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ORIGINAL RESEARCH PAPER

INFLUENCE OF VITAMIN C AND E ENRICHED FEEDING ON GROWTH AND CHEMICAL COMPOSITION OF RED TILAPIA (*OREOCHROMIS* SPP.)

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The objective of this study was determining the influence of enriched feeding with vitamin C and E on growth and chemical composition of red tilapia (*Oreochromis* spp.). A completely randomized design was used in this experiment. No statistically significant differences were observed for humidity, while all other parameters evaluated in this study showed differences. The results obtained showed enhanced growth in terms of size, weight and growth rate, and a 100% survival in the fish using the combination of vitamin C and E. The chemical characterization indicated that protein ranged from 20.36% to 21.56%, humidity from 73.04% to 76.52%, ash from 1.29% to 3.27% and ether extract from1.83% to 3.23% for treatments 1 and 4. The obtained results suggest that the fish growth increased and red tilapia steaks have high nutritional value using the fodder in combination with vitamin C and E.

Keywords: Chemical composition, growth, red tilapia, vitamins C and E

Introduction

Tilapia (*Oreochomis* spp.) occupies one of the main cultures, because it is presented as the most advantageous alternative for the production of healthy proteins and low cost, given their high yields, because their growth is higher than that of other species in intensive culture systems. The red tilapia is a warm water fish, fast growing and with pleasant taste. The chemical composition of the steaks may vary depending on the quality of food and environmental conditions. Their meat is of excellent quality and therefore has good market acceptance. From a

nutritional standpoint contains 19.2% of total protein, 2.3% fat, and low cholesterol levels, 96 Kcal/100 g metabolizable energy, which makes food healthier than pork, poultry and beef (Toledo and García-Pérez-Capote, 1998).

Hernández-Sánchez and Morales-Aguilera (2012) evaluated the nutritional value and importance of the tilapia consumption in the region of Mexico Papaloapan. They concluded that it has a balanced composition of proteins, lipids, minerals and multivitamins. In the case of protein, they contain all essential amino acids for a complete meal. Furthermore, the tilapia having from 1 to 8% of total lipids allows classifying it as a fish with soft flavor because this attribute is closely related to the levels of lipids.

In another study Jover (1998) evaluated the body composition of tilapia (*Oreochomis niloticus*) with feed of different protein concentrations. The researcher observed that the increased protein and fat levels decreased with an increase of food proteins. The value obtained in the study was 29.85% for dry matter, 56.54% for crude protein, 21.79% for crude fat and 17.26% for ash (Jover, 1998).

Izquierdo et al. (2000) studied twelve species of different fish (*Peristedion cataphractum*, *Prochilodus magdalenae*, *Colossoma macropomum*, *Cyprinus carpio*, *Argyrosomus regius*, *Liza saliens*, *Epinephelus marginatus*, *Merluccius hubbsi*, *Pagrus pagrus*, *Centropomus undecimalis*, *Oncorhynchus mykiss* and *Oreochromis* spp), to determine their proximate composition, fatty acid profile, essential amino acids and minerals. They concluded that tilapia is a species that should be included in the daily diet of the people, especially infants, because it contains 72.3% humidity, 22.34% protein, 1.94% ash and 2.26% fat. Moreover, the consumption of these compounds provides essential amino acids, essential fatty acids that significantly reduce plasma triglyceride levels and tend to decrease cholesterol and increase the fraction of cholesterol of high density lipoprotein (HDL).

At the University of Oriente, Núcleo de Monagas, was conducted a study which compared the nutritional value of red phenotypically tilapia with black phenotypically reared in laboratory conditions. The researchers found that the value of physico-chemical analysis were similar to those established by Norma COVENIN (1980a, 1980b, 1980c, 1984, and 1995). The proximal analysis revealed that nutritional value of phenotypically red tilapia was higher than the phenotypically black (Parias, 2008). In agreement with the above, the present study aimed at determining the growth of red tilapia fed with fodder and vitamins C and E. Also, the chemical characterization of steaks was evaluated.

Materials and methods

48 juvenile fish of both sexes provided from the culture tanks of Fish Laboratory with sizes between 12.84 cm and 14.85 cm of total length were selected. After selection, the distribution of the specimens was performed at three fish per tank, for a total of 16 tanks (A-1, A-2, A-3 ..... A-16), either one with 90 L capacity and were prepared one week in advance. For this, the tanks were filled with water from

the culture tanks and were connected to a continuous aeration system to maintain the stable temperature and oxygenation of the water. The growth of tilapia was determined every 7 days by weighing into a Sartorius brand balance and the total length was measured with a graduated rule of 30 cm from the front of the head (snout) to the end of the fin flow. Moreover, the growth rate was determined according to Jover et al. (1998) using the following formula:

Growth rate (GR) = 100 [ln (final weight/initial weight) /time]

Survival (S) was calculated with formula proposed by Pineda (1999):

S = (final number of fish /initial number of fish) x 100

Develop diets and food for fish

Before diet development, it was first necessary to determine the amount of food that was distributed in each aquarium fish. This was adjusted weekly according to the biomass in each tank with a feed rate of 8%. For fish feed there were used dietary supplements (DS) specially formulated for tilapia with 28% of Crude Protein (CP) as control treatment. This value was confirmed by proximal analysis of food performed in the Animal Nutrition and Feed Laboratory (Table 1). The same feed was supplemented with 0.1 g / kg diet of vitamin C (Treatment 2), with 0.05 g / kg of vitamin E (Treatment 3) and with the mixture of 0.1 g of vitamin C and 0.05 g of vitamin E (Treatment 4).The fish were fed for 45 days twice a day.

Table 1. Proximate composition of the fodder to tilapia in the study

IDENTIFICATION	% DM	%OM	% ASH	% CP	% CF	% EE
FOOD FOR TILAPIA	90.36	84.43	5.93	28.85	5.64	3.68

%DM: percentage of dry mass;
%OM percentage of organic matter;
%CP: percentage of crude protein;
%CF: percentage of crude fiber;
%EE: percentage of ether extract

Proximal or body composition of tilapia steak

The determination of the proximal composition of tilapia steaks (*Oreochromis* spp.) fed with dietary supplements and vitamins C and E was performed at the Laboratory of Animal Nutrition and Feed by Faculty of Animal Husbandry. To do this, we removed the scales from fish skin and then the fish was filleted with stainless steel knife. These samples were dried in a conventional oven at 60°C/24 h, and then they were ground in an electric hammer mill (Thomas Wiley Model 4), using a sieve of 2 mm and then stored. Chemical characterization was carried out as follows: Kjeldahl method for protein (COVENIN, 1995), humidity according to COVENIN (1980), Gold fish fat according to COVENIN, (1980b) and ash content by incineration of the sample in a muffle furnace (COVENIN, 1980c).

Experimental design

In this study a completely randomized design was used, where the independent

variables were the treatments. Four treatments (T) with four replications were evaluated using 16 tank fish and 48 aquarium fish. During 45 days were given dietary supplements (DS), as follows: T1 - only DS, T2 – DS and 0.1 g / kg diet of vitamin C, T3 – DS and 0.05 g / kg of vitamin E, T4 – DS and a mixture of 0.1 g of vitamin C and 0.05 g of vitamin E. The dependent variables in the growth of the fish were: height, weight, growth rate and survival. While the dependent variables for tilapia fillets were humidity, crude protein, fat and ash.

Statistical analysis

Statistical analysis was performed using STATGRAPHICS Centurion XV (2007). The data were analyzed by ANOVA and the least significant difference test (LSD) was applied to determine differences among means with a significance level of 5%. All experimental analyses were carried out in triplicate.

Results and discussion

Determination of growth (size, weight and growth rate) and survival of red tilapia fed with fodder and supplements of vitamins C and E.

The table 2 shows the mean values for growth and survival of red tilapia. The values of the size ranged initially between 12.84 and 14.85 cm being the highest value for the T2. For treatments T3 and T4 the greatest values obtained ranged between 14.72 and 15.80 cm, although there was a slight increase in the control treatment. These results indicate that fish growth in the assay with various diets used did not markedly influence the growth of tilapia. Thus, the analysis showed no statistically significant results. However, it can be noted that the control diets obtained better growth in length than those with vitamin C and E individually or combined. So that based on data included in Table 2 the increase was 1.88 cm (T1), 0.61 cm (T2), 1.20 cm (T3) and 1.29 cm (T4). Therefore, vitamin supplements affect growth (length) of the fish. It is important to remark the lack of uniformity in terms of fish length. Lara *et al*. (2002) indicated that the type of food provided and in some cases supplemented with various vitamins may influence the growth of fish. Though, no such effect was very evident in this study. This can be because growth was studied for 45 days and perhaps it is probable that if the test time is extended, it is possible to see some differences. Similar results were obtained by Barreto (2010), who concluded that diets with vitamin C did not markedly affect the growth of tilapia. On the other, Echezuria (2010), who fed tilapia fry with concentrated vitamin E did not found any difference among fish samples with different diets. Castro et al. (2004) cultured *O. mossambicus* in hard water for 90 days and reported for size values of 15.58 cm, comparing the results obtained in this study with those authors we can say that even though the tests are similar, the sizes between the species are different. However, the initial size of the test fish is important for comparison, because it is considered by Brett et al. (1969) and Brett (1979) as a factor that influences food intake and therefore their growth.

Values are means of four replications. T 1: fodder, T 2: Fodder plus 0.1 g/kg of vitamin C, T 3: Fodder plus 0.05 g/kg of vitamin E and T 4: Fodder plus 0.1 g/kg

vitamin C and 0.05 g/kg of vitamin E.

Table 2. Growth (size and weight), growth rate and survival of red tilapia supplemented with vitamins C and E.

Treatments	Initial size (cm ± SD)	Final size (cm ± SD)	Initial weight (g ± SD)	Final weight (g ± SD)	Growth rate (%/día ± SD)	Survival (% ± SD)
T1	12.84 ± 0.72[a]	14.72 ± 0.95[a]	49.40 ± 0.68[b]	55.37 ± 2.27[c]	0.23 ±0.06[b]	58.33 ± 1.00[c]
T2	14.85 ± 2.01[a]	15.46 ± 1.64[a]	55.52 ± 0.98[a]	61.60 ± 0.84[b]	0.25 ±0.02[b]	66.70 ± 1.50[b]
T3	14.60 ± 0.69[a]	15.80 ± 1.30[a]	50.52 ± 1.00[b]	59.74 ±1.50[b]	0.37 ±0.01[a]	66.74 ± 1.49[b]
T4	14.51 ± 1.94[a]	15.80 ±1.70[a]	55.88 ± 1.63[a]	66.57 ±1.84[a]	0.38 ±0.11[a]	100.00 ± 0.00[a]

SD: standard deviation. Letters equal in the same row indicate no statistical differences between treatments at $p \leq 0.05$.

Weight

The two variables quantified for weight (initial and final) showed statistical differences. The initial weight ranged from 49.40 to 55.88 g with lower values for treatment (T1) and the highest for treatment (T4) or diet supplemented with vitamin C and E (Table 2). The statistical results allowed to express that the initial weights of the fish used for the present study were due to two homogeneous groups formed by T2 and T4 treatments with higher weights and the other group consisting of T1 and T3 with lower values than previous. In general the results indicated an increases in weight for each experimental group: 5.97 g (from 49.40 to 55.37g) for T1; 6.08 g (from 55.52 to 61.60 g) to T2; 9.22 g (from 50.52 to 59.74 g) to T3 and 10.69 g (from 55.88 to 66.57 grams) to T4 (Table 2).

The final weights of the samples ranged between 55.37 and 66.57 g, maintaining the lowest value for the control and the highest value for the treatment T4. Thus, three homogeneous groups were evident, where treatment T4 with 66.57 g represented the highest value, followed by the group formed by T2 and T3 with 61.60 g and 59.74 g, respectively. Accordingly, we find that the joint combination of vitamins C and E shows best results in weight while individually with fodder their average results are lowest (Table 2). Castro et al. (2004) reported values of final weight of 55.37 and 66.57 g. The average values reported in this study is comparable with those obtained by Castro et al. (2004), who reported for *O. mossambicus*, at 120 days after the trial, a weight of 46.6 g, followed by *O. niloticus* with 26.1 g and *O. aurea* with 21.5 g. It can be noted that the values obtained by Castro et al. (2004) are below those reported in the present study, and the differences might be due to the different fish species used in the experiments. It is worth mentioning, that the work of Castro-Rivera et al. (2004) was performed on 30 days old fish that were grown for 120 days and reached sizes between 15.21 and

20.17 cm. The tilapia samples used in the present study reached sizes between 14.7 cm and 15.8 cm after the 45 days of growth, and in both cases the specimens between youth and adult stage complied with the classification of Morales (2003).

Growth rate

The growth rate of the fish showed statistical differences with values between 0.23 %/day and 0.38%/day, corresponding to the lowest value and the highest value at T1 and T4 respectively. These results indicate that the dose of vitamin C and E used contribute to the increase of the growth rate in tilapias studied. The results indicate that the concentrations of vitamins C and E used influence on the rate of growth of the tilapias because the higher values were obtained in the T4 and T3. The results of growth rate obtained in this study (0.23 to 0.38 % per day) can be compared with those reported by Mora et al. (1997) who obtained 4.7 % per day of absolute growth rate in the hybrid red tilapia reared in water reservoirs in sugar cane plantations. Moreover, when working on *Oreochromis mossambicus* x *O. niloticus* hybrid, Mena et al. (2002) obtained a percentage of weight gained of 4.27 % per day. It can be noted that our results are below those reported by Mora et al. (1997) and Mena et al. (2002) and might be explained by the different test conditions, the type of food provided and the age of the specimens.

However, the values of this study are above those reported by Salazar and Ascanio (2007), who reported 0.001 and 0 06 %/d growth rate of hybrid tilapia (*Oreochromis* spp.). This might be due to the size of fish, because they used fry and in this study fish with juvenile sizes were considered.

Delgado-Vidal et al. (2009) indicated that by feeding tilapia (*Oreochromis niloticus*) with compensatory growth using banana flour, they found growth rate values ranging from 0.40 %/day to 5.76 %/day as obtained for treatment T4. We can say that although the growth rate was not continuously evaluated in this trial, only at the end of the experiment, the values of this study may be very close to the range that is mentioned in that investigation. The growth rate decrease in time can be associated with the versatility of this species to assimilate and transform nutrients (Delgado-Vidal et al., 2009).

Percent survival

The survival variable showed significant differences between treatments ($p \leq 0.05$). The values ranged between 58.33 and 100.00 %, the lowest and highest values being obtained for T1 and T4, respectively. These samples differed significantly from T2 and T3 which had comparable values (Table 2). These results indicate that the inclusion of vitamins in the diets of tilapia improved its survival. According to Olabuenaga (2000) the combination of vitamin C with E increases in hybrid defence system, resistance to withstand environmental changes and experimental management practices to which they are subjected. In addition, Corredor and Landines (2006) indicate that adding vitamins in fodder promotes growth, improves the immune response, the metabolism of nutrients, the resistance to stress, and the characteristics of the final product. However, vitamins C and E are

among the most important nutrients that have a positive influence on the immune system of fish (Pulsford et al, 1995; Montero et al, 1999). Our results concerning the T4 treatment with 100% survival are comparable with those of Castro-Rivera et al. (2004), who reported mortalities of 3.3%, 0.7% and 0.5% for *O. mossambicus*, *O. aurea* and *O. niloticus*, respectively, equivalent to 96.7, 99.3 and 99.5% survival, respectively.

When working on *O. niloticus*, Marengoni (2006) reported different percentages of survival (98.53, 99.06, 98.93 and 98.27%) in culture at different densities. The values obtained by these authors are below that found in this study, and this is attributed to the combination of the vitamins used.

The benefits of vitamin C can be noted because the effect of promoting the survival and optimal performance of fish (Misra et al, 2007) and because it is used as an immunomodulator and as a nutritional compound key in modern fish farming, in promoting survival and optimum performance of fish (Verlhac and Gabaudan, 2007).

Vitamin C is necessary for collagen synthesis and red blood cells because they contribute to the well-functioning immune system, plays a role in iron metabolism, participates in the formation of certain neurotransmitters like serotonin, in the transformation of noradrenalin and dopamine, in other hydroxylation reactions which include aromatic amino acids and steroids (Chagas and Val 2006).

Vitamin E promotes physiological profile, protects fish under stress and decreases the chance of getting sick (Ortuño et al, 2000; İspir et al, 2011). Moreover, vitamin E can reduce mortality and improve the performance of the fish (Ortuño et al, 2001; Hamre, 2011).

These advantages of vitamins C and E are involved in all the possible benefits that result from the addition to the food concentrate favoring the survival of fish in this investigation and possibly demonstrated improving the fish health and immune system.

Determination of proximal composition of tilapia (Oreochromis spp.) fed with fodder and vitamins C and E.

In Table 3 are presented the averages obtained from the proximal analyzes of tilapia steaks, which were fed with fodder and vitamins C and E. The treatments showed significant differences in most of the evaluated variables.

Table 3. Average approximate composition of tilapia fed with balanced food and vitamin supplements.

Treatment	Protein (%)	Humidity (%)	Ash (%)	Fat (%)
1	20.36^{b}	76.52^{a}	1.29^{d}	1.83^{c}
2	21.56^{ab}	73.47^{a}	2.38^{c}	2.53^{b}
3	20.45^{b}	73.53^{a}	2.92^{b}	3.03^{a}
4	20.45^{b}	73.04^{a}	3.27^{a}	3.23^{a}

Different letters in the same column indicate significant differences ($p< 0.05$)

Crude Protein

The average values of protein ranged from 20.36 to 21.56%, with significant differences ($p < 0.05$) between treatments. These values are reported in tilapia steaks and should be noted that they have high protein content. Stansby (1962) reported that values close to 20% permitted to consider tilapia a species with high protein content, comparable with proteins from other animals such as bovines, sheep and pigs. Farias (2008) and Toledo and García-Pérez-Capote (2000) reported values of 18.95% and 19.2%, respectively; both values are below those found in our experiment. However, Izquierdo *et al.* (2000) indicate values of 22.34% for protein, which is above the values obtain in our study. Also, Hernández-Sánchez and Aguilera-Morales (2012) reported values of 13-25% for protein.

The value of protein found in this study may be related to the quality of the diet provided, the amount of food consumed, nutritional efficiency, tilapia species, age, size, sex, reproduction, among others. These results are in agreement with those reported by Marengori (2006).

Moreover, Izquierdo *et al.* (2000), Gonzales and Brown (2006) reported that tilapia proteins are of excellent quality, because they have all the amino acids and a high biological value.

Humidity

The mean values from humidity ranged between 73.04% and 76.52% (Table 3), no significant difference was found between treatments. The higher value of 76.52% was found in T1 (without vitamins) and the T3 (with vitamin E), indicating that the use of vitamins in feed caused no variation for humidity of red tilapia steaks. The values obtained in this study are compared with those of 77.18%, reported for the same species by Parias (2008). It can be noted that this is similar to the present investigation. Other researchers such as Lawrence (2011) reported 78.12%, Hernández-Sánchez and Morales-Aguilera (2012) indicated values of 72-80% for humidity, which are higher than the present result. However, COVENIN standard (1994), No. 3086 requires humidity values for fish meat between 70 and 82%. The results obtain in the present study are within in the range mentioned. Similarly, Hall (2001) reported that humidity for fish varies between 60 to 84%, with an average of 74%.

Higher or lower values of humidity in foods cause physical state, presence or absence of microorganisms, among others. Furthermore, the available water is a good catalyst for different alteration reactions. Therefore it is considered as one of the factors which must be controlled carefully to prevent spoilage of certain foods (Fennema, 2000). According to the statement, tilapia steaks should be properly preserved to prevent damage.

Ash

As shown in Table 3, the ash percentage values ranged between 1.29 and 3.27% and significant differences ($p < 0.05$) were found between treatments. The treatment

4 had the highest percentage of ash, 3.27% while for treatments 3, 2 and 1 percentage of ash was 2.92%, 2.38% and 1.29%, respectively. These results indicate that the vitamins increased the percentage of ash in tilapia steak. In this case, the increase in the ash was due to the remaining bones after manual filleting. However, fish can absorb dissolved minerals from water through gills. Moreover, vitamin C is known to improve iron absorption, therefore increasing the ash content when diets are supplemented with this vitamin (Chagas y Val, 2006). In the other hand, the vitamin E is used associated with ascorbic acid to enhance growth, improve immune response, metabolism of nutrients and resistance to stress, while improving the characteristic of the final product (Corredor and Landines, 2009).

Muller and Tobin (1990) reported for ash values between 0.5 and 1.8% in fish. Also, Hernández-Sánchez and Aguilera-Morales (2012) obtain values between 0.5 and 1.5%. Instead, for red tilapia Parias (2008) and Lorenzo (2011) reported a rate of 1.14% of ash. It can be seen that only treatment 1 is in agreement with results reported by various researchers. Therefore, treatment 2, 3 and 4 are the given values. However, the values obtain in our study was in the range of up to 7% ash according with COVENIN standard (1994), No. 3086. The determination of ash can give us an idea of the mineral content in food (Fischer, 1991). A mineral varies in different types of fish, in particular, to their habitat.

In *Oreochromis niloticus* minerals found in greater proportion are calcium, phosphorus, iron and magnesium (Gonzales and Brown, 2006). On the other hand, Hernández Sánchez and Morales-Aguilera (2012) agree with the above comment, except for tilapia, noting that the residues of red tilapia species contain more minerals in the body and steaks.

However, iron is produced in large amounts in steaks and is recommended in diets for children, adolescents and adults. Also, Paria (2007) recommends a diet low in minerals for heart patients. Therefore, minerals present in steaks fish are of interest in the diet in order to ensure good nutrition.

Fat

The values of the ether extract or fat ranged between 1.83% and 3.23% (Table 3), statistical differences were showed between the treatments. The highest value was obtained for T4: 3.32%. In case of treatment 3 a fat content of 3.03% was obtained, which is comparable with the value for T4. The lowest values of the fat content were obtained for T2 and T1, being 2.53% and 1.83%, respectively (Table 3). Parias (2008) and Lorenzo (2011) reported values of 1.76% and 1.3% fat lower than that our results. These results indicate that although the treatment was one that did not contain vitamins was superior other studies that compare. Also, when vitamins C and E are added in food increased the percentage of fat in tilapia steaks. Therefore, the addition of vitamins stimulates fatty acid synthesis and despite the fact that the steaks are under stress the fat content increases. Hernández Sánchez and Morales-Aguilera (2012) reported values ranging from 0.79 to 8.5% fat in tilapia. The values obtained in this study are within the range quoted by these authors. These researchers argue that there are many factors that determine the

percentage of fat present in species such as water temperature, habitat, salinity environment, type of diet, and other factors. According to the results obtained, it can be concluded that the above mentioned factors significantly improve the quality of tilapia meat. By supplementation of vitamins C and E in feed given of fish and due to the interaction of these can improve nutritional value of red tilapia steaks.

Conclusions

The combination of vitamins C and E in the fodder increased the size, weight and growth of red tilapia. In addition, the enriched feeding allowed the highest percentage of survival of fish in the study. The use of the vitamin supplements in diets increased values of the chemical composition of red tilapia steaks. Overall the combined treatment of vitamins in the diet was more effective to increase the values of protein, humidity, ash and fat in meat compared with treatments that used vitamins individually. Therefore, the nutritional value of tilapia meat was significantly improved by combined vitamins supplement.

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