência Agron* 46: 72–79.
- Bagheri T, Hedayati SA, Yavari V, Alizade M, Farzanfar A. 2008. Growth, survival and gut microbial load of rainbow trout *Oncorhynchus mykiss* fry given diet supplemented with probiotic during the two months of first feeding. *Turkish J Fish Aquat Sci* 8: 43–48.
- Bergheim A. 2007. Water quality criteria I recirculation system for tilapia. *Int Res Inst Stavanger*, 4068, Norway.
- Boccignone M, Forneris G, Salvo F, Ziino M, Leuzzi U. 1993. Size and meal timing: effect on body composition in rainbow trout. In: Kaushik SJ, Luquet P. (Eds.) *Fish Nutrition in Practice*. Les Colloques INRA, n 61, Paris, France, pp. 293–296.
- Bolliet V, Cheewasedtham C, Houlihan D, Gélinau A, Boujard T. 2000. Effect of feeding time on digestibility, growth performance and protein metabolism in the rainbow trout *Oncorhynchus mykiss* interactions with dietary fat levels. *Aquat Living Resource* 13: 107–113.
- Boujard T, Gélinau A, Corraze G. 1995. Time of a single daily meal influences growth performance in rainbow trout *Oncorhynchus mykiss*. *Aquacul Res* 26: 341–349.
- Davies OA, Inko-Tariah MB, Amachree D. 2006. Growth response and survival of *Heterobranchus longifilis* fingerlings fed at different feeding frequencies. *Afr J Biotechnol* 5: 778–787.
- Dhanaraj M, Haniffa MA, Singh SA, Arockiaraj AJ, Ramakrishanan CM, Seetharaman S, Arthimanju R. 2010. Effect of probiotics on growth performance of koi carp *Cyprinus carpio*. *J Appl Aquacul* 22: 202–209.
- Diab AS, Abdel-Hady YM, Ahmed MH, Saber SF, Aboel-Atta ME. 2006. Out-door study on the use of Echinacea *Echinacea purpurea*, marjoram *Origanum dictamnus* and yeast *Scharomycis cerevisiea* as feed additive for *Oreochromis niloticus*. *Egypt J Agric Res* 84: 537–551.
- El-Zayat AM. 2014. Study the effect of some probiotics on production of fish farms. Ph.D. Thesis, Faculty of agriculture, Al-Azhar Univ. Cairo, Egypt.
- El-Dahhar AA, Salama ME, Essa MA, Elebiary HE, Abd El Rahim M, Lotfy A. 2015. Effect of Probiotic (EM) in the Diet of Gilthead Sea bream *Sparus aurata* post larvae reared in El-Max station on water quality, survival, growth and bacterial load. *J Arab Aquacul Soc* 10: 51–70.
- El-Haroun ER, Goda AS, Kabir Chowdhury MA. 2006. Effect of dietary probiotic Biogen[®] supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus*. *Aquacul Res* 37: 1473–1480.
- El-Husseiny OM, Abdel-Aziz GM, Ashraf MSG, Mabroke RS. 2004. Influence of feeding time of day, feeding frequency and diet composition on Nile tilapia *Oreochromis niloticus* growth performance. *Egypt J Aquat Biol Fish* 8: 155–179.
- EMRO (EM research organization) 2010. Working with EM technology for sustainable environment. Accessed on November 06, 2015 from: <http://www.emsustains.co.uk>

- FAO. 2016. Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome, pp. 200.
- Fernande R, Sridhar M, Sridhar N. 2011. Effect of lactic acid bacteria administered orally on growth performance of *Penaeus indicus* juveniles. *Res J Microbiol* 6: 466.
- Fuller R. 1989. Probiotics in man and animals. *J Appl Bacteriol* 66: 365–378.
- Gao J, Tai X, Shao N, Sun Y, Nie Z, Wang Y, Li Q, Xu P, Xu G. 2021. Effects of effective microorganisms on the growth performance, nutritional composition and flavour quality of the pond-cultured *Eriocheir sinensis*. *Aquacul Res* 52: 871–880.
- Geater A, Tansakul R. 1988. Effects of acid water on survival and growth rate of Nile tilapia (*Oreochromis niloticus*). In 2nd Int. Sym. on Tilapia in Aquacul. World Fish, 15, p. 433.
- Gélineau A, Bolliet V, Corraze G, Boujard T. 2002. The combined effects of feeding time and dietary fat levels on feed intake, growth and body composition in rainbow trout. *Aquat Living Resour* 15: 225–230.
- Goddard S. 1995. Feed Management in Intensive Aquaculture. New York: Chapman and Hall, 194p.
- Gomez-Gil B, Roque A, Turnbull JF. 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* 191: 259–270.
- Guim A, Andrade PD, Iturrino-Schocken RP, Franco GL, Ruggieri AC, Malheiros EB. 2002. Aerobic stability of wilted grass silages *Pennisetum Purpureum* treated with microbial inoculant. *Rev Brasil Zoot* 31: 2176–2185.
- Hassaan MS, Soltan MA, Jarmolowicz S, Abdo HS. 2018. Combined effects of dietary malic acid and *Bacillus subtilis* on growth, gut microbiota and blood parameters of Nile tilapia *Oreochromis niloticus*. *Aquacul Nutr* 24: 83–93.
- Heilman MJ, Spieler RE. 1999. The daily feeding rhythm to demand feeders and the effects of timed meal-feeding on the growth of juvenile Florida pompano *Trachinotus carolinus*. *Aquaculture* 180: 53–64.
- Huang YC, Wang SL, Huang ZM, Li CL. 1999. Effect of effective microorganisms (EM) on the growth of Jian carp and the quality of water. *J Jimei Uni Nat Sci* 1.
- Hussein MS, Yones AM, Ali MW, Abd El-Azem A. 2016. Effect of sorghum replacement and probiotic on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus*. *Egypt J Aquat Biol Fish* 20: 113–121.
- Jarboe HH, Grant WJ. 1996. Effects of feeding time and frequency on growth of channel catfish *Ictalurus punctatus* in closed recirculating raceway systems. *J World Aquacul Soc* 27: 235–239.
- Kolk LJ, Smink W. 2005. Non-specific immune stimulation of Bokashi in head kidney cells of carp. *Feed Innov. Serv. (F.I.S.)* by Wageningen, 1–8.
- Kriangsak P. 2001. Biotechnology for black tiger prawn culture. *Rimbo J* 30: 22–23.
- Lara-Flores M, Olvera-Novoa MA, Guzman-Mendez BE, Lopez-Madrid W. 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia *Oreochromis niloticus*. *Aquaculture* 1/4: 193–201.
- Macfadyen G, Nasr Allah AM, Kenawy DA, Ahmed MF, Hebicha H, Diab A, Hussein SM, Abouzied RM, El Naggar G. 2011. Value-chain analysis of Egyptian aquaculture. Project report 2011-54. *The worldfish center penang*, Malaysia. 84 pp.
- Michalska K, Gesek M, Sokół R, Murawska D, Mikiewicz M, Chłódowska A. 2021. Effective microorganisms (EM) improve internal organ morphology, intestinal morphometry and serum biochemical activity in Japanese quails under *Clostridium perfringens* challenge. *Molecules* 26: 2786.
- Mizanur RM, Bai SC. 2014. The optimum feeding frequency in growing Korean rockfish *Sebastes schlegelii* rearing at the temperature of 15 °C and 19 °C. *Asian-Austr J Ani Sci* 27: 1319.
- Mohapatra S, Chakraborty T, Prusty AK, Das P, Paniprasad K, Mohanta KN. 2012. Use of different microbial probiotics in the diet of rohu *Labeo rohita* fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal micro flora. *Aquacul Nutr* 18: 1–11.
- Mohit AH, Hosseini SA, Lotfollahian H, Shariatmadari F. 2007. Effects of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature. *Int J Poult Sci* 6: 895–900.
- Nahashon SN, Nakau HS, Mirosh LW. 1994. Production variables and nutrient retention in Single Comb White Leghorn laying pullets fed diets supplemented with direct-fed microbials. *Poult Sci* 73: 1699–1711.
- Niang 2013. Communication personnelle. Pour son mémoire en TS CNFPTA.
- Noeske-Hallin TA, Spieler RE, Parker NC, Suttle MA. 1985. Feeding time differentially affects fattening and growth of channel catfish. *J Nutr* 115: 1228–1232.
- NRC (National Research Council). 2011. Nutrient Requirements of fish and shrimp. Washington, DC: National Academy Press.
- Omar WA, Abdel-Salam RG, Mahmoud HM. 2017. The use of effective microorganisms (EM) as a probiotic on cultured Nile tilapia *Oreochromis niloticus*. *Egypt J Zool* 67: 67–90.
- Opiyo MA, Jumbe J, Ngugi CC, Charo-Karisa H. 2019. Composition of Nile tilapia *Oreochromis niloticus* cultured in low input ponds. *Sci Afri* 4: e00103.
- Pond MJ, Stone DM, Alderman DJ. 2006. Comparison of conventional and molecular techniques to investigate the intestinal microflora of rainbow trout *Oncorhynchus mykiss*. *Aquaculture* 261: 194–203.
- Ringø E, Gatesoupe FJ. 1998. Lactic acid bacteria in fish: a review. *Aquaculture* 160: 177–203.
- Robinson EH, Jackson LS, Li MH, Kingsbury SK, Tucker CS. 1995. Effect of time of feeding on growth of Channel Catfish 1. *J World Aquacult Soc* 26: 320–322.
- Sanchez-Vazquez FJ, Tabata M. 1998. Circadian rhythms of demandfeeding and locomotor activity in rainbow trout. *J Fish Biol* 52: 255–267.
- Soltan MA, Fouad IM, Elfeky A. 2016. Growth and feed utilization of Nile tilapia *Oreochromis niloticus* fed diets containing probiotic. *Global Vet* 17: 442–450.
- Soltan M, El-Laithy S. 2008. Evaluation of fermented silage made from fish, tomato and potato by-products as a feed ingredient for Nile tilapia *Oreochromis niloticus*. *Egypt J Aquat Biol Fish* 12: 25–41.
- Spierer RE, Noeske TA. 1984. Effects of photoperiod and feeding schedule on diel variations of locomotor activity, cortisol, and thyroxine in goldfish. *Trans Am Fish Soc* 113: 528–539.
- SPSS 2007. Statistical Package For Social Science (for Windows). Release 16 Copyright ©, SPSS Inc., Chicago, USA.
- Sumi KR, Das M, Siddika I. 2011. Effect of different protein levels of fry feed on the production of the quality of tilapia *Oreochromis niloticus* fry. *J Bangladesh Agric Uni* 9: 365–374.
- Suzer C, Çoban D, Kamaci HO, Saka S, Firat K, Otcucuoğlu Ö, Küçüksarı H. 2008. *Lactobacillus spp.* bacteria as probiotics in Gilthead Sea bream (*Sparus aurata*, L.) larvae: effects on growth performance and digestive enzyme activities. *Aquaculture* 280: 140–145.

- Totou MM. 2014. Use of probiotics and prebiotics as feed additives to stimulate growth of sea bream *Sparus aurata*. PhD Thesis, Fac. Environ. Agri. Sci. Suez Canal University, Egypt.
- Tovar D, Zambonino J, Cahu C, Gatesoupe FJ, Vázquez-Juárez R, Lésel R. 2002. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass *Dicentrarchus labrax* larvae. *Aquaculture* 204: 113–123.
- Van der Meer M, Herwaarden H, Verdeyen M. 1997. Effect of number of meal and frequency of feeding on voluntary feed intake of *Colossoma macropomum* (Cuvier). *Aquacul Res* 28: 419–432.
- Verbeeten BE, Carter CG, Purser GJ. 1999. The combined effect of feeding time and ration on growth performance and nitrogen metabolism of greenback flounder. *J Fish Biol* 55: 1328–1343.
- Webster CD, Tidwell JH, Yancey BA. 1992. Effect of protein level and feeding frequency on growth and body composition of cage reared Channel Catfish. *Prog Fish-Culturist* 54: 92–96.
- Wu G, Saoud IP, Miller C, Davis DA. 2004. The effect of feeding regimen on mixed-size pond-grown channel catfish, *Ictalurus punctatus*. *J Appl Aquacul* 15: 115–125.
- Xu W, Mawolo PY, Gao J, Chu L, Wang Y, Nie Z, Song L, Shao N, Gao J, Xu P, Xu G. 2021. Effects of supplemental effective microorganisms in feed on the growth, immunity, and appetite regulation in juvenile GIFT tilapia. *Aquacul Rep* 19: 100577.
- Yones AM, Hussein MS, Ali MW, Abdel-Azem MA. 2019b. Effect of dietary Lacto cel-con probiotic on growth performance and hematology indices of fingerlings mono-sex Nile tilapia *Oreochromis niloticus*. *Egypt J Aquat Biol Fish* 23: 227–239.
- Yones AM, Hussein MS, Metwalli AA, Lotfy AM. 2019a. Effect of different rates on growth performance, feed utilization and body composition for mixed sex Nile Tilapia *Oreochromis niloticus* Reared in Tanks. *J Ocean Fish* 11: MS.ID. 5557807.

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