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'Effect of dietary vitamin C on growth and development of gilt-head seabream (*Sparus aurata*) fingerlings'

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Abstract

Growing healthy fish with high survival is essential for the sustainable development of aquaculture. Furthermore, improved growth rate and feed conversion ratio have been proven to minimize space water and feed requirement. All kind of vitamins, but more specifically Vitamin C is key nutritional element in promoting optimal survival and performance. However, an exogenous source is required in fish's diet as many teleost fish lack the ability of vitamin C synthesis. In this study gilthead seabream fingerlings were fed a fish meal-based diet supplemented with different amounts of vitamin C (ascorbic acid) of 0, 500 and 2000 mg/kg, for 5 weeks. Although there were no significant differences between growth rates of each group by the end of the experiment, the feed consumption was significantly higher for fish fed vitamin C free diet. During the 5-week experiment a more rapid growth was observed in fish with vitamin C free diet. However, towards the end of the experiment, fish fed 2000 mg/kg of dietary Vitamin C had the highest growth rate as it decreased for fish with 0 and 500 mg/kg of dietary Vitamin C. Lastly, feed conversion ratio, by the end of the experiment, was higher for the group A, although not statistical difference was observed between groups.

1. Introduction

1.1 Literature review

Worldwide food demand is expected to double as world's population is growing and is estimated to increase to 9.1 billion by 2050 (Diouf, 2009). Due to this food crises many discuss the importance of marine-based food production and more specific the importance of mariculture in meeting future food demands along with nutritional security (Duarte C.M., Holmer M., Olsen Y., Soto D., Marba N., Guiu J., Black K., Karakassis N., 2009; Marra, 2005). Mariculture is a specialized wing of aquaculture which is basically the breeding, rearing and harvesting of fish, shellfish, algae and other organisms in an aquatic environment (National Oceanic and atmospheric Administration, 2021). To achieve a sustainable development of future aquaculture production; improvements in growth rate, survival, and feed conversion efficiency have been proven to minimize space, water, and feed requirements, and if extensively implemented, might have a significant impact on global aquaculture production (Gjedrem, T., Robinson, N., & Rye, M., 2012).

In aquaculture, over the past 25 years, the nutritional importance of different vitamins and more specific ascorbic acid (AA), also known as vitamin C has grown steadily. Several studies have been contacted examining the effect of vitamin C in fish resulting in a beneficial output in fish survival, fish growth and especially on immune parameters (Ai, Q., Mai, K., Zhang, C., Xu, W., Duan, Q., Tan, B., & Liufu, Z., 2004). Specifically, previous studies reported that an increased immune response due to high concentrations of vitamin C have been demonstrated in marine fish, such as turbot (*Scophthalmus maximus*), gilthead seabream (*Sparus aurata*) (Henrique MMF, Gomes EF, Gouillou-Coustans MF, Oliva-Teles A, Davies SJ., 1998), Japanese sea bass (*Lateolabrax japonicus*) (Ai Q. et al., 2004) and grouper (*Epinephelus malabaricus*) (Lin MF, Shiau SY. , 2005). As high-density aquaculture has led to excessive stress and contributes to the outbreaks of bacterial infection causing high mortality (Dawood, M., Koshio, S., El-Sabagh, M., Billah, M., Zaineldin, A., Zayed, M., & Omar, A., 2017), an increased immune response in commercially farmed fish is necessary.

Vitamin C intake is beneficial for health in humans, animals and in cultured cells. In fish, vitamin C is an important nutrient for optimum maintenance and growth (Al-Amoudi, M.M.. El-Nakkadi, A.M.N. and El-Nouman, B.M., , 1992). Furthermore, vitamin C plays an important role in response to stressors, healing and immune response (NRC, 2011). Due to the lack of L-gulonolactone oxidase that is responsible for the synthesis of vitamin C, some teleost fish cannot synthesize vitamin C or L- ascorbic acid (Nishikimi M., Yagi K.,, 1991). Therefore, an exogenous source of vitamin C is required in its diet (Fracalossi, D., Allen, M., Yuyama, L., & Oftedal, O. , 2001) as vitamin C deficiency in fish can result in reduced growth, structure deformities, reduced immune responses and slow wound repair (Fracalossi, D.M., Allen, M.E., Nichols, D.K., Oftedal, O.T., 1998; Roberts, M.L., Davies, S.J., Pulsford, A.L.,, 1995).

The gilthead seabream (***Sparus aurata***) belongs to the family of Sparidae and inhabits the Atlantic coasts of Europe, Mediterranean and Black Sea (more rarely). This species, quickly showed a high adaptability to intensive rearing conditions, both in ponds and cages, and its annual production increased regularly until 2000, when it reached a peak of over 87 000 tones. Especially in the Mediterranean, Gilt head seabream is one of the most important marine fish in fishery and aquaculture and are extensively farmed in lagoons or intensively in tanks and cages. Until 2010, most production is from intensive farming, with average densities of 20-100 kg per cubic meter. Fingerling production in Greece is estimated that reached 422.3 million fingerlings in 2019, out of which 56% were gilthead seabream and 41% seabass. Furthermore, adult Seabream production in 2019 amounted to 65 300 tons (Aquaculture in Greece, 2020). From such mass production, up to 78 % of these fish produced in Greece are exported to 32 countries with major destination Italy, France and Spain absorbing about 50%. Big volumes are also oriented to new markets such as United states of America and Canada (Federation of Eurobean Aquaculture Producers, 2015)

For this species, there are many reports on nutritional requirements with the use of either live or artificial diets. Dietary vitamin C requirements are affected by size, age, growth rates and environmental factors (NRC, 2011). Larval gilthead sea bream after they deplete their yolk sacs (3-4 DPH) and after they accomplish metamorphosis, by preying on rotifers and artemia (30-35 DPH), they pass thru the process of weaning (shifting from live to artificial food). At this stage, food given must be enriched with essential fatty acids and vitamins that are critical for good growth, development and survival (FAO, 2005-2021). The National Research Council (1993) recommends 25-50 mg/kg diet as a requirement for an optimal performance in juvenile fish (National Research Council, 1993). According to Dabrowski (1990), larval fish might need a higher dietary ascorbic acid dietary than juveniles to sustain optimal growth as they present a relatively faster growth rate and metabolism (Dabrowski, 1990). Furthermore Dawood (2017), showed that after a 57 days trail on gilthead seabream fingerlings an implementation of 500 mg/kg of vitamin had a significantly higher weight than those with no added vitamin in diet (Dawood et al., 2017).

Fish feed is the number one cost in fish farming production, so fish efficiency plays a vital role in profitability. One of the parameters to be considered for reducing feeding costs but at the same time achieve balance between fast fish growth and cost-effective use of nutrients, is the choice of feeding rate. Restricted rations often result in improved feed efficiency, but low feeding rates lead to decreased growth (Mihelakakis A, Tsolkas C, Yoshimatsu T, 2002). Since feed allowance influence growth of the fish, knowledge about the relationship between growth and feeding rate is an important variable in order to obtain appropriate sizes of juveniles and meet market demands. Furthermore, by implementing the appropriate feeding rate, an increase in output aquaculture products will be achieved, as the demand is increasing due to growing populations (FAO, 2005-2021). Feeding conversion rate is not only important for cutting production costs and having an increased output, but also for limiting nutrient emission to the environment from food loses. By knowing the feeding conversion rate of a fish of a certain age, accordingly, the appropriate amount of feed will be given so no uneaten feed go wasted in the sea, reducing the amount of nitrate and phosphate loss that will affect different parts of the marine ecosystem (Olsen, Lasse & Holmer, Marianne & Olsen, Yngvar., 2008)

However, there is still little information about the effects, of different levels of dietary vitamin C in fingerling growth and development during a short term. Most studies analyzing the effect of dietary Vitamin C in gilthead seabream development, last for at least a period of 8 weeks (Alexis M., Karanikolas, K., & Richards, R., 1997; Saleh, N., Wassef, E., Kamel, M., El-Haroun, E., & El-Tahan, R., 2021; Ortuno, J., Esteban, M., & Meseguer, J. , 1999). Furthermore, little information is present on the effect of Vitamin C in feed consumption and feed conversion rate.

1.2 Study Aim

The aim of this study is to analyze the effects of different vitamin C levels on gilthead seabream fingerling growth by comparing different growth characteristic like total length, fork length, standard length, head length, wet weight, dry weight, specific growth rate (SGR), Viscerosomatic index and Hepatosomatic index. Furthermore, the effect of vitamin C on Feed Conversion Rate (FCR) was analyzed to determine if additional vitamin C has any effect.

2. Material & Methods

2.1 Fish rearing & material used

The experiment was conducted at the Department of Fisheries and Marine Research, in Cyprus, using gilthead seabream (*Sparus aurata*) fingerlings, which were produced from a commercial fish hatchery. For the experiment, nine 300-L cylindrical tanks with conical bottom were used

(Figure 2), with 100 fish randomly distributed in each one. All tanks were connected in a flow-through system, with central drainage and independently supplied with filtered seawater of approximately 39 ppt salinity. Oxygen concentration remained above 90% saturation throughout the duration of the experiment. Temperature of water was recorded every 15 min intervals by a mean of a temperature data logger and fluctuated between 22.06 and 28.5 °C (Figure 1).

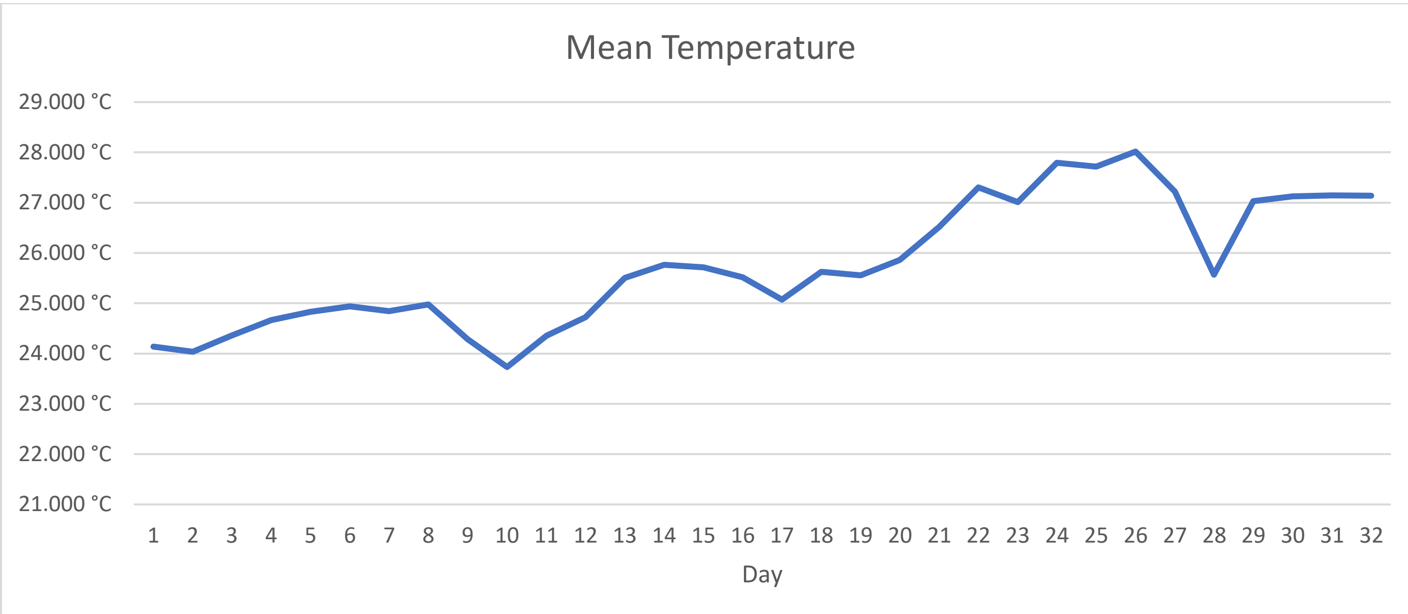


Figure 1: Daily Mean Temperature of water recorded by temperature data logger every 15 minutes.

After fish were transferred in the experimental tanks, an acclimatization period of 24 h was allowed, before starting feeding the fish. Feeding initiated on 23rd of June and the whole experiment lasted 5 weeks.

Figure 2: 300 L tanks used with water supply and overflow drainage.

The experimental design involved three different groups (three tanks per group), in each of which a feed with a different dietary concentration of vitamin C was given. Each diet was provided to 3 tanks, therefore to a total of 300 fish. The three different concentrations of vitamin C were 0, 500 and 2000 mg ascorbic acid per kilogram of feed. Sprayed-dried powder consisting of stabilized Na/Ca ascorbic acid (ROVIMIX® STAY-C®35) was mixed with the feed pellets that had a diameter of 1 mm. The commercial fish feed used throughout the experiment was the Start Premium made by Alltech Coppens BV, based in the Netherlands. The commercial fish feed itself do not contain vitamin C (Table 1).

DIET COMPOSITION	
PROTEIN (%)	54
FAT (%)	15
CRUDE FIBRE (%)	0.1
ASH (%)	10.4
TOTAL P (%)	1.59
VITAMIN A(IE/KG)	12000

Table 1: Diet composition of pellets used to mix with ascorbic acid (Alltech Coppens, Start premium 1.0 mm)

The mixture was let for 48 h prior feeding for better vitamin C absorption. All fish were fed until satiation at each meal, twice a day for four days a week (9 a.m and 1:30 p.m) and once on Monday, Tuesday and the weekend (at 9 am). Monday and Tuesday fish were fed once due to sampling. Duration of each meal was 1 hour. Food containers were weighted before and after feeding to estimate weight of food consumed by each tank.

2.2 Fish sampling & Calculations

Every day the number of dead individuals were recorded from each tank so the overall survival of each tank/group could be estimated. First fish sampling took place before fish transportation in the 9 tanks, where 10 individuals were sampled. Next samplings were done every 7 days; 10 fish were collected randomly from each tank using a mesh net for sampling. Each fish was measured for Wet weight, Standard, Total, Fork and Head length (Figure 3).

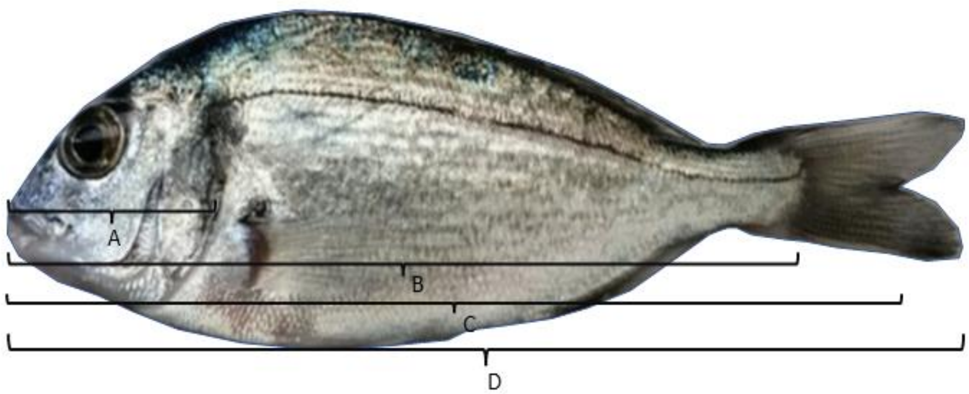


Figure 3 : Gilthead seabream (Sparus aurata) fish with different lengths measured. A= Head Length, B= Standard length, C= Fork length, D= Total length.

The data obtained were used to compare between group's fish mean weight and length, and to also calculate Specific growth rate (SGR) for each group with the following formula:

$$\textit{Specific growth rate (SGR) (\% day−1)} = 100 * \frac{ln\,FBW - lnIBW}{days} ,$$

Where:

FBW and IBW represent the final and the initial body weights

Same 10 individuals were then dissected after euthanized by exposing the fish in a bath of 0.3 mg/L 2-phenoxyethanol solution for at least 10 minutes. The viscera and liver were removed and weighted together and individually (Figure 4) so the Viscerosomatic index (VSI) and hepatosomatic index (HSI) can be estimated.

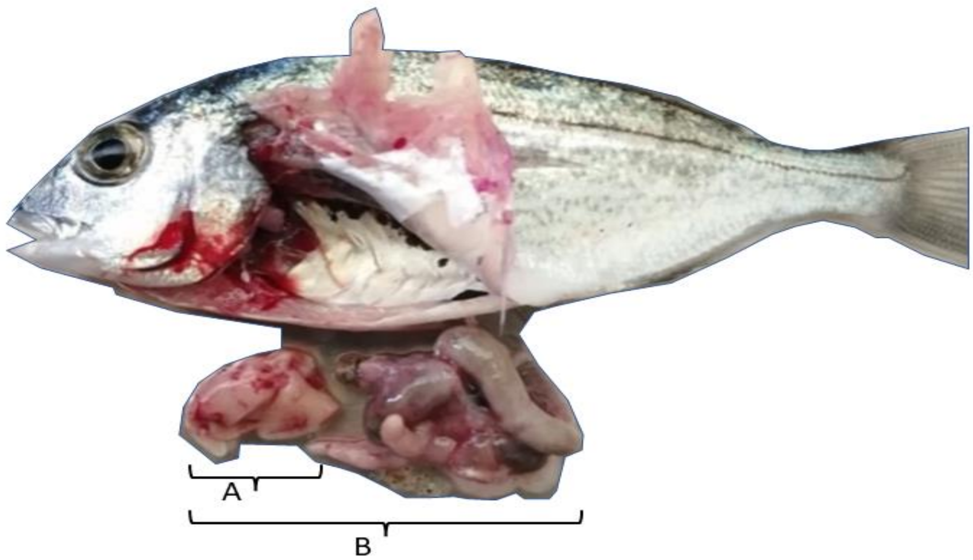


Figure 4: Dissected Gilthead seabream (Sparus Aurata) fish with removed viscera. A=Liver and B= Viscera

Lastly whole fish with viscera was kept in an oven for 24h at 100 ˚C to dry out so the dry weight was also measured.

The above indices were calculated with the following formulas:

$$\text{Viscerosomatic index (VSI) (\%)} = 100 * \frac{\text{viscera weight (g)}}{\text{body weight (g)}}$$

$$\text{Hepatosomatic index (HSI) (\%)} = 100 * \frac{\text{liver weight (g)}}{\text{body weight (g)}}$$

Lastly, feed conversion ratio (FCR) was calculated in order to gain information on the relationship between the input of the feed that has been fed and the weight gain by a population during a certain period of time. For the estimation of FCR, the number of deaths and the number of fish removed due to sampling was taken into consideration. FCR was calculated using the following formula:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed given (g)}}{\text{weight gained (g)}}$$

2.3 Statistical analysis

All results are presented as Mean ± Standard deviation. All statistical analysis that occurred for evaluating the difference between the three groups were analyzed in RStudio software. Firstly, one sample Kolmogorov- Smirnov test, was appropriate to determine whether samples were normally distributed (goodness of fit). After the normality test, One-way Anova was used between the three groups for each sampling to determine if the three groups had a significant difference in food consumption, growth characteristics (Total length, fork length, head length, standard length, wet weight, dry weight and Specific Growth Rate) and in Feed conversion rate (FCR). Furthermore, characteristics that shown a significant different, Tukey test was conducted to indicate between which groups the difference was present.

3. Results

3.1 Survival and food consumption

The experimental diets were accepted by fish although some mortalities were present. After 5 weeks of feeding three diets containing different vitamin C levels, Group A with no added vitamin C had a significantly higher survivability (99.3%; n=298) than the other two groups (P= 0.025, P<0.05). Groups B and C with 500mg/kg and 2000 mg/kg had a lower survivability of 97% (n=291) by the end of the experiment. Most of the mortalities recorded in all groups were seen in the first 24 hours during the acclimatization of the fish with 2, 5 and 4 fish respectively (Figure 5). Afterwards, mortalities were only observed in group B and C.

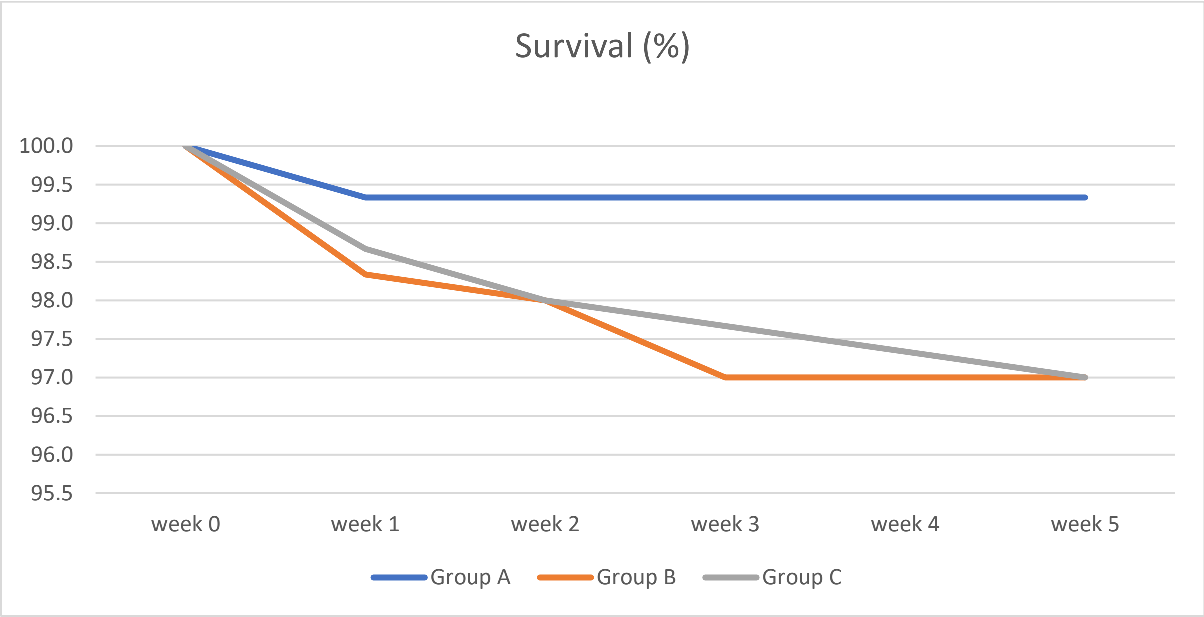
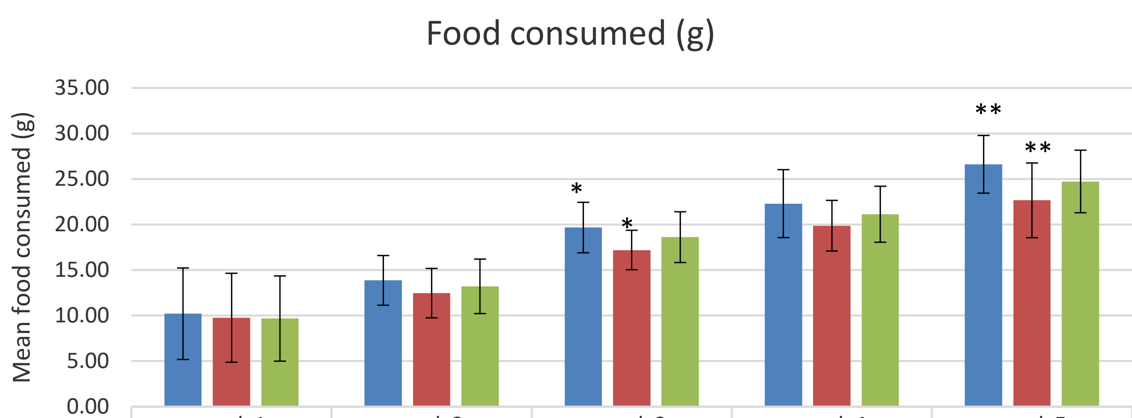


Figure 5: Survival of fish Gilthead seabream (Sparus aurata) during experiment. Group A= 0 mg/kg, Group B= 500 mg/kg, Group C= 2000 mg/kg Vitamin C

As far as feed consumed by the fish is concerned, for week 1, 2 and 4 fish from all three groups fed approximately the same amount (week 1; P=0.895, week 2; P= 0.28 and week 4; P= 0.056).

Week 3 and 5 of the experiment, One-way Anova showed that Group A consumed the most food (Week 3 P= 0.01 and week 5 P=0.003, P<0.001) although a significant difference was seen only between group A and group B (Tukey test, p= 0.009 & P=0.002). Mean food consumed can be seen in Figure 6.



	week 1	week 2	week 3	week 4	week 5
Group A	10.21	13.87	19.67	22.30	26.61
Group B	9.77	12.47	17.20	19.88	22.66
Group C	9.68	13.22	18.62	21.13	24.73

Figure 6: Mean food consumption of fish for 5 weeks of the experiment presented as Mean ± standard deviation. Group A= 0 mg/kg, Group B= 500 mg/kg, Group C= 2000 mg/kg Vitamin C. Poles with Asterisk '' indicate significant difference between groups (P<0.05).*

3.2 Growth and development

By the end of the 5 weeks experiment, growth of fish did not seem to change significantly between the three groups. Growth characteristics were observed to be significantly different between the three groups, during the experiment, right after week 1 (sampling 2) and week 3 (sampling 4). Specific growth rate, during the first sampling seems to be really low, whereas during the 2nd and 3rd sampling, SGR increases rapidly. After the first week, Group C had the highest specific growth rate (SGR) of 2.65 % despite group A and B with 1.55 % and 1.2 % respectively. During the same sampling (after week 1) Group C had a significantly larger total (Tukey test p=0.047) and head length (Tukey test; p=0.0048) than group A. Except 2nd sampling, after week 3 (sampling 4) group's A specific growth rate (SGR) was the highest with 7.32 %. Also in sampling 4, all growth characteristic shown to be significantly different between groups (Anova; P< 0.05). Furthermore, Tukey test indicated that wet weight, dry weight, total length, standard length and fork length of group's C fish were significantly higher of those of group A and B (p<0.05) (Figure 7).

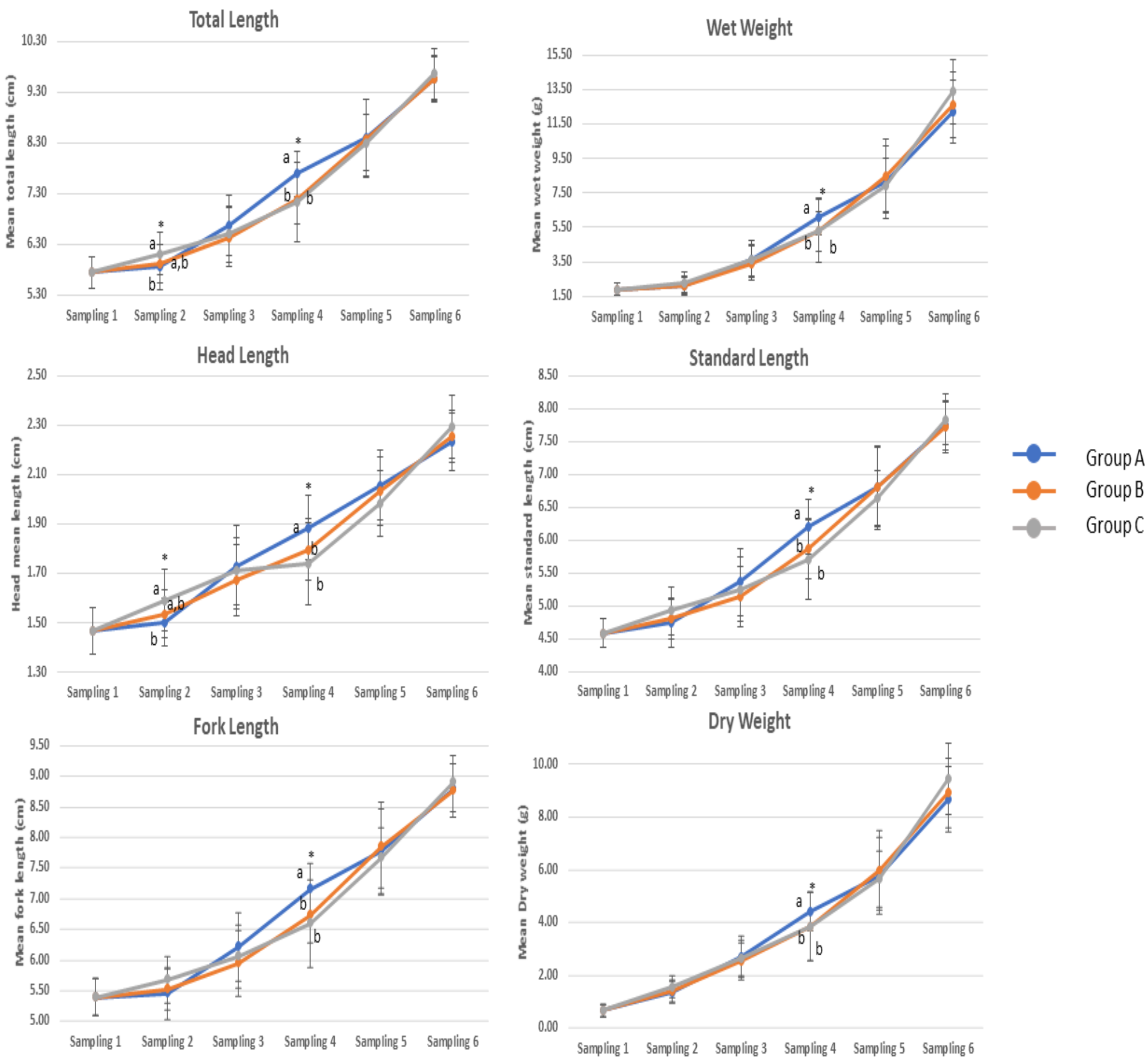


Figure 7: Growth characteristics graphs for gilthead seabream during the experiment. Group A= 0 mg/kg, Group B= 500 mg/kg, Group C= 2000 mg/kg Vitamin C. Asterisk (*) indicates significant difference between groups. Different letters on such points indicates which groups are significant different.

Despite that fish from group A shown to have a significantly higher growth during week 3 (sampling 4), the Viscerosomatic (VSI) and Hepatosomatic (HMI) index group C was not significantly different. Although, during sampling 5, group C shown to have a significantly lower VSI and HMI than the two other groups (Tukey test, $p<0.05$). VSI and HMI for all groups shown to have their maximum value during sampling 4 as following to week 4 indices tend to decrease. Fish fed diet with 2000 mg vitamin C had the lowest FCR with an overall mean of 1.09± 0.68. Higher FCR was seen for group B with 1.36 ± 1.35 and group A with 2.05 ± 3.54. Despite this, there was no significant different between groups for the FCR (Anova; P=0.48, P>0.05). Relatively to time, feed conversion ratio had greater values only in week 1 as by week 2 it decreased and stayed approximately Constand (Table 2).

		Group A (0 mg/kg vitamin C)	**Group B** (500 mg/kg vitamin C)	**Group C** (2000 mg/kg vitamin C)
Feed conversion ratio (FCR)	sampling 1	-	-	-
	sampling 2	6.71 ± 6.84	3.82 ± 1.16	2.09 ± 0.97
	Sampling 3	0.72 ± 0.08	0.79 ± 0.25	0.96 ± 0.48
	sampling 4	0.81 ± 0.16	0.85 ± 0.11	0.99 ± 0.11
	sampling 5	1.22 ± 0.43	0.68 ± 0.17	0.86 ± 0.09
	sampling 6	0.80 ± 0.25	0.67 ± 0.11	0.56 ± 0.04
Viscerosomatic index (VSI)	sampling 1	6.63 ± 1.02	6.63 ± 1.02	6.63 ± 1.02
	sampling 2	7.06 ± 2.20	7.37 ± 1.80	8.14 ± 3.01
	sampling 3	10.92 ± 0.99	10.62 ± 1.57	11.02 ± 1.42
	sampling 4	11.31 ± 0.93	11.17 ± 0.82	11.03 ± 0.86
	sampling 5 *	10.48 ± 1.18 ^{a}	10.25 ± 0.90^{a}	9.52 ± 0.79^{b}
	sampling 6	9.70 ± 1.02	9.76 ± 0.84	9.58 ± 0.98
Hepatosomatic index (HSI)	sampling 1	1.43 ± 0.29	1.43 ± 0.29	1.43 ± 0.29
	sampling 2	1.19 ± 0.55	1.22 ± 0.66	1.09 ± 0.57
	sampling 3 *	2.68 ± 0.72 ^{a,b}	2.53 ± 0.58 ^{a}	2.94 ± 0.57^{b}
	sampling 4	2.99 ± 0.50	2.88 ± 0.46	2.86 ± 0.48
	sampling 5 *	2.62 ± 0.41 ^{a}	2.63 ± 0.37 ^{a}	2.40 ± 0.38^{b}
	sampling 6	2.66 ± 1.20	2.47 ± 0.33	2.48 ± 0.41
Specific Growth rate (SGR %)	sampling 1	-	-	-
	sampling 2	1.56	1.21	2.65
	sampling 3	7.68	7.07	6.41
	sampling 4	7.32	6.19	5.54
	sampling 5	4.16	6.84	5.75
	sampling 6	5.81	5.66	7.44

Table 2: Specific growth rate (SGR), Feed conversion rate (FCR), Viscerosomatic index (VSI) and Hepatosomatic index (HSI) for gilthead seabream during the 5-week experiment presented as Mean ± standard deviation. Asterisk (*) indicated significant difference between groups. Different letters on such points indicates which groups are significant different.

4. Discussion

4.1 Growth, Development and Survival

By the end of the 5-week experiment, gilthead seabream did not show a significant difference in growth characteristics among the three groups fed with diets different in Vitamin C concentration. For the same species and same age, Henrique et al. (1998) observed similar results during a 12-week period; with seabream showing no significant difference between four groups (0, 25, 50, 100 and 200 mg/g Vitamin C). Despite the results Henrique stated that the lack of significant difference for growth in his study cannot be associate with the high levels of vitamin C in diets, as vitamin C concentrations present in liver and spleen were significantly lower in groups with Vitamin C free diets. For this study, such conclusions cannot be made as vitamin C concentration analyses in body parts, was not examined.

For this study, the lack of significant difference can be due to short duration of the experiment that was contacted. All studies that showed a significant difference between groups with and without Vitamin C in diet, were contacted for a period of at least 8 weeks. For example, similar aged fish but different species like the Japanese parrot fish (*Oplegnathus fasciatus*), Japanese flounder (*Paralichthus olivaceus*) and hybrid Tilapia, after 8, 9 and 10 weeks respectively, a vitamin C free diet resulted in poorer growth and deficiency signs (Ishibashi, Y., Ikeda, S., Murata, O., Nasu, T., & Harada, T., 1992; Teshima, S.I., Kanazawa, A., Koshio, S., Itoh, S.,, 1993; Shiau, S.Y., Hsu, T.S., 1995). Furthermore, the lack of significant difference between groups can be also due to the way vitamin C was mixed with the basic dietary formula pellets. In current study Vitamin C, in powder form, was mixed in pellets and let for 48 hours for better absorption. Several studies contacted, examining the effects of dietary vitamin C or other key ingredients in fish performance, used feed was created by scratch as the mixture was blended and cooked with vitamin C. Furthermore, the use of kitchen mixer and mincer, help them achieve the desired size (Alexis et al., 1997). Another method used by Ortuno et al. (1998) who analyzed the non-immune response of gilthead seabream by the intake of high dietary Vitamin C, suspended the vitamin C in fish oil daily and then sprayed it on the feed (Ortuno, Esteban & Meseguer, 1998).

Significant difference for growth characteristics was observed during the experiment in sampling 2 & 4. Group A showed a significant increase in both wet weight, dry weight and length measurements. This difference, is not associate with the concentration of Vitamin C in the feed as it was only observed in sampling 4. The significantly more feed consumption by group A, during week 3, in conjunction with the better FCR, can be the factor triggering this increased growth. In contrast, the significantly higher consumption of feed during week 5, did not result in an increased growth because FCR of group A was the worst. Fish fed the vitamin C free diet showed to grow more rapidly, during the experiment as SGR reached a peak of 7.68% during sampling 3. Considering that group A ate more feed, SGR of fish had declined rapidly to 5.81% by the end of the experiment as fish from group C showed to increase their SGR from

6.41% (sampling 3) to 7.44%. Similarly, Hepatosomatic (HSI) and Viscerosomatic (VSI) index had their peak values for all groups (Table 2) during sampling 4 as by the end of the experiment showed to decline. Furthermore, Group C despite having an increasing growth rate for the last two samplings, its hepatosomatic and viscerosomatic index decreased more rapidly than the other two groups, resulting in a significantly lower VSI and HSI at sampling 5 (Table2).

Despite that fish growth was not affected by different vitamin C levels, fish with a vitamin C free diet showed a significantly lower mortality than the other two groups (Figure 5). Fish from Group A showed mortalities only during the first 24 hours, that fish were still acclimating before feed was supplied. On the other hand, group B and C had mortalities throughout the duration of the experiment. In the majority of other studies; survival of fish was not affected by the dietary vitamin C (Dawood et al., 2016; Henrique et al.,1998; Saleh et al., 2021). However, in the study of Alexis et al. (1997), after 30 days, gilthead seabream suffered heavy mortalities on a vitamin C free diet.

4.2 Growth, Development and Food consumption

Food consumption by fish was approximately similar between groups during the first two weeks, with a slight increase between week 1 to week 2. Apart from the first week where all groups ate approximately the same amount of feed, for the rest of the experiment, fish from group B ate the least food between the three groups. Group A consumed the highest amount of feed, although only for week 3 and 5 consumption was statistically higher for group A. As far as food consumption is concerned, similar results were observed by Henrique et al. (1998), where fish fed Vitamin C free diet consumed significantly more feed than fish fed with a diet enriched by 100 and 200 mg/kg of Vitamin C.

Food conversion ratio (FCR), during the first week, is shown to be very high, which means that the efficiency of the feed (in terms of how much feed is required to produce 1 Kg of fish) was worse than the rest duration of the experiment. These increased values must be due to acclimatization of fish. Despite the absent of significant difference between group's FCR, by the end of the experiment Group with Vitamin C free diet had a higher FCR than groups consumed 500 and 2000 mg/kg of Vitamin C (Table 2). Higher FCR for fish consumed Vitamin C free diet was also seen by Saleh et al. (2021) after 60 days of experimenting on gilthead seabream with a slightly lower initial body weight (0.5 g). Saleh et al. (2021) after feeding fish with 0 and 500 mg/kg of Vitamin C observed a significant higher FCR for fish feeding on Vitamin C free diet.

As far as this study is concerned, associating the results of FCR with feed consumption and growth rate, can explain why despite the higher food consumption for group A, there was no significant difference in growth characteristics by the end of the experiment. Henrique et al. (1998) observed similar results with fish fed vitamin C free diet, which, although, consumed significantly more feed than fish with vitamin C in their diet, did not show any significant difference in growth between groups. This result indicates the need of more feed intake for fish without additional vitamin C to achieve same performance as fish feeding with Vitamin C. Furthermore, the late (week 5) increase in Specific Growth Rate and growth characteristics of fish fed 2000 mg/kg Vitamin C in their diet (fish from group C have higher but not statistically different growth characteristics than the other two groups in sampling 6), can be a sign that Vitamin C, as it plays a significant role in other biological functions, needs a certain period of time in order to affect the growth characteristics of fish. Several studies have shown that dietary Vitamin C intake in gilthead seabream for a duration lower than 5 weeks, had a positive effect in innate immunity after exposed to different diseases. For example, Ortuno et al. (1999) after 2 weeks of feeding 3000 mg/kg of Vitamin C in diet had a positive effect in fish against phagocytosis. Furthermore, according to Trichet et al. (2015), increased survival and growth will be achieved by good health status followed by a good immune response (Trichet, V., Santigosa, E., Cochin, E., & Gabaudan, J. , 2015)

5. Conclusion

Living in a world with growing population, demand for food will increase and marine-based food will be the key to future food security. In aquaculture, the fast production of healthy fish is essential for meeting future demands as growth rate, survival, and feed conversion efficiency have been proven to minimize space, water, and feed requirements. Vitamin C is key nutritional element in promoting optimal survival and performance for all living creatures and especially in fish. Due to the fact that teleost fish lack the ability in synthesizing Vitamin C (ascorbic acid), an exogenous source is essential in their diet. The aim of this study is to investigate the effect of different levels of dietary Vitamin C in growth characteristics and development of gilthead seabream. Furthermore, the effect that Vitamin C has on feed conversion ratio (FCR) was observed in order to observe if additional Vitamin C improve FCR. Three groups (three tanks/group) of 100 gilthead seabream fingerlings per tank were fed diets, which contained different Vitamin C concentrations. The concentrations of vitamin C used were 0, 500 and 2000 mg/kg of feed.

By the end of the experiment, fish between groups did not show any significant difference in growth characteristics, although fish fed Vitamin C free diet showed to have a better increase in growth. Despite the lack of different in growth characteristics, fish fed Vitamin C free diet consumed more feed than the other two groups. Furthermore, feed conversion ratio of fish has been observed to be slightly lower for fish fed a diet supplemented with Vitamin C, but not statistically different. Taking into account the feed conversion ratio, feed consumption and lack of difference in growth characteristics, it can be concluded that fish fed vitamin C free diets need more feed in order to achieve same performance and growth with fish fed Vitamin C diets. Being able to reduce the amount of feed given to fish without effecting performance; not only feed expenses will reduce but also a reduction in the amount of uneaten feed will go wasted in the sea, reducing the amount of nitrate and phosphate loss that will affect different parts of the marine ecosystem.

A limitation of this study was the fact that the experiment lasted only for 5 weeks and such duration may not be enough to determine the effect of Vitamin C in growth characteristics.

Lastly, for future research on the effects of Vitamin C in fish, it is strongly suggested to undertake a Vitamin C body and serum concentration analysis. Such analysis is more detailed and sensitive as it can be used to observe Vitamin C concentrations in fish body parts before and after Vitamin C supplementation. Furthermore, such analysis can be used before and after fish exposure to stress factors (example: hypoxia) to observe Vitamin C secretion in body parts and blood as serum Vitamin C enhances immune responses.

6. References

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