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Impact of dietary protein levels and feeding regimes on growth performance and biochemical profile of European sea bass (*Dicentrarchus labrax*) reared in a brackish water recirculating aquaculture system

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ABSTRACT

A 16-week trial was conducted to assess the effects of dietary protein levels and feeding regimes on the growth performance and biochemical profile of European sea bass (*Dicentrarchus labrax*), initial weight 56 ± 1.0 g reared under brackish water conditions (7 ppt) in a recirculating aquaculture system. The experiment was designed as a completely randomized layout of six duplicated treatments: T1 (42 % protein, fed once daily), T2 (42 % protein, fed twice daily), T3 (42 % protein, fed thrice daily), T4 (44 % protein, fed once daily), T5 (44 % protein, fed twice daily), and T6 (44 % protein, fed thrice daily). Our results indicated improved growth performance regarding the feed conversion ratio and specific growth rate in T2 compared to the other treatments. Weight gain was significantly higher in T2, T4, T5, and T6 than in T1 and T3. Additionally, T2 and T4 achieved the highest survival percentages compared to the other treatments. For fish health assessment, our findings revealed significantly lower metabolic enzyme activity of alanine transaminase in T2 and T4 compared to the different treatments, and alkaline phosphatase activity in T2 and T3 compared to T4, T5, and T6. However, T1 recorded significantly the lowest values for aspartate transaminase activity. Regarding kidney function, significantly lower and higher values for glucose, uric acid, and hemoglobin, respectively, were noted in T2. In conclusion, feeding *Dicentrarchus labrax* with 42 % protein twice daily is an optimal feeding strategy for enhancing its growth performance and health status in brackish water (7 ppt) conditions within a recirculating aquaculture system.

1. Introduction

Recirculating Aquaculture Systems (RAS) have emerged as an innovative and highly efficient fish production technique and a key area of focus for aquaculture research, aiming to meet the increasing global protein demands (Gupta et al., 2024). As an intensive aquaculture technique, feeding in RAS is a crucial component, representing almost 60 % of the overall production expenses (Hossain et al., 2024). The goal of optimal feed management is to avoid overfeeding, which leads to a decrease in feed costs, increases productivity by enhancing growth and conversion efficiency, and reduces waste accumulation, which maintains water quality (Abdel-Aziz et al., 2021a). In addition, different feeding management practices can improve water quality and promote an increase in production efficiency through compensatory growth (Nebo et al., 2018; Roa et al., 2019). However, when implementing any feed management practice, it is important to consider the individual

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nutritional requirements of the reared species (Laczynska et al., 2020). Furthermore, to fulfil the dietary nutritional requirements of fish and improve their growth performance, it is essential to determine the ideal feeding rate, feeding frequency, feed ingredients, and nutritional content of the feed (Habib-Ul-Hassan et al., 2022a). As such, extensive research has been done to examine the optimum protein requirements of various fish species, as demonstrated by Basto et al. (2020) in European sea bass (*Dicentrarchus labrax*), Liu et al. (2023) in largemouth bass (*Micropterus salmoides*), Cai et al. (2020) in spotted seabass (*Dicentrarchus punctatus*), and Nguyen et al. (2021) in Nile tilapia (*Oreochromis niloticus*).

The optimum dietary protein requirements of fish are influenced by the interaction of several factors. These factors include the species and size, frequency of feeding, amount of non-protein energy sources, protein quality (digestibility and amino acid balance), and environmental conditions such as water temperature, pH, dissolved oxygen, ammonia, nitrite, and nitrate concentrations in rearing water (Cai et al., 2020). Most of the research on the protein needs of sea bass has been done on juveniles, which estimated that the optimum requirements for sea bass growth are between 40 % and 50 % protein (Dias et al., 2003a, 2003b; Oliva-Teles, 2000; Ballestrazzi et al., 1994). Another study done by Habib-Ul-Hassan et al. (2022b) demonstrated that the optimum protein levels are between 45 % and 45 % protein. According to Hassan et al. (2021). Accurate feeding frequency (FF) reduces size variance and aggressive social behavior between sea bass. Additionally, it increases the effectiveness of the fish's digestive system and minimizes competition for nutrition. Some researchers demonstrate that the easy FF improves feed utilization and accelerates fish growth, for example, *Oreochromis niloticus* (Daudpota et al., 2016a) and Asian seabass (*Lates calcarifer*) (Hassan et al., 2021).

RAS is known to maintain better water quality due to its water recirculation and purification in several compartments, thus permitting wastewater reuse while increasing production (Modeel et al., 2024). Because of its advantages over traditional aquaculture production systems, which include requiring less water and land, controlling water quality, dealing with erratic environmental conditions, applying good food practices, minimizing the seasonality effect, lowering the risk of disease infection, and providing high-quality fish production (Oliva-Teles, 2000). An indoor RAS was chosen in this trial for the intensive production of European seabass (*D. labrax*).

European sea bass (*D. labrax*) is one of the most reared marine finfish species in Europe and the Mediterranean region (Rigos et al., 2021). However, mortality and disease outbreaks have negatively impacted the production of these species, resulting in lower yields (Montero et al., 2023). These issues may be linked to several factors, such as poor feeding management practices, water quality parameters, and salinity. Feeding practices, specifically the feeding frequency (FF), if handled well, can, however, improve growth and reduce feeding costs by boosting feed efficiency and growth by minimizing the amount of uneaten feed, leaching of feed ingredients into water, and deterioration of water quality. Reduced feed frequency causes an increase in food quantity and uneaten feed follow-up, which raises the feed conversion ratio (FCR) (Espinoza-Ortega et al., 2024). Numerous studies have assessed the significance of FF in enhancing the FCR and the specific growth rate (SGR) under various rearing conditions. Abdel-Aziz et al., 2021b and Abutalb (Abutalb, 2023) mentioned that, as fish may control their feed intake to meet their needs, SGR and FCR should increase. If FF reaches maximal feed utilization, then there will be a strong negative correlation between SGR and FCR. Furthermore, studies on tilapia (*Oreochromis niloticus*) and carp fish (*Cyprinus* sp.), respectively, showed that SGR increases with increased FF, while FCR did not (Daudpota et al., 2016a).

The objective of this study, therefore, is to investigate the effects of feed quality (dietary protein levels) and feeding frequency on the growth performance, survival, and health status of European sea bass (*Dicentrarchus labrax*) reared in a brackish water-recirculating aquaculture system. This study's results will greatly benefit aquaculture farmers without direct access to the sea and those in regions with rich sources of brackish water.

2. Materials and methods

2.1. Fish acclimation

European sea bass (*Dicentrarchus labrax*) was obtained from a governmental hatchery (Marine 21 hatchery) in Alexandria governorate and carefully transported to the Center for Applied Research on the Environment and Sustainability (CARES), The American University in Cairo (AUC). Before transportation, the fish were starved for 24 h to avoid any stress during transportation. Fish were carefully transferred to well-aerated plastic containers (1000 L) filled with seawater of salinity level 35 ppt. The transportation was done in the early morning to avoid heat stress. Upon reaching the CARES facility, all fish were transferred to other 1000 L tanks in a Recirculating Aquaculture System (RAS) and fed on a commercial feed with 40 % protein for 45 days to reach acclimation. During the acclimation period, the salinity level gradually decreased by adding fresh water until it reached brackish water salinities of 7 ppt.

2.2. Experiment design

After acclimation, fish were redistributed into 6 groups/treatments of 1200 healthy European seabass (initial weight 56 ± 1.0 g) at a stocking density of 100 fish/m^3. The treatments were arranged in a completely randomized design in duplicates as follows. T1(42 % protein, fed once/day), T2 (42 % protein, fed twice/day), T3 (42 % protein, fed thrice/day), T4 (44 % protein, fed once/day), T5 (44 % protein, fed twice/day), and T6 (44 % protein, fed thrice/day). Fish were fed until satiation on pellets purchased from the Skretting Egypt company. The water in the RAS was running at a flow rate of 2 L/min, measured and maintained automatically by the sensors every minute. The water temperature, pH, dissolved oxygen, and salinity levels were maintained at 25 ± 1 °C, 7–8, 7 mg/ L, and 7 ppt, respectively. For the total ammonia monitoring, the HANNA Aquaculture photometer (HI83303) was used, which showed an optimal level of < 0.05 mg/L.

2.3. Experimental termination and growth performance

At the end of the trial (110 days), all fish were starved for 24 h, and then the final weight and number were taken to calculate growth performance [i.e., weight gain (WG), specific growth rate (SGR), feed intake (FI), daily feed intake (DFI), and feed conversion ratio (FCR)] and survival rate as follows:

1. WG (g) = [final body weight, FBW (g) – initial weight, IBW (g)]/ IBW (g)
2. SGR (%/day) = (ln FBW – ln IBW) / duration (60 days) × 100
3. FCR = FI (g)/ WG (g)
4. Survival (%) = 100 × [final fish number/initial fish number]

2.4. Feed economic efficacy

To estimate the economic value for every treatment, follow the equation:

Feed cost with Egyptian Pound (EGP) =

5. The cost of one kg of each diet X the amount of total feed intake (kg) during the experimental period (110 days).
6. Feed cost/kg gain with Egyptian Pound (EGP) =Total feed cost/Total weight gain (kg).

2.5. Blood sampling and biochemical analysis

The fish were anesthetized with 50 ppm tricaine methane sulfonate (MS-222) according to Topic Popovic et al. (2012). Two fish per replicate were sampled, and blood was obtained from the caudal vein using a syringe and stored in a refrigerator for 3 – 4 h until a serum layer was formed. The samples were centrifuged at 3000 rpm for 10 min at 4°C, and the supernatant (serum) was purely isolated and stored at −20°C until further analysis (Dong et al., 2017). Biochemical analysis of liver function (i.e., alanine transaminase "ALT", aspartate transaminase "AST", alkaline phosphatase "ALP", total Bilirubin, and Albumin) and kidney function (i.e., urea, creatinine, and uric acid), as well as total protein, glucose, and hemoglobin, were conducted using biochemical kits purchased from Spectrum company, and the samples were prepared following Spectrum protocol, then measured in triplicates using the ELIZA device (Tecan infinite200pro).

2.6. Statistical analysis

Data was analyzed statistically and visualized using GraphPad Prism software package version 8.4.2. Data sets were subjected to normality and equality of variances tests using the Shapiro-Wilk and Bartlett's tests, respectively. A two-way analysis of variance (ANOVA) was performed, along with a Tukey post hoc test to determine the significance of the results. The significance of the results obtained was judged at the 5 % level.

3. Results

3.1. Growth performance and survival

Growth performance and feed utilization parameters revealed significant differences between different treatments in all measured parameters except for the feed intake (FI), feed conversion ratio (FCR), and specific growth rate (SGR), as shown in Table 1. However, the highest weight gain (WG) was noted in T2, without any significant differences among T4, T5, and T6. On the other hand, T1 and T3 recorded the lowest WG with a significant difference compared to all other treatments. Furthermore, the FCR was better under T2 as indicated by lower values compared to other treatments. Likewise, T2 recorded the best SGR as indicated by high values, followed by T6, T5, T4, T3, and T1, respectively. For the survival percentage, T2 and T4 significantly recorded the highest values compared to T6, T5, and T1.

3.2. Feed economic efficacy

Table 2 shows feed costs of using feeding frequency and different protein levels, where T2 showed a significant decrease in feed cost compared with other treatments, Furthermore, there was a significant difference in cost among all treatments, which reflected that feeding frequency affected cost in 42 % protein level without any difference in 44 % protein level.

3.3. Biochemical Profile

3.3.1. Liver function

To assess the liver function, metabolic enzyme activities for Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline phosphatase (ALP), as well as total bilirubin and albumin levels were measured, and the results were presented in Fig. 1. T2 and T4 significantly recorded the lowest values for ALT activity compared to other treatments, without any significant differences between each other ($P > 0.05$). (Fig. 1A). In contrast, there was a significant decline in AST activity in T1 compared to other treatments ($P < 0.05$) (Fig. 1B). No significant differences in AST activity were noted among T2, T3, T4, T5, and T6 ($P > 0.05$). However, the ALP activity was significantly higher in T6, T5, and T4 compared to other treatments, with T1 recording the lowest activity ($P < 0.05$) (Fig. 1C).

In total albumin, there were no significant differences among all treatments ($P > 0.05$). (Fig. 2A). In contrast, the total bilirubin was significantly higher in T3 than in other treatments (Fig. 2B). Likewise, the total bilirubin was significantly higher in T1 and T2 than in T4, T5, and T6 ($P < 0.05$)

3.3.2. Kidney function

Fig. 3 represents the serum biochemical parameters for assessing kidney function. As shown in Fig. 3A, there was a variation in the concentration of urea among the treatments ($P < 0.05$), with T3 significantly recording the highest values compared to T1, T2, T5, and T6, respectively ($P < 0.05$). However, no significant differences in the concentration of urea were noted across T1, T2, T4, T5, and T6 ($P > 0.05$). For uric acid, there was a significant decline in the uric acid concentration in T2 compared to other treatments (Fig. 3B). Furthermore, there was a decline in the concentration of creatine, except with T1, which significantly recorded higher values relative to other treatments ($P < 0.05$) (Fig. 3C).

3.3.3. General health status biomarkers

Total protein, glucose, and hemoglobin are among the most common biomarkers used for assessing the health status of fish, and these indices are summarized in Fig. 4. As shown in Fig. 4A, no significant differences in total protein were noted across all the treatments ($P > 0.05$). However, results on the glucose content indicated a significant increase in T1 compared to other treatments, while T2 and T4 significantly recorded the lowest values ($P < 0.05$) (Fig. 4B). The total hemoglobin content was significantly higher and lower in T2 and T3, respectively, compared to other treatments($P < 0.05$) (Fig. 4C).

4. Discussion

In this study, dietary protein levels and feeding frequency had significant effects on the growth performance and survival of European

Table 1
Growth performance and survival of European sea bass (*Dicentrarchus labrax*) at 110 days.

Parameters	T1	T2	T3	T4	T5	T6	P
IBW (g)	57.35 ± 0.06	55.45 ± 0.35	55.4 ± 0.28	57.15 ± 0.35	56.1 ± 0.28	57.05 ± 0.35	ns
FBW (g)	102 ± 6.36^{b}	113 ± 4.24^{a}	101 ± 2.12^{b}	104 ± 1.41^{b}	112 ± 1.41^{a}	115 ± 1.41^{a}	< 0.05
WG (g)	37.0 ± 3.32^{c}	57.4 ± 4.67^{a}	41.5 ± 4.17^{b}	46.8 ± 0.42^{a}	48 ± 0.42^{a}	47.0 ± 0.42^{a}	< 0.05
FI (g)	93.3 ± 0.28	87.5 ± 2.8	85.8 ± 3.96	86.6 ± 0.50	85.7 ± 0.50	91.05 ± 0.50	*ns*
FCR	2.6 ± 0.21	1.6 ± 0.21	2.1 ± 0.14	2.0 ± 014	1.9 ± 0.14	2.0 ± 0.14	ns
SGR (%/day)	0.48 ± 0.09	0.62 ± 0.04	0.51 ± 0.00	0.52 ± 0.01	0.60 ± 0.01	0.61 ± 0.01	ns
SR (%)	92.5 ± 3.50^{b}	100 ± 0.00^{a}	97.0 ± 4.24^{ab}	100 ± 0.00^{a}	94.5 ± 0.71^{b}	91.5 ± 0.71^{b}	< 0.05

Values are presented as Means ± Standard Deviation (SD), and different superscript letters for each parameter within rows indicate significant differences. ($P < 0.05$). IBW: initial body weight, FBW: final body weight, WG: weight gain, FI: feed intake, FCR: feed conversion ratio, SGR: specific growth rate, SR: Survival percentage. Treatments: T1(42 % protein, fed once/day), T2 (42 % protein, fed twice/day), T3 (42 % protein, fed thrice/day), T4 (44 % protein, fed once/day), T5 (44 % protein, fed twice/day), and T6 (44 % protein, fed thrice/day).

Table 2
Effect of feeding frequency and different protein levels on economic efficiency.

Items (EGP)	T1	T2	T3	T4	T5	T6	P
Feed cost	396 ± 0.5^{a}	358 ± 2.8^{c}	378 ± 2.8^{b}	368 ± 2.8^{bc}	365 ± 4.95^{bc}	389 ± 4.24^{ab}	< 0.05
Feed cost/kg gain	127 ± 1.09^{a}	75.0.3^{c}	104 ± 6.4^{b}	93 ± 0.7^{b}	90 ± 1.4^{b}	98 ± 1.4^{b}	< 0.05

Values are expressed as Means ± SD with different superscript letters for each parameter within rows indicating significant differences. ($P < 0.05$). Treatments: T1 (42 % protein, fed once/day), T2 (42 % protein, fed twice/day), T3 (42 % protein, fed thrice/day), T4 (44 % protein, fed once/day), T5 (44 % protein, fed twice/day), and T6 (44 % protein, fed thrice/day).

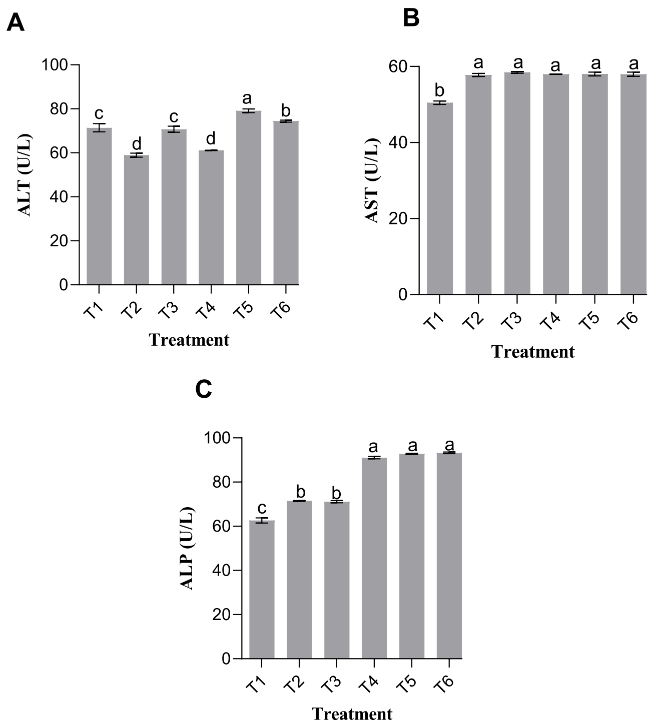

Fig. 1. Enzyme activity for (A) alanine transaminase (ALT), (B) aspartate transaminase (AST), and (C) alkaline phosphatase (ALP) under different treatments. Data are presented as mean ± standard deviation. Treatments: T1(42 % protein, fed once/day), T2(42 % protein, fed twice/day), T3(42 % protein, fed thrice/day), T4(44 % protein, fed once/day), T5(44 % protein, fed twice/day) and T6(44 % protein, fed thrice/day).

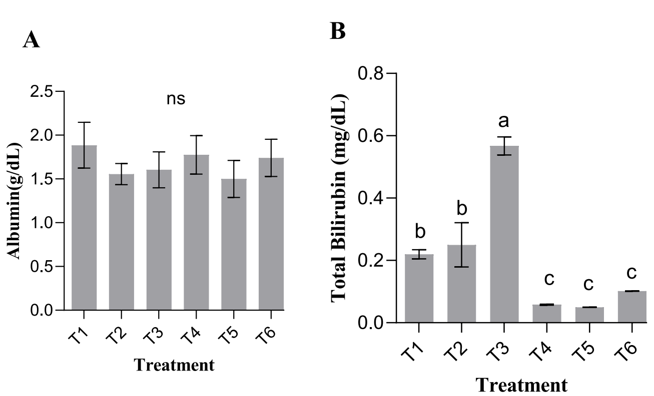

Fig. 2. Concentrations of (A) albumin and (B) total bilirubin under different treatments. Data are presented as mean ± standard deviation. Treatments: T1 (42 % protein, fed once/day), T2(42 % protein, fed twice/day), T3(42 % protein, fed thrice/day), T4(44 % protein, fed once/day), T5(44 % protein, fed twice/day) and T6(44 % protein, fed thrice/day).

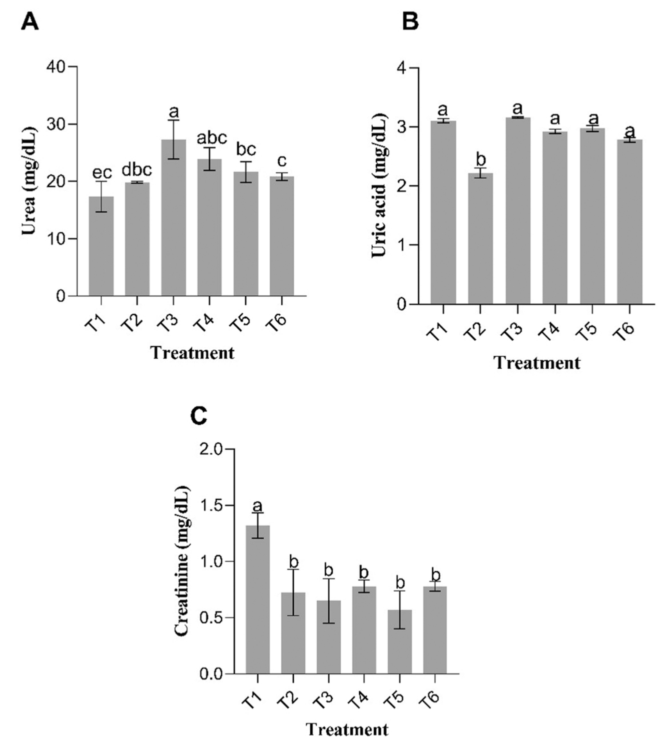

Fig. 3. Concentrations of (A) urea, (B) uric acid, and (C) creatinine under different treatments. Data are presented as mean ± standard deviation. Treatments: T1(42 % protein, fed once/day), T2(42 % protein, fed twice/day), T3 (42 % protein, fed thrice/day), T4(44 % protein, fed once/day), T5(44 % protein, fed twice/day) and T6(44 % protein, fed thrice/day).

seabass (*Dicentrarchus labrax*) reared under a brackish water RAS. Feeding fish on a diet containing 42 % protein twice/day (T2) improved the growth performance in terms of (WG), (FCR), (SGR), and survival percentage compared to other treatments. This means that lower protein levels and feeding frequency can support *D. labrax* to reach its optimum growth performance and survival under brackish water conditions. The influence of dietary protein levels and feeding frequency has been assessed in different species and experimental conditions. El-Dahhar et al. (2016) reported that 38 % protein was optimal for feed utilization and growth performance of *D. labrax* fingerlings reared under seawater flow conditions. Karapanagiotidis et al. (2022) conducted a second-degree polynomial regression analysis and reported that a dietary protein level of 41.4 % and 43.8 % delivered the maximum WG and the higher feed efficiency, respectively in sharp snout sea bream (*Diplodus puntazzo*) juveniles (Chen et al., 2023) showed that feeding rainbow trout (*Oncorhynchus mykiss*) with dietary protein levels of 44 % enhanced the growth performance and feed utilization of fish. For feeding frequency, (Dwyer et al., 2002) suggested that feeding juvenile yellowtail flounder (*Limanda ferruginea*, initial weight 6.80 ± 0.20 g) twice/day significantly improved the weight and FCR of fish. In Nile tilapia (*Oreochromis niloticus*, initial weight 1.0 g) reared in low salinity water, (Daudpota et al., 2016b) showed that a feeding frequency of 4 – 5

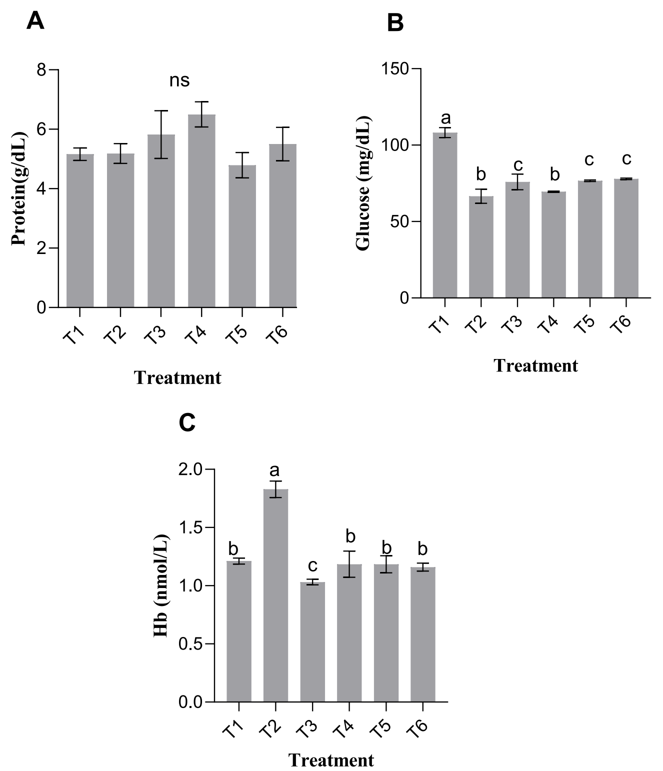

Fig. 4. Concentrations of (A) total protein, (B) glucose, and (C) hemoglobin under different treatments. Data are presented as mean ± standard deviation. Treatments: T1(42 % protein, fed once/day), T2(42 % protein, fed twice/day), T3(42 % protein, fed thrice/day), T4(44 % protein, fed once/day), T5(44 % protein, fed twice/day) and T6(44 % protein, fed thrice/day).

times/day enhanced the WG, SGR, and FCR of fish (Okomoda et al., 2019) recommended a feeding frequency of five times/day for better growth performance and nutrient utilization in African catfish (*Clarias gariepinus*) fry and fingerlings. Generally, the growth performance of fish increases with the increasing feeding frequency up to a certain level and then declines (Li et al., 2014; Russo et al., 2017; Oh et al., 2018). However, it is imperative to note that the optimum feeding frequency is dependent on several factors, such as fish size and species, rearing conditions, and the nutrient content of the feed (Huang et al., 2025). Nonetheless, fish must be fed to satiation to reach optimum growth. Lower or higher feeding frequencies can negatively impact the growth performance of fish as indicated in this study (i.e., T1 (42 %, fed once/day), T3 (42 %, fed thrice/day), and T6 (44 %, fed thrice/day)) and this is similar to previous studies on juvenile dolly varden char (*Salvelinus malma,* average weight: 9.40 ± 0.30 g) reared under hypoxia stress by Guo et al. (2018). Juvenile blunt snout bream (*Megalobrama amblycephala,* average weights: 9.92 ± 0.06 g and 8.87 ± 0.03 g) by Li et al. (2014). This poor growth performance is attributed to the faster gastrointestinal evacuation rate and reduced residence time in the stomach and hence resulting in less effective digestion and assimilation of nutrients (Wang et al., 2020). However, the study results on better growth performance and survival under T2 agree with previous studies by Wang et al. (2020) and Yúfera et al. (2014) who have shown that feeding gilthead seabream (*Sparus aurata*) continuously or twice/day not only allowed better and prolonged feed residence time in the stomach but also improved the acid digestion efficiency.

As reported in the previous section, feed is one of the major inputs in commercial aquaculture, contributing approximately 60 – 80 % of all the production costs (Verdegem et al., 2023; Ali et al., 2023). Reducing the feeding frequency and dietary protein levels without compromising the growth performance and survival of fish could, in the long run, lower the production costs. The lower feeding frequency under similar protein percentages (i.e., T1, T2, and T3) lowered the production cost in the order T2 < T3 < T1. However, the feeding regime affected the growth performance, which in turn affected the production cost under our experimental conditions. This contradicts earlier reports, which show that feeding frequency does not affect production costs due to compensatory growth (El-Araby et al., 2020; Amer et al., 2020).

In this study, the serum biochemical profile was used to assess the liver and kidney function as well as the general well-being of fish as reported by Bavia et al. (2024). Serum ALT and AST play an important role in protein and amino acid metabolism and are indicators of liver health. They may be released into plasma following tissue damage and dysfunction occurrence (Zhang et al., 2021; Zheng et al., 2022). ALP has a close relationship with the immune function of animals (Lallès, 2014). Serum Albumin plays a crucial role in nutrient transport and osmotic regulation. Furthermore, Serum Bilirubin is used as an indicator of liver function and is excreted as a waste product (Chien et al., 2017). Our study showed that dietary protein levels and feeding regimes influence the activity of these enzymes, as indicated by the high concentration of ALT in T5 and T6 and low concentrations of AST in T1 relative to other treatments. The high levels of dietary protein resulted in the increase of protein catabolism by these metabolic enzymes, thus diverting the nutrients to energy production rather than being used for growth, and this is supported by the inferior growth performance regarding WG in T5 and T6 treatments. Similar results have been previously reported in *O. niloticus* (Gaye-Siessegger et al., 2006; Abdel-Tawwab et al., 2010) and *Solea aegyptiaca* juveniles (Yones et al., 2018). Furthermore, the rise of serum protein with dietary protein could likely be due to the enhanced levels of digested protein; however, lower values for serum glucose were observed in high dietary protein diet treatments and those with frequent feeding. This means that fish were using proteins for energy metabolism rather than using glucose as the source of energy, a process known as gluconeogenesis (Melo et al., 2016). Moreover, elevated hemoglobin levels in T2 compared to other treatments indicated that feeding fish at moderate dietary protein levels twice/day improves the health status of fish under stress conditions, thus in line with a previous study on common carp (*Cyprinus carpio*) (Ahmed Khan and Maqbool, 2017), African catfish (*C. gariepinus*) (Aderolu et al., 2017), and Tambaqui (*Colossoma macropomum*) (Rodrigues et al., 2024).

When fish are stressed, they release large volumes of certain compounds such as carbon dioxide, ammonia, and a small amount of uric acid (Nasr-Eldahan et al., 2024). The accumulated ammonia is then converted into a relatively non-toxic form, such as uric acid or urea, which is subsequently excreted by the kidneys (Wilkie, 2002; Zimmer et al., 2017). High levels of serum urea and uric acid indicate kidney dysfunction (Dawood et al., 2023; Busti et al., 2020) In this study, although high levels of serum urea and uric acid were noted in T3, T4, T5, and T6, no significant differences were noted with T1; thus, our experimental conditions did not negatively affect the kidney function of *D. labrax*. This is further supported by significantly lower serum creatinine and total bilirubin levels that were within the normal range in healthy fish, as well as non-significant differences in albumin levels across all the treatments.

5. Conclusion

The current study focuses on the effect of dietary protein levels and feeding regime on the growth performance and biochemical profile of European sea bass (*Dicentrarchus labrax*) reared under brackish water conditions (7 ppt) in a RAS. The collective results demonstrated that there was a significant difference in the growth performance and survival of fish depending on the feeding regime and dietary protein levels, with better growth performance in terms of FCR, SGR, WG, and survival percentage recorded in fish fed on 42 % protein twice/day which reflected that using appreciate protein level with good food strategy improve the growth performance and reduce food cost. Moreover, our experimental conditions did not show a negative impact on the liver and kidney function as well as the blood biochemical profile. Furthermore, showed improvement in liver function and hemoglobin in using 42 % protein twice per day which reflected in health situation of fish. Hence, European sea bass showed the possibility of growth with normal health status under brackish water (7 ppt) conditions in a RAS, provided that fish are fed on 42 % protein twice/day.

CRediT authorship contribution statement

Sameh Nasr-Eldahan: Writing – original draft, Methodology, Formal analysis, Conceptualization, Data curation, Writing – review & editing. **Mahmoud A.O. Dawood:** Investigation, Methodology. **Muziri Mugwanya:** Writing – review & editing, Data curation, Writing – original draft. **Fahad Kimera:** Writing – review & editing, Writing – original draft. **Hani Sewilam:** Supervision, Resources, Conceptualization.

Ethical approval and consent to participate

This experimental protocol was approved by the Institutional Review Board of the American University in Cairo. All methods applied were carried out following relevant guidelines and regulations approved by the Animal Ethics Committee / American University in Cairo.

Consent for publication

Not applicable.

Funding

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Abdel-Aziz, M.F.A., Hassan, H.U., Yones, A.M., Abdel-Tawwab, Y.A., Metwalli, A.A.A.T., 2021a. Assessing the effect of different feeding frequencies combined with dtocking density, initial weight, and dietary protein ratio on the growth performance of tilapia, catfish and carp (Jul.). Sci. Afr. 12, e00806. https://doi.org/10.1016/J.SCIAF.2021.E00806 (Jul.).

Abdel-Aziz, M.F.A., Hassan, H.U., Yones, A.M., Abdel-Tawwab, Y.A., Metwalli, A.A.A.T., 2021b. Assessing the effect of different feeding frequencies combined with stocking density, initial weight, and dietary protein ratio on the growth performance of tilapia, catfish and carp (Jul.). Sci. Afr. 12, e00806. https://doi.org/10.1016/J.SCIAF.2021.E00806 (Jul.).

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E., Shalaby, A.M.E., 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *oreochromis niloticus* (L.) (Jan.). Aquaculture 298 (3–4), 267–274. https://doi.org/10.1016/J.AQUACULTURE.2009.10.027 (Jan.).

Abutalb, A.A., 2023. Effect of different feeding times on growth performance and blood parameters of African catfish (*clarias gariepinus*) under different stocking densities (Jun.). AlAzhar J. Agric. Res. 48 (1), 153–167. https://doi.org/10.21608/AJAR.2023.316065 (Jun.).

Aderolu, A., Lawal, M., Eziefula, P., Ahaiwe, E., 2017. Feeding frequency and feeding regime in catfish: effects on nutrient utilization, growth, biochemical and haematological parameters. J. Agric. Sci. Belgrade 62 (4), 395–410. https://doi.org/10.2298/jas1704395a.

Ahmed Khan, I., Maqbool, A., 2017. Effects of dietary protein levels on the growth, feed utilization and Haemato-Biochemical parameters of freshwater fish, *cyprinus carpio var. specularis*. Fish. Aquac. J. 08 (01). https://doi.org/10.4172/2150-3508.1000187.

Ali, H., Belton, B., Haque, M.M., Murshed-e-Jahan, K., 2023. Transformation of the feed supply segment of the aquaculture value chain in Bangladesh (Nov.). Aquaculture 576. https://doi.org/10.1016/J.AQUACULTURE.2023.739897 (Nov.).

Amer, S.A., Ahmed, S.A.A., Ibrahim, R.E., Al-Gabri, N.A., Osman, A., Sitohy, M., 2020. Impact of partial substitution of fish meal by methylated soy protein isolates on the nutritional, immunological, and health aspects of Nile tilapia, *oreochromis niloticus* fingerlings (Mar.). Aquaculture 518, 734871. https://doi.org/10.1016/J.AQUACULTURE.2019.734871 (Mar.).

Ballestrazzi, R., Lanari, D., D'Agaro, E., Mion, A., 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing seabass (*dicentrarchus labrax*) (Nov.). Aquaculture 127 (2–3), 197–206. https://doi.org/10.1016/0044-8486(94)90426-X (Nov.).

Basto, A., Matos, E., Valente, L.M.P., 2020. Nutritional value of different insect larvae meals as protein sources for european sea bass (*dicentrarchus labrax*) juveniles (May). Aquaculture 521, 735085. https://doi.org/10.1016/J.AQUACULTURE.2020.735085 (May).

Bavia, L., et al., 2024. Health status biomarkers and Hemato-Biochemical indices in Nile tilápia (Dec.). Comp. Immunol. Rep. 7, 200168. https://doi.org/10.1016/J.CIREP.2024.200168 (Dec.).

Busti, S., et al., 2020. Effects of different feeding frequencies on growth, feed utilisation, digestive enzyme activities and plasma biochemistry of gilthead seabream (*sparus aurata*) fed with different fishmeal and fish oil dietary levels (Dec.). Aquaculture 529, 735616. https://doi.org/10.1016/J.AQUACULTURE.2020.735616 (Dec.).

Cai, L.Sen, Wang, L., Song, K., Lu, K.Le, Zhang, C.X., Rahimnejad, S., 2020. Evaluation of protein requirement of spotted seabass (*lateolabrax maculatus*) under two temperatures, and the liver transcriptome response to thermal stress (Feb.). Aquaculture 516, 734615. https://doi.org/10.1016/J.AQUACULTURE.2019.734615 (Feb.).

Chen, Y.F., Cao, K.L., Huang, H.F., Li, X.Q., Leng, X.J., 2023. Dietary effects of lipid and protein levels on growth, feed utilization, lipid metabolism, and antioxidant capacity of triploid rainbow trout (*oncorhynchus mykiss*) (vol). Aquac. Nutr. 2023. https://doi.org/10.1155/2023/8325440.

Chien, S.C., Chen, C.Y., Lin, C.F., Yeh, H.I., 2017. Critical appraisal of the role of serum albumin in cardiovascular disease (Nov.). Biomark. Res. 5 (1), 1–9. https://doi.org/10.1186/S40364-017-0111-X/FIGURES/2 (Nov.).

Daudpota, A.M., et al., 2016a. Effect of feeding frequency on growth performance, feed utilization and body composition of juvenile Nile tilapia, *oreochromis niloticus* (L.) reared in low salinity water. Pak. J. Zool. (s).

A.Malik Daudpota *et al.*, "Effect of Feeding Frequency on Growth Performance, Feed Utilization and Body Composition of Juvenile Nile Tilapia, *Oreochromis niloticus* (L.) Reared in Low Salinity Water," 2016a.

A.Malik Daudpota *et al.*, "Effect of Feeding Frequency on Growth Performance, Feed Utilization and Body Composition of Juvenile Nile Tilapia, *Oreochromis niloticus* (L.) Reared in Low Salinity Water," 2016b.

Dawood, M.A.O., Madkour, K., Sewilam, H., 2023. Polyculture of european seabass and Nile tilapia in the recirculating aquaculture system with brackish water: effects on the growth performance, feed utilization, and health status (Nov.). Aquac. Fish.. https://doi.org/10.1016/J.AAF.2023.11.001 (Nov.).

Dias, J., Arzel, J., Aguirre, P., Corraze, G., Kaushik, S., 2003a. Growth and hepatic acetyl Coenzyme-A carboxylase activity are affected by dietary protein level in european seabass (*dicentrarchus labrax*) (May). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 135 (1), 183–196. https://doi.org/10.1016/S1096-4959(03)00078-2 (May).

Dias, J., Arzel, J., Aguirre, P., Corraze, G., Kaushik, S., 2003b. Growth and hepatic acetyl Coenzyme-A carboxylase activity are affected by dietary protein level in european seabass (*dicentrarchus labrax*) (May). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 135 (1), 183–196. https://doi.org/10.1016/S1096-4959(03)00078-2 (May).

Dong, M., et al., 2017. Proteomic characterization of the interactions between fish serum proteins and waterborne bacteria reveals the suppression of Anti-Oxidative defense as a Serum-Mediated antimicrobial mechanism (Mar.). Fish. Shellfish Immunol. 62, 96–106. https://doi.org/10.1016/J.FSI.2017.01.013 (Mar.).

Dwyer, K.S., Brown, J.A., Parrish, C., Lall, S.P., 2002. Feeding frequency affects food consumption, feeding pattern and growth of juvenile yellowtail flounder (*limanda ferruginea*) (Oct.). Aquaculture 213 (1–4), 279–292. https://doi.org/10.1016/S0044-8486(02)00224-7 (Oct.).

El-Araby, D.A., Amer, S.A., Khalil, A.A., 2020. Effect of different feeding regimes on the growth performance, antioxidant activity, and health of Nile tilapia, *oreochromis niloticus* (Nov.). Aquaculture 528, 735572. https://doi.org/10.1016/J.AQUACULTURE.2020.735572 (Nov.).

El-Dahhar, A., El-Zaeem, S., Khalifa, G., 2016. Effect of dietary protein level on survival, growth performance and body composition of european sea bass (*dicentrarchus labrax*) fingerlings under sea water flow conditions (Dec.). J. Arab. Aquac. Soc. 11 (2), 121–138. https://doi.org/10.12816/0050314 (Dec.).

Espinoza-Ortega, M., Molina-Poveda, C., Jover-Cerdá, M., Civera-Cerecedo, R., 2024. Feeding frequency effect on water quality and growth of *litopenaeus vannamei* fed extruded and pelleted diets (Feb.). Aquac. Int. 32 (1), 413–429. https://doi.org/10.1007/S10499-023-01166-9/FIGURES/2 (Feb.).

Gaye-Siessegger, J., Focken, U., Becker, K., 2006. Effect of dietary Protein/Carbohydrate ratio on activities of hepatic enzymes involved in the amino acid metabolism of Nile tilapia, *oreochromis niloticus* (L.) (Dec.). Fish. Physiol. Biochem 32 (4), 275–282. https://doi.org/10.1007/S10695-006-9000-1 (Dec.).

Guo, Z., et al., 2018. Effect of feeding frequency on growth performance, antioxidant status, immune response and resistance to hypoxia stress challenge on juvenile dolly varden char salvelinus malma (Feb.). Aquaculture 486, 197–201. https://doi.org/10.1016/J.AQUACULTURE.2017.12.031 (Feb.).

Gupta, S., et al., 2024. Recent developments in recirculating aquaculture systems: a review (vol). Aquac. Res (1), 6096671. https://doi.org/10.1155/ARE/6096671.
Habib-Ul-Hassan, Q.M., Ali, M.A.M., Siddique, M.R. Hasan, Hossain, M.Y., 2022a. Effects of dietary protein levels on growth, nutritional utilization, carcass composition and survival of asian seabass *lates calcarifer* (Bloch, 1790) fingerlings rearing in net cages (Apr). Thalassas 38 (1), 21–27. https://doi.org/10.1007/S41208-021-00371-8/TABLES/3.
Habib-Ul-Hassan, Q.M., Ali, M.A.M., Siddique, M.R. Hasan, Hossain, M.Y., 2022b. Effects of dietary protein levels on growth, nutritional utilization, carcass composition and survival of asian seabass *lates calcarifer* (Bloch, 1790) fingerlings rearing in net cages (Apr). Thalassas 38 (1), 21–27. https://doi.org/10.1007/S41208-021-00371-8/TABLES/3.
Hassan, H.U., et al., Dec. 2021. Effect of feeding frequency as a rearing system on biological performance, survival, body chemical composition and economic efficiency of asian seabass *lates calcarifer* (Bloch, 1790) reared under controlled environmental conditions. Saudi J. Biol. Sci. 28 (12), 7360–7366. https://doi.org/10.1016/J.SJBS.2021.08.031.
Hossain, M.S., Small, B.C., Kumar, V., Hardy, R., 2024. Utilization of functional feed additives to produce Cost-Effective, ecofriendly aquafeeds high in Plant-Based ingredients (Jan.). Rev. Aquac. 16 (1), 121–153. https://doi.org/10.1111/RAQ.12824 (Jan.).
Huang, M., et al., 2025. Optimizing feeding frequencies in fish: a meta-analysis and machine learning approach (Jan.). Aquaculture 595. https://doi.org/10.1016/J.AQUACULTURE.2024.741678 (Jan.).
Karapanagiotidis, I.T., et al., 2022. The effect of different dietary protein levels on growth performance and nutrient utilization of snarpsnout sea bream (*diplodus puntazzo*) (Apr). Aquac. Res. 53 (5), 1909–1917. https://doi.org/10.1111/ARE.15719.
Laczynska, B., Siddique, M.A.M., Ziomek, E., Shelton, W.L., Fopp-Bayat, D., 2020. Early weaning effects on survival, growth, and histopathology of larval sterlet *acipenser ruthenus* (Apr.). N. Am. J. Aquac. 82 (2), 181–189. https://doi.org/10.1002/NAAQ.10141 (Apr.).
Lallès, J.P., 2014. Intestinal alkaline phosphatase: novel functions and protective effects (Feb.). Nutr. Rev. 72 (2), 82–94. https://doi.org/10.1111/NURE.12082 (Feb.).
Li, X.F., Tian, H.Y., Zhang, D.D., Jiang, G.Z., Liu, W.Bin, 2014. Feeding frequency affects stress, innate immunity and disease resistance of juvenile blunt snout bream *megalobrama amblycephala* (May). Fish. Shellfish Immunol. 38 (1), 80–87. https://doi.org/10.1016/J.FSI.2014.03.005 (May).
Liu, Y., Wang, J., Ao, H., Liu, L., Chen, Y., 2023. Effects of extruded and pelleted diets with different protein levels on growth performance and nutrient retention of largemouth bass (*micropterus salmoides*) (Apr.). Aquac. Rep. 29, 101479. https://doi.org/10.1016/J.AQREP.2023.101479 (Apr.).
Melo, J.F.B., Lundstedt, L.M., Inoue, L.A.K., Metón, I., Baanante, I.V., Moraes, G., 2016. Glycolysis and gluconeogenesis in the liver of catfish fed with different concentrations of proteins, lipids and carbohydrates. Arq. Bras. Med. Vet. Zootec. 68 (5), 1251–1258. https://doi.org/10.1590/1678-4162-8337.
Modeel, S., Dolkar, P., Siwach, S., Yadav, P., Negi, R.K., 2024. Role of science and technology for sustainable aquaculture development and aquatic ecosystem management. Role Sci. Technol. Sustain. Future 277–301. https://doi.org/10.1007/978-981-97-0710-2_17.
Montero, D., et al., 2023. Nutritional innovations in superior european seabass (*dicentrarchus labrax*) genotypes: implications on fish performance and feed utilization (Jul.). Aquaculture 572, 739486. https://doi.org/10.1016/J.AQUACULTURE.2023.739486 (Jul.).
Nasr-Eldahan, S., Attia Shreadah, M., Maher, A.M., El-Sayed Ali, T., Nabil-Adam, A., 2024. New vaccination approach using Formalin-killed *streptococcus pyogenes* vaccine on the liver of *oreochromis niloticus* fingerlings (Aug.). Sci. Rep. 2024 141 14 (1), 1–16. https://doi.org/10.1038/s41598-024-67198-0 (Aug.).
Nebo, C., Gimbo, R.Y., Kojima, J.T., Overturf, K., Dal-Pai-Silva, M., Portella, M.C., 2018. Depletion of stored nutrients during fasting in Nile tilapia (*oreochromis niloticus*) juveniles (Apr.). J. Appl. Aquac. 30 (2), 157–173. https://doi.org/10.1080/10454438.2017.1420516 (Apr.).
Nguyen, H.Y.N., Trinh, T.L., Baruah, K., Lundh, T., Kiessling, A., 2021. Growth and feed utilisation of Nile tilapia (*oreochromis niloticus*) fed different protein levels in a Clear-Water or Biofloc-RAS system (Apr.). Aquaculture 536, 736404. https://doi.org/10.1016/J.AQUACULTURE.2021.736404 (Apr.).
Oh, S.Y., Venmathi Maran, B.A., Park, J.W., 2018. Effect of feeding frequency on growth, food consumption, proximate composition, and blood chemistry of juvenile Dark-banded rockfish, sebastes inermis (Dec.). J. World Aquac. Soc. 49 (6), 994–1001. https://doi.org/10.1111/JWAS.12512 (Dec.).
Okomoda, V.T., Aminem, W., Hassan, A., Martins, C.O., 2019. Effects of feeding frequency on fry and fingerlings of African catfish *clarias gariepinus* (Sep.). Aquaculture 511. https://doi.org/10.1016/J.AQUACULTURE.2019.734232 (Sep.).
Oliva-Teles, A., 2000. Recent advances in european seabass and gilthead seabream nutrition. Aquac. Int. 8 (6), 477–492. https://doi.org/10.1023/A:1009236517555/METRICS.
Rigos, G., et al., 2021. Best therapeutic practices for the use of antibacterial agents in finfish aquaculture: a particular view on european seabass (Dicentrarchus labrax) and Gilthead Seabream (Sparus aurata) in mediterranean aquaculture (Jun.). Rev. Aquac. 13 (3), 1285–1323. https://doi.org/10.1111/RAQ.12523 (Jun.).
Roa, F.G.B., Silva, S.D.S., Hoshiba, M.A., da Silva, L.K.S., de Barros, A.F., de Abreu, J.S., 2019. Performance of tambaqui juveniles subjected to short Feed-Deprivation and refeeding cycles (Dec.). Bol. do Inst. De. Pesca 45 (4). https://doi.org/10.20950/1678-2305.2019.45.4.466 (Dec.).
Rodrigues, A.P.O., de Freitas, L.E.L., Maciel-Honda, P.O., Lima, A.F., de Lima, L.K.F., 2024. Feeding rate and feeding frequency during the Grow-out phase of tambaqui (*colossoma macropomum*) in earthen ponds (Apr.). Aquac. Rep. 35. https://doi.org/10.1016/J.AQREP.2024.102000 (Apr.).
Russo, D.J., Watanabe, W.O., Kinsey, S.T., Seaton, P.J., 2017. Effects of feeding frequency of live prey on larval growth, survival, resistance to hyposalinity stress, Na+/K+ ATPase activity, and fatty acid profiles in black seabass centropristis striata (Mar.). Aquaculture 470, 56–67. https://doi.org/10.1016/J.AQUACULTURE.2016.12.005 (Mar.).
Topic Popovic, N., et al., 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia (Aug.). J. Appl. Ichthyol. 28 (4), 553–564. https://doi.org/10.1111/J.1439-0426.2012.01950.X (Aug.).
Verdegem, M., Buschmann, A.H., Latt, U.W., Dalsgaard, A.J.T., Lovatelli, A., 2023. The contribution of aquaculture systems to global aquaculture production (Apr.). J. World Aquac. Soc. 54 (2), 206–250. https://doi.org/10.1111/JWAS.12963 (Apr.).
Wang, Y., et al., 2020. Optimum feeding frequency of juvenile largemouth bass (*micropterus salmoides*) reared in-Pond raceway recirculating culture system (Dec.). Fish. Physiol. Biochem 46 (6), 2197–2212. https://doi.org/10.1007/S10695-020-00866-W/TABLES/4 (Dec.).
Wilkie, M.P., 2002. Ammonia excretion and urea handling by fish gills: present understanding and future research challenges (Aug.). J. Exp. Zool. 293 (3), 284–301. https://doi.org/10.1002/JEZ.10123 (Aug.).
Yones, A.M.M., M. Nazmi, H., A. Metwalli, A., 2018. Influence of several dietary protein and lipid levels on nutritional parameters and liver functions of *solea aegyptiaca* juveniles (Special Issue). Egypt J. Aquat. Biol. Fish. 22 (5), 433–446. https://doi.org/10.21608/EJABF.2018.23926.
Yúfera, M., et al., 2014. Effect of feeding frequency on the daily rhythms of acidic digestion in a teleost fish (*gilthead seabream*) (Nov.). Chrono. Int 31 (9), 1024–1033. https://doi.org/10.3109/07420528.2014.944265 (Nov.).
Zhang, Y., et al., 2021. Study of dietary curcumin on the restorative effect of liver injury induced by carbon tetrachloride in common carp, *cyprinus carpio* (Nov.). Aquac. Rep. 21. https://doi.org/10.1016/J.AQREP.2021.100825 (Nov.).
Zheng, Y., et al., 2022. Seasonal changes of growth, immune parameters and liver function in wild Chinese sturgeons under indoor conditions: implication for artificial rearing (Apr.). Front. Physiol. 13. https://doi.org/10.3389/FPHYS.2022.894729/FULL (Apr.).
Zimmer, A.M., Wright, P.A., Wood, C.M., 2017. Ammonia and urea handling by early life stages of fishes (Nov.). J. Exp. Biol. 220 (21), 3843–3855. https://doi.org/10.1242/JEB.140210 (Nov.).