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Full length Research Paper

Effects of selenium and vitamin E supplementation in the diet on the pacu´s productive performance

***Romário Jobim Machida, Sérgio Barrichello and Eduardo Vieira**

Post Graduate in Aquaculture and Sustainable Development, Universidade Federal do Paraná, Rua Pioneiro, 2153, Palotina, Paraná 85950-000 Brazil.

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Current study evaluates the supplementation of selenium and vitamin E in the diet on performance, body yields and lipid stability of pacu. Seven hundred and twenty juveniles were distributed in 36 cages installed in a masonry tank. During 100 days, the animals were fed on diets supplemented with four selenium levels (0, 1, 2 and 4 mg/kg), combined with three levels of vitamin E (0, 100 and 200 mg/kg) in a 4x3 factorial arrangement. Selenium combined with vitamin E levels did not affect (P>0.05) growth and body yield of pacu. The interaction between levels of selenium and vitamin E did not influence (P>0.05) the lipid stability of main trunks. When the levels of vitamin E only are evaluated, 200 mg vitamin E/kg diet decreases lipid oxidation in 90 and 120 days of storage. The combination of 0 Se + 0 vitamin E / kg diet was influenced by storage time, and the highest rate of lipid oxidation was observed with 120 days of storage. Level 1 mg selenium + 200 mg vitamin E/kg in the diet of the pacu decreased meat lipid oxidation after 90 days of storage under freezing, coupled to the maintenance of performance and body yields.

Key words: Aquaculture, dietary antioxidants, lipid oxidation.

INTRODUCTION

In Brazilian aquaculture, the pacu *Piaractus mesopotamicus* (Holmberg, 1887) stands out for its high growth rate, easy adaptation to aquaculture systems and high fecundity (Sampaio et al., 2008), coupled to other factors such as its meat quality, with good acceptance by consumers (Jomori et al., 2003). Small quantities of vitamins and minerals are required for the animals normal growth, reproduction, health and metabolism (Lovell, 1998). Vitamin E is the most important metabolic antioxidant in the cell membrane, protecting it from oxidation by fatty acids and cholesterol, and reducing or inhibiting the production and action of free radicals

*Corresponding Author. Email: jobim.mchida@gmail.com

Table 1. Percentage composition and nutritional requirements of the basal diet used.

Food[1]	(%)	Calculated composition	(%)
Corn grain	40.59	Starch	31.24
Wheat bran	20.00	Total arginine	1.79
Soybean meal	20.00	Calcium	0.98
Poultry viscera meal	13.10	Digestible energy (kcal / kg)	3000.00
Commercial fish meal	5.00	Phenylalanine	1.20
Vitamin and mineral supplement[2]	0.50	Crude fiber	3.99
Salt	0.30	total phosphorus	0.93
DL-Methionine	0.18	Fat	4.40
Soybean oil[4]	0.17	Histidine	0.64
Antifungal (calcium propionate)	0.10	Isoleucine	1.09
L-Lysine HCl	0.04	Leucine	2.13
Antioxidant (B H T)	0.02	Linoleic acid	1.18
TOTAL	100.00	Total lysine	1.43
		Methionine + total Cystine	1.07
		Total methionine	0.65
		Crude protein	26.00
		Total threonine	1.03
		Total tryptophan	0.29
		Total valine	1.29

[1]Available nutrients based on Abimorad and Carneiro (2004). [2]Supplementation levels per kg feed: 12 000 IU vitamin A, vitamin D3 3000 IU, Vitamin K3 MNB 15 mg / kg, 20 mg vitamin B1 / kg, 20 mg vitamin B2 / kg vitamin B6, 18 mg / kg vitamin B12 0.04 mg / kg, vitamin C 300 mg / kg, niacin 100 mg / kg, Calcium Pantothenate 50 mg / kg; biotin 1 mg / kg, 6 mg Folic Acid / kg Inositol 150 mg / kg, Choline chloride 500 mg / kg, copper sulfate pentahydrate, 18 mg / kg, iron sulfate monohydrate 80 mg / kg, Manganese Sulfate 50 mg / kg, Zinc Sulfate 120 mg / kg; calcium iodate 0.8 mg / kg, cobalt sulfate 0.6 mg / kg, 12 combinations of selenium and vitamin E, selenium 0, 1, 2 and 4 mg / kg vitamin E and 0, 100 and 200 mg / kg.

(Sampaio et al., 2004). Besides being a component of several selenoproteins, Selenium is a co-factor and an integral part of the glutathione peroxidase enzyme (GPx) (Rotruck et al., 1973).

Vitamin E is included in the non-enzymatic antioxidant system of the animal organism, while selenium, as member of GPx, integrates the enzymatic system for antioxidant protection. Selenium and vitamin E act in synergy, since selenium cannot protect the cell or tissue components that have low GPx concentrations (Rotruck et al., 1973). However, these cell or tissue components may be protected by vitamin E, which acts as antioxidant by different mechanisms. Combined selenium and vitamin E are the main antioxidants in the organism.

Antioxidants from the diet do not have only important functions in living organisms but they may decrease the lipid oxidation in tissues after death. Studies on vitamin E supplementation in diets for fish revealed its antioxidant action in *in vivo* and in the reduction of oxidation after death, with an improvement in conservation during storage (Onibi et al., 1996; Pirini et al. 2000; Shiau and Shiau, 2001; Ruff et al., 2002; Hamre et al., 2004; Fogaça and Santana, 2007).

However, the literature reports no studies involving the relationship between selenium and vitamin E for pacu, both in performance and lipid oxidation after death. Current analysis evaluates the antioxidant effects of selenium and vitamin E supplementation in the diet on the pacu´s productive performance, body parameters and lipid stability.

MATERIALS AND METHODS

The experiment was conducted at the Institute of Research in Environmental Aquaculture (InPAA), Universidade Estadual do Oeste do Paraná (UNIOESTE), Toledo campus, Toledo PR Brazil, from March to July 2011, for 100 days. Seven hundred and twenty pacu juveniles (*P. mesopotamicus*), retrieved from a commercial fish farm, with initial average weight = 43.52±1.03 g and total length = 12.33±1.65 cm, were used in current assay. Fish were distributed in 36 cages and installed in a 200m² masonry tank. The 1 m³ cages were made of polyester coated with flexible PVC and with a 5 mm mesh. The experimental unit was a tank with 20 juveniles. Fish were kept in the experimental structures for seven days previously to adapt themselves to the experimental conditions.

The experiment comprised twelve treatments with three replicates per diet. The experimental design was completely randomized, in a 4x3 factorial arrangement, with four selenium inclusion levels (0, 1, 2 and 4 mg / kg) and three vitamin E inclusion levels (0, 100 and 200 mg/kg diet).

The experimental diets were formulated to obtain 26% crude protein and 3000 kcal digestible energy/kg (Table 1), where composition of feed was calculated with software SuperCrac (SUPERCRAC, 2004). DL-α-tocopherol was the additional source of vitamin E, with activity of 50% vitamin E, while sodium selenite

with 45% selenium availability was employed for selenium supplementation.

The ingredients for the processing of rations were initially ground in a hammer-type mill with a 0.5 mm sieve; they were then milled, weighed and mixed for the preparation of the experimental diets. Different levels of vitamin and mineral supplements were added to the mixture. The mixtures were humidified (28% water) and extruded through a EX-MICRO® mill with 10 kg/h production capacity. The diets were dried in a forced-air oven for 12 h, at 55°C, resulting in a product with approximately 10% moisture. Feeding was carried out twice a day, at 10:00 am and 5:00 pm, by apparent satiation and the amount of diet provided was weighed to estimate apparent feed conversion.

The water tank temperature was measured daily at 10:00 am and 5:00 pm and the water physical and chemical parameters (pH, dissolved oxygen and electrical conductivity) were measured once a week at 6:00 am and 4:00 pm with portable meters (YSI Model 55 Dissolved oxygen, YSI Incorporated, Yellow Springs, USA and Alkafit pHmeter AT315 SP, Alkafit, Florianópolis, Brazil). At the end of the experiment, fish were fasted for 24 h to empty the digestive tract. The fish were then removed from the experimental units and anesthetized in benzocaine at 250mg /l (euthanasia), packed in ice inside a cooler and transported to the Fish Technology Laboratory (UNIOESTE), campus Toledo, Toledo PR Brazil.

Individual measures of final weight, total and standard lengths were taken for weight gain calculation (WG = final weight - initial weight), apparent feed conversion (FCR = feed intake / weight gain) and fish survival in each experimental unit. Afterwards, the animals were then opened at the ventral abdominal cavity, from the urogenital hole up to the jaw bones, followed by careful viscera removal to prevent contamination of meat with fecal material. The head was cut and the fish, without head and viscera, were washed in chlorinated water, removing fins and skin, leaving only the trunk. Intraperitoneal fat and liver were manually removed from the viscera, and weighed. Data was used to calculate the hepatosomatic index [HSI = (liver weight / fish weight) x (100)] and intraperitoneal fat index [IGI = (intraperitoneal fat weight / fish weight) x (100)].

The main trunks were placed in plastic bags and stored in a freezer (18±2°C) for 30 days, when chemical analyzes were performed (moisture, protein, lipids and ash), according to methodology proposed by AOAC (2005).

Lipid oxidation was analyzed by the thiobarbituric acid reactive substances method (TBARS) (Vyncke, 1970) to investigate the lipid stability of meat after 60, 90 and 120 days of storage. The amount of malondialdehyde (MDA), the main substance formed during the oxidation and which reacts with thiobarbituric acid, was calculated by the standard curve equation: $y = 73.689 + 0.0223 x$ ($r^2 = 0.9968$). Results were expressed as mg MDA per kg of sample.

Data underwent analysis of variance (ANOVA) at 5% significance in a factorial arrangement, verifying the interaction between selenium and vitamin E. When significant interaction was reported, Duncan test´s was applied at 5% significance to compare means. Data were also evaluated for homogeneity of variances (Levene´s test) with statistic program SAEG 9.1 (UFV, 2007).

RESULTS AND DISCUSSION

Water temperature in the tanks averaged 20.8±2.62°C during the experiment. There was a linear temperature decrease throughout the experimental days ($y = -0.074 + 23.291 x$ $r^2 = 0.7325$) since the experiment began in March and ended in July (period of low temperatures). The physical and chemical parameters of the tank water were 4.67±0.75 mg $O_2D \cdot L\text{-}1$; 7.26±0.64 and 40.08±2.63 mS cm^{-1} for dissolved oxygen, pH and electrical conductivity, respectively. Selenium and vitamin E levels did not affect ($P>0.05$) the parameters total and standard length, weight gain, feed conversion and survival (Table 2).

There was no significant interaction ($P> 0.05$) between selenium and vitamin E on performance and survival, and selenium and vitamin E separately did not affect productive performance.

Similar results in current study were reported for tilapia (*Oreochromis niloticus*) fed on different levels of organic selenium (0, 0.25, 0.50, 1.0, and 1.5 mg Se / kg) in the diet, with no significant difference in performance (weight gain and apparent feed conversion) (Gomes, 2008). Further, in a study on juvenile *Sparus aurata* evaluating the effect of supplementation of 250 mg vitamin E/kg diet in a system of high stocking density (40 kg/m^3), Montero et al. (1999) did not report any effect ($P> 0.05$) on weight gain and survival. There was no difference in growth for the Atlantic halibut (*Hippoglossus hippoglossus*) between treatments with 189 and 613 mg vitamin E / kg in the diet (Ruff et al., 2002).

Current study confirms results by Sampaio (2003) who evaluated levels of selenium (0, 0.25, 0.50 and 1.00 mg / kg diet) and vitamin E (0,100, 200 and 300 mg / kg) for the tilapia (*O. niloticus*). The author failed to register any effect of the interaction of these nutrients on weight gain, feed conversion and survival rate. Studying largemouth bass (*Micropterus salmoide*) fed on graded levels of vitamin E (160, 280, and 400 mg/kg) associated with either 1.2 or 1.8 mg/kg selenium (Se), Chen et al. (2013) reported that vitamin E and selenium inclusion could protect largemouth bass from the oxidative damage challenged by dietary oil oxidation, although none could enhance growth and feed utilization.

The absence of any significant effect of diets on production performance differs from results by Cavichiolo et al. (2002), who evaluated the effect of vitamins C and E on Nile tilapia larvae (*O. niloticus*). They reported that treatment with 300 mg vitamin E per kg diet provided increased weight and final length of larvae, and reduced the occurrence of the ectoparasite *Trichodina* sp in tilapia larvae. In the case of hybrid tilapia (*O. niloticus* x *O. aureus*) fed on increasing levels of vitamin E, Huang and Huang (2004) found greater weight gain in tilapia fed on 62.5 IU vitamin E / kg diet. Likewise, Gonçalves et al. (2010) registered that supplementation with 400 mg / kg vitamin E improved the standard length and weight gain of tambacus (*Colossoma macropomum* × *P. mesopotamicus*).

Rainbow-trout (*Oncorhynchus mykiss*) fed on 50 ppm de vitamin E and on 0.35 ppm selenium improved gain weight and food conversion. There was also an improvement of the fillet´s functional quality when compared to control (Rodríguez and Rojas, 2014).

The highest selenium level in current experiment (4 mg/kg diet) did not cause any damage on fish performance.

Table 2. Performance and survival data of pacu *P. mesopotamicus* fed on diets supplemented with different levels of selenium and vitamin E.

Selenium (mg/kg)	Vitamin E (mg/kg)	TL[1]	SL[2]	WG[3]	AFC[4]	Survival (%)
0	0	14.07±0.18	11.63±0.08	34.15±4.85	3.30±0.60	71.67±14.43
	100	13.80±0.10	11.40±0.21	28.31±2.02	4.17±0.39	63.33±22.55
	200	14.17±0.22	11.69±0.20	38.00±4.21	2.78±0.22	83.33±7.64
1	0	14.05±0.25	11.61±0.08	31.81±4.18	2.98±0.25	71.67±20.21
	100	13.92±0.60	11.37±0.49	30.50±8.38	3.45±0.86	80.00±8.66
	200	13.95±0.17	11.51±0.27	30.87±7.78	3.71±0.63	78.33±10.41
2	0	14.11±0.28	11.55±0.24	30.18±3.77	3.79±0.62	80.00±20.00
	100	13.88±0.51	11.53±0.44	30.86±7.42	3.91±0.64	73.33±7.64
	200	14.05±0.25	11.66±0.25	32.94±4.37	3.21±0.48	83.33±5.77
4	0	13.83±0.50	11.30±0.33	27.84±7.00	3.56±1.06	86.67±15.28
	100	14.12±0.58	11.67±0.50	32.57±8.26	3.43±1.05	76.67±16.07
	200	14.02±0.40	11.66±0.21	33.54±4.78	3.34±0.51	76.67±10.41
Selenium (mg/kg)						
0		14.01±0.22	11.57±0.20	33.49±5.40	3.42±16.41	72.78±16.41
1		13.97±0.34	11.50±0.30	31.06±6.12	3.38±0.63	76.67±12.75
2		14.01±0.33	11.58±0.29	31.32±4.86	3.64±0.60	78.89±11.93
4		13.99±0.45	11.54±0.36	31.36±6.49	3.44±0.79	80.00±13.23
Vitamin E (mg/kg)						
0		14.01±0.30	11.52±0.23	31.00±4.97	3.41±0.67	77.5±16.45
100		13.93±0.44	11.49±0.38	30.56±6.20	3.74±0.74	73.33±14.35
200		14.05±0.25	11.63±0.21	33.87±5.41	3.26±0.54	80.42±8.11
Selenium x Vitamin E		ns	ns	ns	ns	ns
Selenium effect		ns	ns	ns	ns	ns
Vitamin E effect		ns	ns	ns	ns	ns

[1]Total length (cm), [2]Standard length (cm), [3]Weight gain (g), [4]Apparent feed conversion. ns= not significant (P>0.05). Data expressed as mean ± standard deviation.

In a study with *Pogonichthys macrolepidotus* juveniles fed on 0.4, 0.7, 1.4, 2.7, 6.6, 12.6, 26.0 and 57.6 mg selenomethionine / kg diet levels, Teh et al. (2004) observed a histopathologic effect only when concentrations were greater than or equal to 6.6 mg Se / kg diet. The selenium poisoning occurred in the rainbow-trout when diets contained selenium levels exceeding 13 mg / kg (Hilton et al., 1980). Thus, the level of 4 mg selenium / kg diet for pacu (*P. mesopotamicus*) does not seem to cause poisoning and deterioration in fish performance. However, Lin and Shiau (2005) emphasized that the minimum selenium level required by fish diet differed according to the species, or rather, between 0.25 and 0.80 mg / kg.

Although selenium supplementation in the diet of pacu did not cause any damage on fish performance, the concentration of 1.5 mg Se / kg diet improved growth and increased antioxidant defenses of matrinxã (*Brycon cephalus*) by increasing the activity of the enzymes glutathione peroxidase and increased the level of reduced glutathione (Monteiro et al., 2007). Trunk yield, intraperitoneal fat index and hepatosomatic index of pacu fed on diets with different selenium and vitamin E levels did not differ significantly (P>0.05) when treatments were assessed (Table 3). Moreover, selenium and vitamin E separately did not affect (P>0.05) main trunk yields, hepatosomatic index and intraperitoneal fat.

Signor et al. (2010) reported approximately a 60% main trunk yield, or rather, above the average value of 50.60% found in current analysis. An increase in fish size provided a higher main trunk yield.

The intraperitoneal fat index was lower than that reported by Bittencourt et al. (2010) and Signor et al. (2010), although similar to that observed by Hilbig et al.,(2012) for the pacu. Change in the intraperitoneal fat index occurred if fish required fat to obtain energy, a

Table 3. Main trunk yield (%), intraperitoneal fat index (% IGI) and hepatosomatic index (HSI%) of pacu *P. mesopotamicus* fed on different levels of selenium and vitamin E.

Selenium (mg/kg)[1]	Vitamin E (mg/kg)	% Main trunk	IGI	IHS
0	0	49.22±0.69	4.10±2.21	2.77±0.23
	100	49.34±4.44	4.62±2.03	2.79±0.23
	200	46.91±4.96	4.99±0.70	2.60±0.82
1	0	51.14±5.49	4.46±0.10	2.33±0.26
	100	52.82±6.16	5.84±3.30	2.85±0.53
	200	52.84±2.68	5.58±1.42	2.90±0.23
2	0	46.32±3.40	4.24±0.61	3.34±0.15
	100	52.30±1.94	5.31±1.47	2.96±0.59
	200	52.23±0.98	4.99±0.57	2.55±0.25
4	0	52.55±3.94	5.05±2.02	2.46±0.32
	100	52.41±6.60	4.54±1.23	2.67±0.71
	200	49.17±0.28	5.72±1.02	2.72±0.12
Selenium (mg/kg)				
0		48.49±3.55	4.57±1.59	2.72±0.45
1		52.27±4.42	5.29±1.91	2.69±0.42
2		50.28±3.59	4.85±0.97	2.95±0.47
4		51.38±4.19	5.10±1.39	2.62±0.41
Vitamin E (mg/kg)				
0		49.81±4.05	4.46±1.35	2.72±0.46
100		51.72±4.60	5.08±1.93	2.82±0.48
200		50.28±3.50	5.32±0.91	2.69±0.41
Selenium × Vitamin E			ns	ns
Selenium Effect			ns	ns
Vitamin E effect			ns	ns

ns = not significant (P>0.05). Data expressed as mean ± standard deviation.

fact that occurred during long fasting (Hilbig et al., 2012). However, since this experiment had an apparent satiation food supply, the fish probably did not need to mobilize lipids for energy production.

Despite the lack of significant difference (P> 0.05) in the hepatosomatic index of pacu fed on different levels of selenium and vitamin E, fish with selenium accumulation in tissues tended to have a higher hepatosomatic index (Pyle et al. 2005). However, the latter was not observed in this experiment, since the hepatosomatic index was equal for the minimum selenium level and for a greater inclusion of dietary selenium. Checking the above, Gomes (2008), who evaluating levels of organic selenium (0, 0.25, 0.50, 1.0, and 1.5 mg Se/kg) in the diet of tilapia (*O. niloticus*), reported no significant difference in the hepatosomatic index. For *P. mesopotamicus* with average weight of 377g, Hilbig et al. (2012) found a 0.77% average hepatosomatic index, a lower rate than that in that in current assay, which averaged 2.75%.

Moisture, protein, lipids and ash contents of pacus were not significantly different (P>0.05) among supplementations with selenium and vitamin E (Table 4), neither was there any difference when selenium and vitamin E levels were evaluated separately. In fact, results corroborated those by Otani (2009) who did not register any significant difference in moisture, protein and ash contents in fillets of tilapia fed on 0 and 100 mg α-tocopherol per kg diet. However, the same author observed increased lipid contents in these fillets when compared to diet without antioxidant addition, or rather, the use of antioxidants in the diet may protect the lipids of fillets from lipid oxidation, during freezing.

In a study evaluating supplementation with organic selenium (0.0, 0.25, 0.50, 0.75 and 1.0 mg / kg) in the diet of Nile tilapia matrices, Pereira et al. (2009) did not report any significant differences in moisture, protein and

Table 4. Proximate composition of pacu *P. mesopotamicus* fed on different levels of selenium and vitamin E.

Selenium (mg/kg)	Vitamin E (mg/kg)	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)
	0	52.38±20.91	33.41±13.90	12.23±5.56	4.22±1.68
0	100	58.89±15.45	28.86±9.84	10.56±4.73	3.71±1.29
	200	53.42±17.91	32.71±12.47	12.32±4.73	3.80±1.21
	0	61.36±12.59	27.46±7.97	10.10±4.04	3.60±0.89
1	100	66.22±12.51	22.62±8.02	9.43±3.01	2.91±1.26
	200	59.91±15.53	28.97±10.82	9.18±4.73	3.70±1.33
	0	63.16±18.71	26.75±12.61	10.89±5.09	3.20±1.46
2	100	66.54±16.17	24.31±10.31	8.24±4.11	2.93±1.20
	200	68.91±8.97	21.96±6.95	7.86±2.04	2.71±0.82
	0	56.19±19.16	30.78±13.17	10.58±4.84	4.13±1.85
4	100	66.19±13.86	24.81±11.37	8.15±2.84	3.09±1.66
	200	62.57±12.81	27.84±8.76	8.08±3.63	3.55±1.18
Selenium (mg/kg)					
0		54.90±16.08	31.66±10.76	11.71±4.43	3.91±1.24
1		62.50±12.14	26.35±8.34	9.57±3.48	3.40±1.08
2		66.20±13.39	24.34±9.09	9.00±3.71	2.94±1.05
4		61.65±14.14	27.81±10.08	8.94±3.56	3.59±1.45
Vitamin E (mg/kg)					
0		58.27±16.08	29.60±10.72	10.95±4.27	3.79±1.36
100		64.46±12.87	25.15±8.82	9.10±3.36	3.16±1.21
200		61.20±13.43	27.87±9.41	9.36±3.84	3.44±1.08
Selenium × Vitamin E		ns		ns	ns
Selenium Effect		ns		ns	ns
Vitamin E effect		ns		ns	ns

ns = not significant (P>0.05). Data expressed as mean ± standard deviation.

lipid contents of fish fed on different selenium levels. Results corroborated those in current study.

Current assay revealed there was no difference in the proximate composition of pacu for different levels of vitamin E. However, Sau et al. (2004) obtained significant differences (P<0.05) in protein rates of rohu carcass (*Labeo rohita*) at different levels of vitamin E supplementation in the diets. In this study, carcasses of the group fed on diets with 200 mg / kg had lower average percentage of crude protein (57.17%) when compared to groups with 100 mg/kg (58.34%) and 0 mg/kg (58.34%). There were no differences in proximate composition with means 70.9 and 71.2% moisture, 9.7 and 9.5% lipids, 18.4% crude protein and 1.5% ash, respectively, for groups 300 and 1500 mg / kg, in rainbow-trout fed on 300 and 1500 mg vitamin E/kg diet (Chaiyapechara et al., 2003). Results of lipid oxidation for the main trunks of pacus fed on different levels of selenium and vitamin E are shown in Table 5.

In the case of lipid oxidation in different evaluation times, only level 0 Se + 0 vit. E / kg diet was affected by storage time, and the highest oxidation rate was observed for 120 days. However, when selenium levels were evaluated separately, levels 0 and 4 of Se / kg diet were significantly influenced by storage time, and the lipid oxidation was higher after 120 days (P<0.05) when compared to that after 60 and 90 days. Vitamin E supplementations (0, 100 and 200 mg vit. E / kg diet) were also affected by different storage periods, with significant increase (P<0.05) of TBARS overtime, as shown in Table 5.

This observation was also made by Otani (2009) for tilapia fillets and by Weber et al. (2008) for silver catfish fillets, perhaps due to an increase of compounds formed by lipid oxidation which reacts with thiobarbituric acid, thereby increasing TBARS rates. However, these rates tended to decrease overtime since there was a reduction of the substrates which reacted with thiobarbituric acid, as reported by Otani (2009).

The combination of selenium and vitamin E did not affect (P>0.05) lipid oxidation of fillets, neither did selenium levels separately affect this parameter.

Table 5. Mean rates of lipid oxidation by the formation of thiobarbituric acid reactive substances (mg malonaldehyde / kg of main trunk) of pacus supplemented with different concentrations of selenium and vitamin E in the diet during storage under freezing (-18±2°C).

Selenium (mg/kg)	Vitamin E (mg/kg)	Time (days)			Time effect
		60	90	120	
0	0	0.31±0.10 ^{b}	0.48±0.16 ^{b}	1.19±0.59 ^{a}	P<0.05†
	100	0.28±0.02	0.67±0.27	1.00±0.58	ns
	200	0.23±0.08	0.34±0.16	0.54±0.24	ns
1	0	0.37±0.12	0.72±0.53	0.89±0.69	ns
	100	0.37±0.26	0.52±0.42	0.88±0.62	ns
	200	0.25±0.05	0.34±0.09	0.51±0.21	ns
2	0	0.61±0.32	0.75±0.28	0.94±0.29	ns
	100	0.38±0.15	0.63±0.16	0.93±0.40	ns
	200	0.34±0.31	0.40±0.35	0.47±0.35	ns
4	0	0.39±0.03	0.55±0.28	0.88±0.64	ns
	100	0.26±0.21	0.67±0.39	0.98±0.45	ns
	200	0.20±0.08	0.31±0.11	0.52±0.32	ns
Selenium (mg/kg)					
0		0.27±0.07 ^{b}	0.49±0.23 ^{b}	0.91±0.52 ^{a}	P<0.05†
1		0.33±0.16	0.53±0.38	0.76±0.51	ns
2		0.44±0.25	0.59±0.23	0.78±0.32	ns
4		0.28±0.20 ^{b}	0.51±0.33 ^{ab}	0.79±0.51 ^{a}	P<0.05†
Vitamin E (mg/kg)					
0		0.42±0.21 ^{b}	0.63±0.32 ^{bA}	0.98±0.50 ^{aA}	P<0.05†
100		0.32±0.17 ^{c}	0.62±0.29 ^{bA}	0.95±0.45 ^{cA}	P<0.05†
200		0.26±0.16 ^{b}	0.35±0.18 ^{abB}	0.51±0.25 ^{aB}	P<0.05†
Selenium × Vitamin E[2]		ns	ns	ns	
Selenium effect[2]		ns	ns	ns	
Vitamin E effect		ns	P<0.05^{TT}	P<0.05^{TT}	

[1]mg/kg of diet; [2]ns = not significant. † different lowercase letters on the same line indicate significant difference (P<0.05) between different storage times; ^{TT} different uppercase letters in the same column indicate significant difference (P<0.05) between the different levels of vitamin E. Data expressed as mean ± standard deviation.

However, when the levels of vitamin E were assessed separately (Figure 1), levels of vitamin E influenced lipid oxidation after 90 and 120 days of storage and the level of 200 mg vitamin E / kg diet provided a lower rate (P<0.05). This finding showed the postmortem anti-oxidant effect of adding vitamin E on the diet of pacu (*P. mesopotamicus*). Cheah et al. (1995) claim that vitamin E is effective in fish conservation during processing and storage, since it inhibits the degradation of lipids by oxidation.

The antioxidant properties of vitamin E were also observed by Huang and Huang (2004), who found that dietary supplementation of vitamin E for hybrid tilapia (*O. niloticus x O. aureus*) decreases lipid peroxidation of postmortem tissue, whereas increase of dietary vitamin E causes decrease in muscle TBARS. The above authors observed that tilapia fed on 300 IU vitamin E / kg diet had the lowest TBARS rates in the muscle: 1.47 nmol MDA / mg. Moreover, Huang and Huang (2004) also found that vitamin E supplementation increased the glutathione level in the liver.

This is probably due to the contents of vitamin E stored in the muscle, which are greater in fish fed on higher doses of vitamin E. Vitamin E is actually a potent biological antioxidant: its high levels in tissues inhibit lipid peroxidation and decrease the formation of malondialdehyde. This trend has also been reported in the Atlantic salmon (Onibi et al., 1996) and sea bass (Gatta et al., 2000).

Results similar to current study were provided by Fogaça and Santana (2007) with tilapia fed on 0, 100 and 200 vit.E mg / kg diet and after 63 days processed in burgers. The authors noted that the burgers in the control group (without the addition of vitamin E) had higher TBARS rates than treatments with vitamin E supplementation. In fact, vit.E 200 mg / kg was the best to decrease

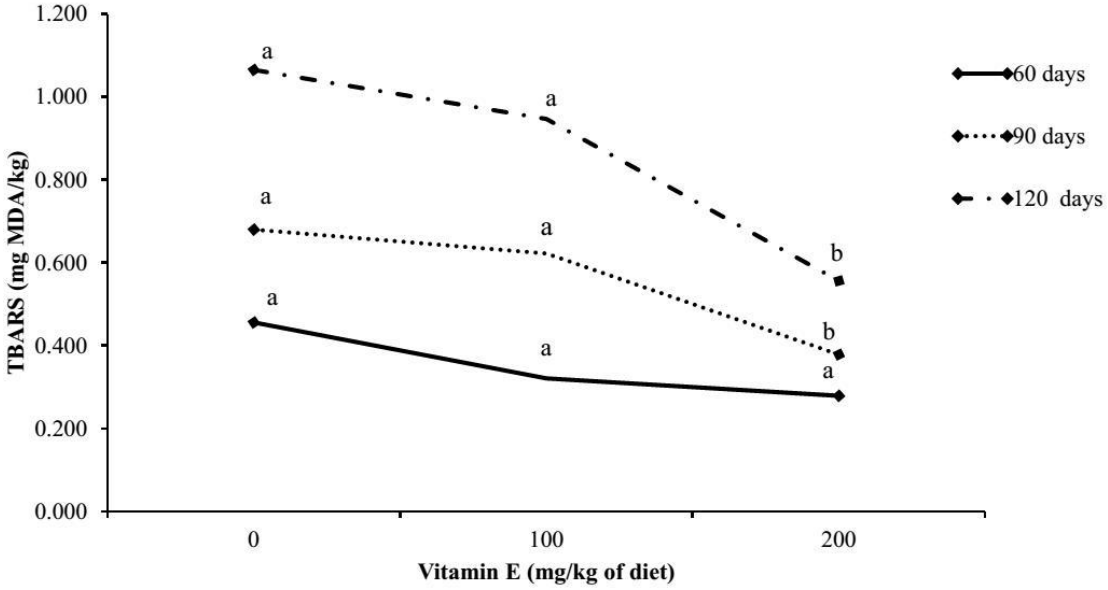

Figure 1. Relationships between vitamin E levels and the formation of thiobarbituric acid reactive substances at different storage times. Different letters indicate significant difference (P <0.05) between the different levels of vitamin E.

to decrease lipid oxidation since it indicated Vitamin E´s antioxidant activity.

Thus, the addition of 200 mg vitamin E / kg diet in pacus is ideal to reduce lipid oxidation after 90 and 120 days of storage, under freezing. This is due to the fact that vitamin E supplementation in the diet makes it incorporated into lipid membranes, protecting the tissue from any post-mortem oxidation.

Level 1 mg selenium + 200 mg vitamin E / kg in the diet of juvenile pacu (*P. mesopotamicus*) decreases lipid oxidation of meat after 90 days of storage, under freezing, and maintains performance and body yields.

Conflict of Interests

The authors have not declared any conflict of interests.

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