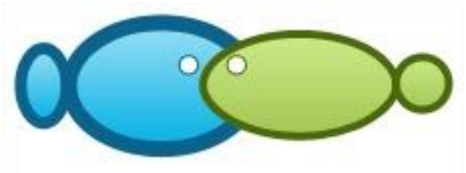

Heterotrophic biofloc as a promising system to enhance nutrients waste recycling, dry diet acceptance and intestinal health status of European eel (*Anguilla anguilla*)

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Abstract. A preliminary study was conducted to assess feeding behavior and health status of European eel *Anguilla anguilla* fed dried diets under biofloc system conditions. Two dietary protein levels were examined (T1: 35%, T2: 47%). Six circular polyethylene tanks (working capacity of 300 L) were used as experimental units, with three replicates per treatment. Each tank was stocked with ten fish with a total biomass between 572-578 g. Eels were observed to consume dry diet pellets successfully from the water surface and showed ability for floc grazing after the first month during the experiment that lasted for 66 days. High-protein level increased total suspended solids (TSS) values ($p<0.05$) and negatively altered gills morphology. Intestinal villi length, width, and absorption surface were increased ($p<0.05$) in fish fed low-protein diet. Liver antioxidant enzymes did not differ significantly between treatments. Higher crude protein (14.73%) and ash content (2.15%) were recorded for T1 ($p<0.05$). The results suggested the eel had the ability to graze floc particle aggregates without impacts to its health status when feeding on low-protein diet. Biofloc technology could be a promising sustainable system for all industrial sectors of European eel.

Key Words: antioxidant enzymes, biofloc system, intestinal histopathology, zooplankton.

Introduction. European eel (*Anguilla anguilla*) is a valuable species in the aquaculture industry because of its high economic value (Melia et al 2006). The global production of *A. anguilla* reached 6994 tonnes in 2016. Netherlands, Italy, and Denmark are the main producing countries (FAO 2020). *A. anguilla* is one of the most commercial and precious species with a wide market. However, the total production does not meet the demand of consumers (Shiraishi & Crook 2015). Dealing with eel culture problems and finding economical solutions are a must to improve productivity.

Management problems as dry feed acceptance, cannibalism, high feed costs and effluent management are the main obstacle facing eel culture. Water quality parameter values are the main indicators of the success or failure of the aquaculture process and are directly correlated with the diet quality. The crude protein requirement of eel has been suggested to range between 45-55% (Degani et al 1985) and, typically, fish carcass retains only 20–25% of the dietary protein (Avnimelech 2006), the unutilized nitrogen becoming waste and directly affecting water quality. Moreover, eel feeding behavior and feeding on wet pastes cause high feed losses that negatively impact water quality. Eel culture systems should overcome these limitations, enhance productivity, and reduce production costs. The main conventional culture systems for European eel are: the outdoor system, the greenhouse system, and the intensive recirculating system (RAS). The biofloc system (BFT) could be a proper system for culturing eels, as it is expected to overcome the culture limitations and convert the cons to pros. Nowadays, BFT has been proven as an important

mechanism to recycle non retained nutrients in fish farming units, maintain water quality, and prevent diseases (Ballester et al 2010). By adding carbohydrate sources to the farming units, heterotrophic bacterial growth is stimulated and nitrogen uptake takes place through the production of microbial proteins (Avnimelech 1999). Nitrogen uptake by heterotrophic bacteria decreases the ammonium concentration more rapidly than nitrifying bacteria (Hargreaves 2006). In addition to bacteria, bioflocs are composed of algae, aggregates of organic matter, protozoa, rotifers, microalgae, cyanobacteria, nematodes, and many others (Hargreaves 2006; Martínez-Córdova et al 2015), which may serve as a source of natural food for eels. The use of biofloc as a food source may imply a decrease in the dietary protein requirement for fish and shrimp (Azim & Little 2008; Xu et al 2012) and maximize the utilization of dietary nitrogen (Avnimelech 2006). In additions to being a tool for managing water quality and optimize feed utilization, biofloc is a hygiene system that protects the cultured organisms and limit economic losses in aquaculture (Crab et al 2010a; Khalil et al 2016; El-Husseiny et al 2017; Zidan et al 2017; El-Shafiey et al 2018; Mabroke et al 2021). Flocs turbidity in BFT may provide better growth conditions for eel by impairing cannibalism behavior. Turbidity can reduce visibility in the water column by scattering and absorption of light (Bin Omar & Bin MatJafri 2009). Culturing of European eel under biofloc systems is linked to its ability to tolerate the system conditions. Dietary protein is the main source of nitrogen for the development of heterotrophic bacteria and indirectly affects floc volume and total suspended solids (TSS) level along with the effect of the feed ration. Although biofloc may provide production benefits for eel culture, turbidity and TSS tolerance of eel must be examined to optimize the culture conditions. Turbidity tolerance may differ among species (Avnimelech 2006). Increased turbidity and TSS concentration could decrease the dissolved oxygen in the water body, augment oxygen demand, may clog the fish gills, limit the development of heterotrophic bacteria and negatively affect the performance of cultivated organisms (Hargreaves 2006; Van Wyk 2006; Hatem et al 2013). TSS values between 200 and 400 mg L^{-1} were recommended for tilapia (*Oreochromis niloticus*) culture (Avnimelech 2012), while TSS values between 400 and 600 mg L^{-1} were suitable to super intensive culture of whiteleg shrimp (*Litopenaeus vannamei*) (Schveitzer et al 2013). No data on the optimum TSS for eel culture under biofloc conditions were found. Determination of efficient dietary protein level along with the consideration of suitable TSS levels for eel culture is needed.

The main objective of the present study was to test the suitability of biofloc technology for culturing European eel, to examine feeding on dry diets with different protein levels and subsequent tolerance to the different levels of TSS. Moreover, liver oxidative enzymes, gill and intestinal histology, body chemical composition, and health status were also investigated.

Material and Method. This study was conducted at the Fish Nutrition Lab, Animal Production Department, Faculty of Agriculture, Cairo University. The study took place at the end of 2017.

Experimental fish and acclimatization period. No ethical approval was officially required for conducting animal experiment in the Faculty of Agriculture by the time our experiment was established. Two hundred European eels were brought from El-Hamoul, Kafr El-Sheikh Governorate, Egypt. The experimental fish were apparently healthy. They were carefully transferred to the laboratory. On arrival, they were acclimatized to laboratory conditions. Fish were stocked in four plastic tanks, 1 m^3 each, with a water capacity of 900 L for one month of acclimatization. The acclimatization tanks were supplemented with previously fermented biofloc (floc volume: 2-4 mL L^{-1}; TSS: 43-47 mg L^{-1}).

The acclimatization period was divided into three stages (ten days per each). Fish were fed on tilapia carcasses in the form of a paste during the first acclimatization stage. In the second stage, eels were fed on an artificial paste diet (50% crude protein). During the last stage, European eels were fed a dried diet (50% crude protein). Fish was fed twice daily at 9 am and 3 pm) for six days weekly. Four polyethylene tubes (50 cm length and 10 cm diameter) were placed inside each tank to provide an adequate resting area (Tesch

2003). Tanks were aerated by an air pump to supply needed oxygen (5 mg L^{-1}). Tanks were always covered with lids to prevent escaping.

Experimental units, design and diets. Six circular polyethylene tanks with a working water capacity of 300 L were used and set inside a greenhouse. Each tank was stocked with ten fish with a total biomass between 572-578 g (initial average body weight of 57.45 g). Continuous mechanical aeration existed in all tanks to provide dissolved oxygen (around 5 mg L^{-1}). No water exchange was performed throughout the experimental period. Underground well water was added only to sustain the water level on a weekly basis.

Two isocaloric dry diets with different crude protein levels (35 and 47%) were formulated and fed to eels to perform two treatments for 66 days. The different protein level was used as a tool to manipulate the floc volume and, subsequently, the TSS value. The formulation and proximate composition of the experimental diets are presented in Table 1.

Table 1

Ingredients	*T1 (% in feed)*	*T2 (% in feed)*
Fish meal[a]	40	50
Gluten	9	26
Soybean meal[b]	7.5	1
Corn[c]	23	2.5
Fish oil[d]	12	12
Carboxy methyl cellulose[e]	6	6
Vitamin C[e]	0.5	0.5
BHT[e]	0.05	0.05
Premix[f]	1.45	1.45
Salt	0.5	0.5
Total	100	100
Proximate analysis		
Moisture	6	6.95
Crude protein	35.78	47.59
Lipid	16.32	17.51
Ash	10.17	10
Total carbohydrates[g]	31.73	17.95
Gross energy (Kcal $100g^{-1}$)[h]	487.858	507.364

Note: T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein; BHT - Butylated Hydroxytoluene; a - local fishmeal (62% crude protein); b - Soy Factory. Food Technology Research Institute, Ministry of Agriculture, Giza, Egypt; c - imported yellow corn from Argentina; d - imported fish oil form India; e - Algomhuria Pharmaceutical Chemical Co. Cairo, Egypt; f - retinyl acetate per kg of diet, 3000 IU; cholecalciferol, 2400 IU; all-rac-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49% ascorbic acid), 120 mg; cyanocobalamine, 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine, HCl, 6 mg; riboflavin, 7.2 mg; thiamine HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3.077 mg; ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 20% Fe), 65 mg; manganese sulfate ($MnSO_4$, 36% Mn), 89 mg; zinc sulfate ($ZnSO_4 \cdot 7H_2O$, 40% Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1000 mg Agri-Vet Co., Cairo, Egypt; g - total carbohydrate [100-(CP+EE+Ash)]; h - gross energy calculated using the factors (5.6, 9.45 and 4.2 Kcal per g of crude protein, ether extract and carbohydrate, respectively) as listed by Young et al (2005).

Diets were prepared using locally available feed ingredients and pelleted using a house meat grinder. 6% carboxymethyl cellulose (CMC) was added during diet formulation to enhance the water stability of the pellets and facilitate diets traceability during feeding, in the gut and wastes. Siphoning was performed at the end of week 6 to control the organic matter, as well as the checking for the presence of dried diet particles.

The eels were fed until satiation during the first three days of the experiments. Experimental diets were administered to fish twice daily (at 9 am and 3 pm) in the form of crumble. A fixed feeding ration of 1.3% of the total stocked biomass was calculated and

was adjusted biweekly according to fish total biomass. The same feeding rate was recommended by Kafuku & Ikenoue (1983). Water C:N ratio was 10:1 (De Schryver et al 2008) was maintained in BFT tanks using corn starch. Corn starch was completely mixed with tank water and spread to the tank surfaces in the afternoon. Fish were checked for any skin or fin damage, and the body length and body weight were measured biweekly.

Physical and chemical water parameters. Water temperature and dissolved oxygen (DO) were detected using Senso Direct Oxi 200, while pH was measured using MilwaukeePH600 Digital pH meter tester pocket pen every three days. Total ammonia nitrogen (TAN) and nitrite (NO_2-N) values were detected using a water analysis photometer (Multi Direct Lovibond) once a week. Biofloc volume was reported using the Imhoff cone and the volume of floc on the bottom of the cone was measured after 15 minutes of sedimentation twice a week (Avnimelech 2009). Alkalinity was monitored once a week by titration with sulfuric acid, until the pH reached 4.5 (APHA 1998). TSS were monitored twice a week by a water analysis photometer (Multi Direct Lovibond).

Microbial count of water biofloc samples. 600 mL plastic bottles were used to collect water samples up to 2/3 from the experimental units and were stored in a freezer (-20°C). Bacterial count and detection for the bacterial groups in the water samples included the following: *Lactobacillus* spp. (NMKL 2007), *Streptococcus* spp. (NMKL 2011), *Bacillus* spp. (Nikiforova et al 2016), *Staphylococcus* spp. (NMKL 2009), total yeast count (NMKL 2005), *Serratia* spp. (Mendpara et al 2013) and *Vibrio parahaemolyticus* (Applied biosystem PCR AB7000). The latter analyses were performed in the regional center for food and feeds, Agricultural Research Center (ARC), Egypt.

Collection of zooplankton samples. Zooplankton samples were collected from different experimental units using zooplankton net (55 µm, 25 cm diameter, and 80 cm length). The water was agitated and 3 L of water sample was filtered through the zooplankton net. After filtration, the samples were fixed immediately using a formaldehyde solution (4-7%). After fixation, 2 mL of Rose Bengal stain (0.5 %) was added.

Analysis of samples. In the laboratory, the filtrated samples were examined with an optic research microscope. Three sub-samples (1 mL for each) of the homogenized plankton samples were transferred to a counting cell and the different plankters were counted. Zooplankton population was calculated as the number of individuals of the different species per cubic meter. The organisms were identified and counted on the counting try with magnification varying from 100X to 400X. Planktonic organisms were identified according to description and keys constructed by Pennak (1989), Edmondson & Ward (1959), Jeje (1988) and Fernando (1994).

Zooplankton calculation. Zooplankton was calculated after examination for all the recorded species in each sample and expressed as organisms per liter depending on the following equation according to APHA (2005).

No. of organisms per liter = N*D/S*C

Where: N -number of organisms for the calculated species; D - volume of the sample after filtration; S -number of subsamples; C -total volume of the collected water sample.

Proximate composition of fish and diets. The proximate composition of the whole body of fish and diets were determined according to AOAC (1995). Fish samples and diets were dried in an oven at 80°C until constant weight was obtained, then grounded, and stored at –20°C for subsequent analysis. Nitrogen content was estimated by the Kjeldahl method and crude protein level was calculated by multiplying the nitrogen percentage by 6.25. Crude lipid was determined by Soxhlet extraction with ether (boiling point at 40-60°C) as a solvent. Ash was determined by incineration at 550°C for 4 h (Azim & Little 2008).

Antioxidants and oxidative biomarker of eel liver. Liver lipid peroxidation (LPO) was examined by a colorimetric method (Satoh 1978). A thiobarbituric acid reactive substance (TBARS) was used for the assessment of LPO and expressed in terms of malondialdehyde (MDA) content. For this purpose, 0.5 mL of trichloroacetic acid (10%) solution was added into a 0.5 mL sample in a test tube. The mixture was centrifuged at 600 × g for 10 min and 0.2 mL of the supernatant was transferred into a new test tube containing 1 mL of TBA (25 mmol L^{-1}) solution and was boiled for 30 min. The solution was then cooled and samples and standards were measured at a wave length of 534 nm using a spectrophotometer. The MDA values were expressed as nmol of MDA g^{-1} tissue.

Superoxide dismutase (SOD) was determined spectrophotometrically at 560 nm according to the method of Nishikimi et al (1972). The method is based on the ability of the SOD enzyme to inhibit the phenazinemethosulphate-mediated reduction of nitro blue tetrazolium dye. Briefly, 0.05 mL of sample was mixed with 1.0 mL buffer (pH 8.5), 0.1 mL nitro blue tetrazolium (NBT) and 0.1 mL NADH. The reaction was initiated by adding 0.01 mL phenazinemethosulphate (PMs) and then the increase in absorbance was read at 560 nm for 5 min. SOD activity was expressed as units per g of protein.

Liver total antioxidant capacity (TAC) was assayed by the method of Koracevic et al (2001). The determination of the antioxidative capacity is performed by adding a defined amount of exogenous hydrogen peroxide (H_2O_2). The added hydrogen peroxide reacts with the sample antioxidants and the residuals of H_2O_2 were then determined. The residual H_2O_2 was determined colorimetrically by an enzymatic reaction which evolves the conversion of 3,5,dichloro-2-hydroxy benzene sulphonate to a colored product. The TAC values were expressed as mM g^{-1} tissue. Livers were also weighted to determine the hepatosomatic index (HIS):

HSI=(liver weight/body weight)x100

Histopathological examination of eel gills and guts. At the end of the experiment, two fish in each BFT treatment unit were sacrificed. Tissue samples from gills and guts were fixed in 10% neutral buffered formalin. Then they were processed by paraffin embedding technique. Tissue sections of 3-4 µm were made using a microtome (Leica2135, Germany) and were stained by hematoxylin and eosin stain. Images were captured using a digital camera (Olympus XC30, Tokyo, Japan). Histomorphometry of intestinal villi length, width and crypt depth was performed using the image analyzer (Image J software) in five captured images per fish at magnification 40X to calculate a mean for each fish. The measurements from each group were then analyzed statistically.

Statistical analysis. All statistics were performed using SPSS Statistics 18.0. Data were analyzed using independent-samples t-test to determine whether there were significant differences between treatments. The level of significance was chosen at $p<0.05$, and the results were presented as the mean±SEM (standard error of means).

Results and Discussion

Water quality parameters. The recorded water quality parameters are presented in Table 2. Water quality parameters were within the optimal range for the culture of European eel. The DO was kept above 5 mg L^{-1} for the two groups. The average DO level was 5.30 for T1 and 5.31 mg L^{-1} for T2. The average water temperature in T1 and T2 was 26.44 and 26.45°C ($p>0.05$), respectively, while the pH value stayed 8.1, with small variations. TAN and NO_2-N values were within the normal ranges (being 0.46 and 0.1 mg L^{-1}, respectively). Floc volume (FV) values for T1 and T2 did not differ significantly, (28.66 and 33 mL L^{-1}, respectively). The high protein-diet showed a higher TSS value ($p<0.05$) than T2 (179.53 and 213.33 mg L^{-1}, respectively). Periodical FV (Figure 1) did not differ significantly between treatments, but a sudden drop was observed after the 6^{th} and 8^{th} weeks in both treatments. Periodical TSS values (Figure 2) showed no significant differences until the 7^{th} week of the experiment. Starting with week 8 to the end of the experiments, the TSS value increased gradually and differed significantly between

treatments. The average concentration of alkalinity was approximately 349.31 mg $CaCO_3$ L^{-1}.

Table 2

Water quality parameters of different experimental groups (mean±SEM)

Water quality parameters	*T1*	*T2*	*T value*	*P value*
Floc volume (mL L^{-1})	28.66±1.76	33.00± 3.51	-1.1	0.35
TSS(mg L^{-1})	179.53±5.54^b	213.33±8.23^a	-3.41	0.03
DO(mg L^{-1})	5.3±1	5.31±8.82	-0.5	0.64
Temperature (°C)	26.44±8.82	26.45±3.33	-1.06	0.37
pH	8.07±3.33^b	8.1±3.33^a	-4.95	0.001
TAN (mg L^{-1})	0.46±0.03	0.46±0.02	-0.15	0.88
Nitrite (mg L^{-1})	0.08±6.67^b	0.13±5.77^a	-4.91	0.001
Alkalinity (mg $CaCO_3$ L^{-1})	351.35±1.54	347.27±2.45	1.4	0.24

Note: TSS - total suspended solids; DO - dissolved oxygen; TAN - total ammonia nitrogen; means in the same row with different superscripts are significantly different ($p<0.05$); T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

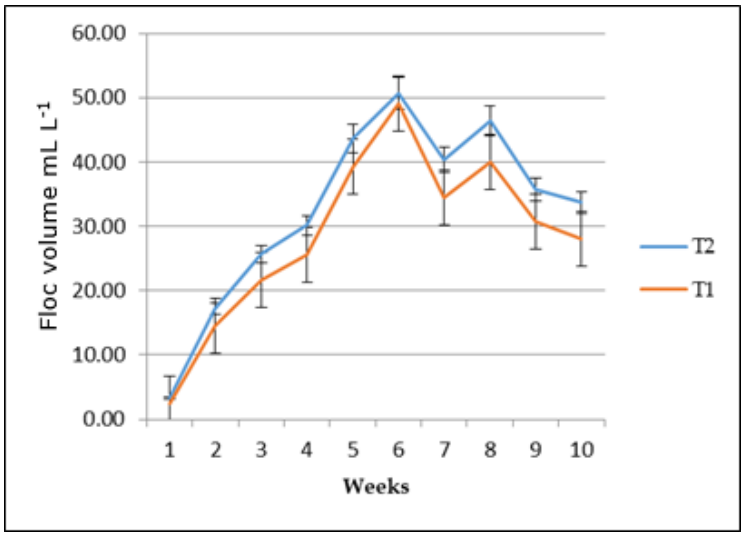

Figure 1. Periodical biofloc volume of experimental groups during the experiment. T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

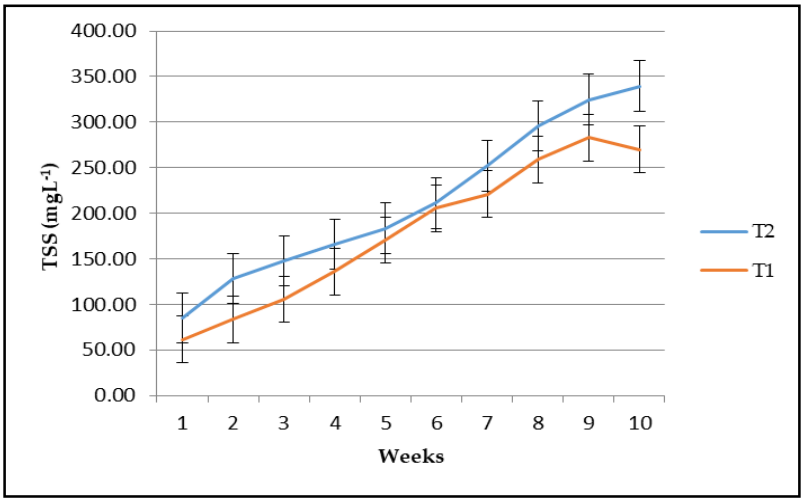

Figure 2. Periodical TSS of experimental groups during the experiment. T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

Microbial count of biofloc water samples. The microbial count of water samples is presented in Table 3. Water samples generally showed no significant difference between treatments regarding counts of detected microbial organisms *Lactobacillus* spp. and *Staphylococcus* spp. *Streptococcus* spp., *Bacillus* spp., total yeast count, *Serratia* spp., and *Vibrio* spp. were not detected. A numerical increasing pattern in beneficial bacteria like *Lactobacillus* spp. was noticed in parallel with increasing dietary protein level. *Vibrio* spp. were not detected in the water samples.

Table 3

Bacterial count of biofloc water samples

Bacteria	*T1*	*T2*	*T value*	*P value*
Lactobacillus spp. $\geq 10^2$ cfu g^{-1}	9.33±0.33	26.66±0.88	-1.96	0.18
Streptococcus spp.	ND	ND		
Bacillus spp.	ND	ND		
Staphylococcus spp. $\geq 10^1$ cfu g^{-1}	6.5±0.86	12.33±5.2	-1.11	0.37
Total yeast count	ND	ND		
Serratia spp.	ND	ND		
Vibrio spp. (PCR)	ND	ND		

Note: ND - not detected; means in the same row with different superscripts are significantly different ($p<0.05$); T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

Biofloc zooplankton community. The zooplankton distributions in different experimental biofloc treatments were detected at the end of the experimental period and the results are represented in Table 4. Total zooplankton count showed a higher value in the high protein diet treatment. The same pattern was noticed for all species, except for class Ostracoda. Elevation of the protein level resulted in a decrease of Ostracoda by 10% in comparison with tanks with the low protein-diet. It seems that different protein levels may had caused different amino acid profiles and availability and led to different balances among organisms in the floc community. Rotifers appeared to be the most abundant biofloc microorganisms, with 53.25% and 48.80% in T1 and T2, respectively. They were followed by both protozoa (T1: 21.24%; T2: 22.3%) and nematodes (T1:18.7%; T2: 23.12%).

Table 4

The most dominant biofloc zooplankton community of the experimental groups (organisms L^{-1} presented as mean±SEM)

Zooplankton	*T1*		*T2*	
	Average	*%*	*Average*	*%*
Rotifers				
Lecane bulla	30777±7139.6	43.9	31977±13931	40.37
Philodina spp.	6555±3422.8	9.35	6677±3178.8	8.43
Total rotifers	37333±8838	53.25	38655±10763	48.8
Protozoa				
Arcella spp.	889±484.41	1.27	1000±192.26	1.26
Centropyixus spp.	9000±5500.8	12.84	11333±4765	14.31
Vorticella spp.	5000±1539.8	7.13	5333±2603.4	6.73
Total protozoa	14889±7286.3	21.24	17667±2404	22.3
Ostracoda	2889±1889	4.12	2600±896.91	3.28
Oligochaeta	1889±728.54	2.69	1982±1660	2.5
Nematoda	13111±8620.1	18.7	18311±4893.6	23.12
Total zooplankton	70111±24424	100	79215±12981	100

Note: T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

Chemical composition of eel carcass. Table 5 reveals the effect of different dietary protein levels on the body chemical composition of European eel. Eel fed high protein diet had a higher content of dry matter (41.34%) and ether extract (25.02%) than fish fed low

protein diet, with 39.42% and 20.80%, respectively. However, the differences are not significant (p>0.05).The low protein diet treatment had higher crude protein (14.73%) and ash content (2.15%) (p<0.05) compared to those fed a high protein diet, with 13.11% and 1.49%, respectively.

Table 5

Effect of dietary protein level on body chemical composition of European eel (% of wet basis)

Parameter	*T1*	*T2*	*T value*	*P value*
Dry matter (%)	39.42±3.46	41.34±1.51	-0.51	0.65
Crude protein (%)	14.73±0.3[a]	13.11±0.28[b]	3.87	0.01
Lipid (%)	20.8±3.68	25.02±1.02	-1.1	0.37
Ash (%)	2.15±0.03[a]	1.49±0.15[b]	4.17	0.04

Note: means in the same row with different superscripts are significantly different (p<0.05); T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

Eel feeding behavior under BFT. Eels consumed dry diet pellets successfully from the water surface. Eel behavior did not change with different dietary protein levels. No pellets were noticed in the water body during siphoning by the end of the experiment, but were recognized in the eel digestive tract while dissecting. Eels showed the ability to graze floc particles, as showed for T1 and T2 after the weekly fasting day (Figure 1).

Eel health status. The cumulative mortality of eels in the current study is presented in Figure 3. T2 had a higher mortality than T1.

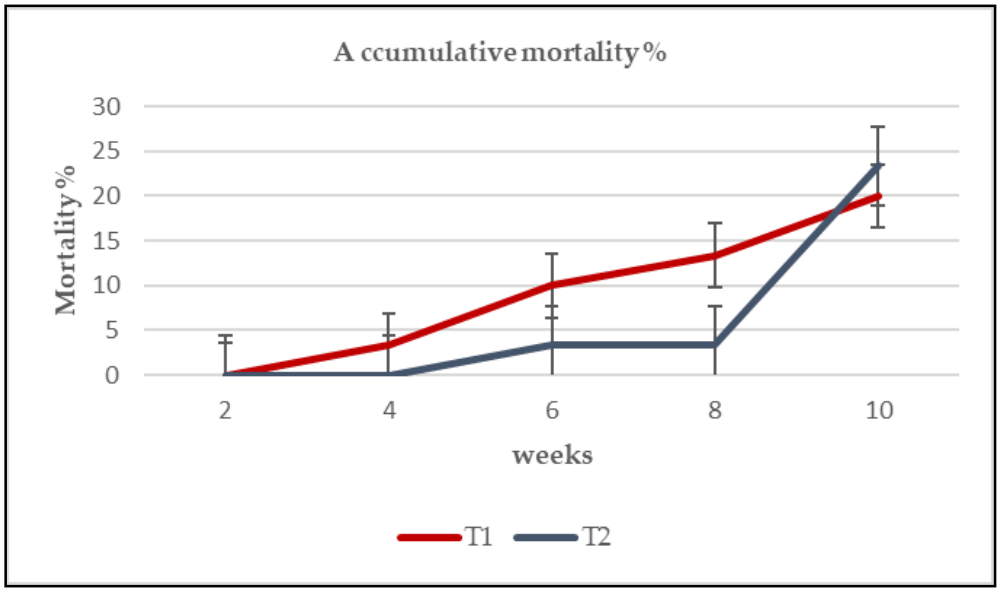

Figure 3. Accumulative mortality % during experimental period. T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

The antioxidant enzymes activity and oxidative biomarkers of different experimental groups are presented in Table 6. The malondialdehyde (MDA) level was not significantly different between the two groups. T2 had higher MDA values of MDA (7.51 nmol g^{-1} tissue) than T1 (5.96 nmol g^{-1} tissue), but the difference was not significant (p>0.05).

Table 6

Antioxidant enzymes of different experimental groups (mean±SEM)

Antioxidant biomarker	*T1*	*T2*	*T value*	*P-value*
MDA (nmol g^{-1} tissue)	5.96± 0.99	7.51±0.30	-1.49	0.25
SOD (units g^{-1})	65.97±8.44	66.25±12.32	-0.02	0.98
TAC (mM g^{-1} tissue)	3.96±0.06	3.56±0.36	1.05	0.39
HSI(%)	1.50± 0.08	1.39± 0.05	1.10	0.33

Note: HSI - hepatosomatic index; SOD - superoxide dismutase; TAC - total antioxidant capacity; means in the same row with different superscripts are significantly different ($p<0.05$); T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

High levels of dietary fish oil (12%) in the current study did not stimulate the elevation of MDA values (5.96 and 7.51 nmol g^{-1} tissue) in eel. The concentrations of antioxidant enzymes (SOD) did significantly differ between treatments, but an increase was noticed in parallel with the increasing protein level. T2 exhibited a higher value for SOD activity compared to T1 (78.58±0.08 and 58.33±6.25 units g^{-1}, respectively). TAC levels did not significantly differ between T1 (3.96 mM g^{-1} tissue) and T2 (3.56 mM g^{-1} tissue) ($p>0.05$). The mean value of the HSI of eel ranged from 1.39 to 1.5 (Table 6). No significant differences were noted between the treatments regarding HSI.

Histopathological examination of eel gills and guts. By the end of the experiment and while dissecting, sampled fish from different tanks showed infection with *Anguillicoloides crassus* parasites. This was not recognized by the general examination of the eel at the beginning and during the experiment.

The gills exhibited mild mucous cells hyperplasia and a slight curling of the second-gill lamellae (Figure 4a) in T1. In T2, the gills of fish had moderate mucous cells hyperplasia and a curling of the primary gill lamellae (Figure 4b). Therefore, the alteration in gill morphology was seen in the group receiving a high protein diet. In the current study, the intestine showed a normal histological structure with long intestinal villi in T1 (Figure 4c) and had a normal histological structure with moderately long villi in T2 (Figure 4d). There was a significant increase in the length, width, and absorption surface of intestinal villi in T1 compared to T2 (Table 7).

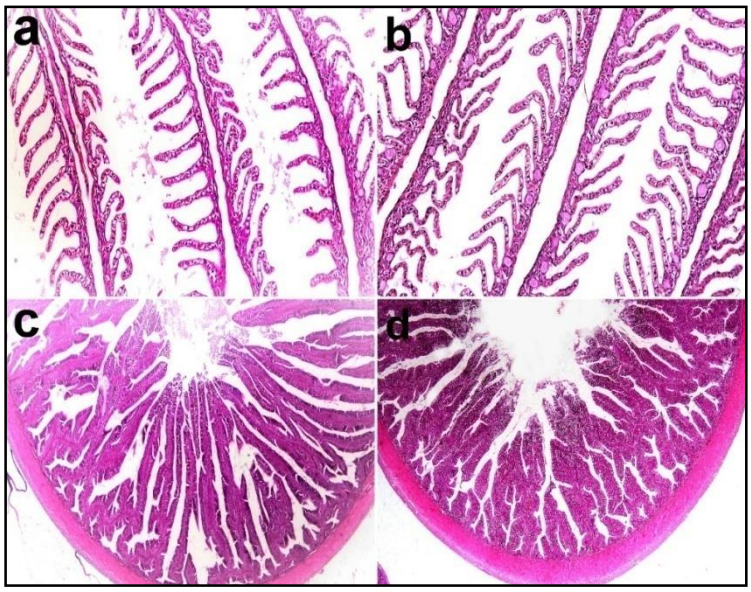

Figure 4. Histopathology of gills and intestine of eel; a - mild mucous cells hyperplasia and slight curling of the second-gill lamellae in T1; b - moderate mucous cells hyperplasia and curling of primary gill lamellae in T2 (H and E stain, X200); c - normal histological structure of the intestine with long intestinal villi in T1; d - normal histological structure of intestine with moderately long villi in T2 (H and E stain, X40).

Table 7
Length, width, crypt depth, length/crypt depth, and absorbance surface of intestinal villi in the group receiving 35% protein diet and 47% protein diet

Measurement	*T1*	*T2*	*P value*
Length (µm)	814.96±22.03[a]	563.1281±22.96 [b]	1.742E-10
Width (µm)	84.44±4.16	82.0256±4.12515	0.68
Crypt depth (µm)	47.03±3.86[a]	38.55±1.63[b]	0.04
Length/Crypt depth (µm)	19.63±1.27[a]	15.2756±0.93[b]	0.008
Absorption surface (mm)	69.25±4190.45[a]	47.02±3484.54[b]	0.001

Note: T1 - treatment with 37% crude protein; T2 - treatment with 47% crude protein.

The biofloc system afforded a good water quality for eel culture (Degani et al 1985; Fekri et al 2018) and limited the water exchange needs during our experimental period. A DO concentration of 4 ppm or above, ammonia concentration of 1-2 ppm, pH of 6.8-7.9, and temperatures between 24 and 30°C are optimal for eel culture. Supplementing experimental tanks with organic carbon sources enhanced the heterotrophic bacterial growth and subsequently increased nitrogen fixation and decreased ammonia and nitrite (Avnimelech 1999; Ekasari & Maryam 2012).

Eels consumed dry diet pellets successfully from the water surface and were recognized in the digestive tract while dissecting. Eel showed ability to graze biofloc particles as the reduction in interval FV values was recorded. The gradual increase in FV for the first 6 weeks could illustrate the adaption period of eel for grazing flocs. Paste feed is the typical form of commercial diets used in the eel farming industry (De la Higuera et al 1989). Dealing with dry feed is easier than with wet feed, because of depositing complications. Nowadays, due to useful characteristics, dry diets are usually replaced by wet paste. Several authors used dry diet to feed European eel (*Anguilla anguilla*) during their studies (Degani et al 1985; De la Higuera et al 1989).

The low protein diet (35% crude protein) seems to be efficient for European eel culture under biofloc conditions. No severe alterations were noticed in gill histology, while the gut health condition (increase in intestinal villi length, width, and absorption surface) was better compared to that from T2 ($p<0.05$). The high-protein diet (47%) resulted in higher TSS values that may negatively affect gill histology, but no effect on liver oxidative stress levels was noticed. Gills morphology is affected by the quality of water and feed (Mohammed et al 2020). Similarly, in a previous study on grass carp (*Ctenopharyngodon idella*), gill physical barrier function improved at the optimal level of dietary protein (Xu et al 2016). Different types of feed affect the length of the villi, especially the protein concentration in Indonesian shortfin eel (*Anguilla bicolor)* (Ratucoreh & Retnoaji 2018).

Elevation of dietary protein up to 47% did not improve the protein content of eel carcass, instead, more lipid deposition ($p>0.05$) was noted. The unutilized amino acid was catabolized into ammonia. Wasted nitrogen was successfully recycled in our study, as rich zooplankton communities were detected (rotifers: *Lecanebulla*, *Philodina* spp.; protozoa: *Arcella* spp., *Centropyixus* spp., *Vorticella* spp.; Ostracoda, Oligochaeta and Nematoda). Biofloc particles including zooplankton provide important nutrients such as proteins, lipids, carbohydrates and vitamin C that represent a considerable fraction of the nutritional requirements of several aquaculture species (Tacon et al 2002; Crab et al 2010b). Nematodes are an important group in the biofloc systems because of its nutritional value (48.3% crude protein, 17.3% fat, 31.3% carbohydrates of dry weight (Biedenbach et al 1989; Focken et al 2006). Generally, it was suggested by Ekasari et al (2014) that biofloc is rich in valine, lysine, leucine, phenylalanine, and threonine, deficient in methionine and contains no cysteine. Carbohydrate levels of biofloc particles could reach 36.4-50% (Kuhn et al 2009; Crab et al 2010b; Mabroke et al 2019). A high percentage of carbohydrates in lower protein diets can influence hypothalamus appetite control centers, and, subsequently, affect the feed intake (Martin & Garcia Gallego 1987). European eels showed a more efficient adapted digestive enzyme system to high carbohydrates in diet than rainbow trout (*Oncorhynchus mykiss*) (Bulnheim 1974; Garcia-Gallego et al 1995). Garcia-Gallego et al (1994) state that eels have a high ability to utilize dietary carbohydrate levels

up to 40%. This suggests the effective utilization of biofloc carbohydrates by the eel in our study. Based on our results, the dietary protein level of 35% is sufficient under the conditions of bilofloc, which afford more economical culture conditions for eel. Under the condition of clear water, it was suggested that the dietary protein requirement for eel was 45% (Degani et al 1985), while 30% protein was suggested (casein as a protein source) by Arai et al (1986).

It seems that biofloc conditions moderate the harmful effect of high-protein diet (47%) and elevation of TSS values, as liver oxidative stress and survival rate were not influenced. The last could be explained by the fact that biofloc particles were not only a supplemental nutrition source (protein, lipid, mineral, and vitamin) for the growth of cultured fish, but also a source of abundant natural microbes and bioactive compounds (antioxidant, carotenoids, and fat-soluble vitamins) (Ju et al 2008a, 2008b). Carotenoids have been reported to improve animal immune systems (Liñán-Cabello et al 2002; Babin et al 2010; Xu et al 2013) and perform an antioxidant function. The enhancement of the immune system response and antioxidant capabilities could positively affect health status (Xu & Pan 2014). The antioxidant enzyme levels in our study were obtained under a low level of TSS that did not exceed 400 mg L^{-1}. Further studies should be conducted to determine the ideal TSS concentration for growing eel under BFT.

BFT conditions also limited the proliferation of *Vibrio* in our experiments. Thus, it might have minimized the risk of infection, as suggested by Cardona et al (2016). Biofloc may display biocontrol activity against *Vibrio harveyi*, by a cell-to-cell communication called quorum sensing (Defoirdt et al 2008; Crab et al 2010a). On the other hand, beneficial bacteria such as *Lactobacillus* spp. was recognized in our work. The bacteria may reduce the pathogenic bacteria growth, lead to healthier gut conditions and better survival rates, as reported by Crab et al (2010a). A lower survival rate of juvenile *L. vannamei* that was linked with high dietary protein level under biofloc conditions was documented (Xu & Pan 2014), which in accordance with our results for eel. Some studies suggested that using low protein feeds in biofloc-based culture systems could achieve good survival of cultured shrimp (Wasielesky et al 2006; Ballester et al 2010; Megahed 2010). Gaona et al (2015) revealed that high TSS levels affected negatively the survival rate of *L. vannamei.*

Conclusions. The present study revealed that European eel (*A. anguilla*) has the ability to feed on dried diets under a biofloc system. Cultivation of eel under biofloc may be considered as a promising approach to the better sustainability of eel production sectors due to its advantages: lower cost of feed, water treatment and waste recycling. A dietary protein level of 35% was adequate for cultured eel and sustained a positive health status under the condition of biofloc. Further studies are required to detect the optimal dietary protein level and TSS for eel optimal growth performance and feed utilization in the presence of biofloc.

Conflict of Interest. The authors declare that there is no conflict of interest.

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