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Essential oils in the initial phase of broodstock diets of Nile tilapia

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ABSTRACT - This study evaluated the effect of feed supplementation (at doses of 0, 50, 100, 150, and 200 mg/kg) with SALUTO®, a microencapsulated blend of essential oils including carvacrol, cinnamaldehyde, 1,8-cineol, and pepper oleoresin, in Nile tilapia (*Oreochromis niloticus*) broodstock on reproductive and growth parameters during the initial phase of rearing. The growth parameters, somatic indexes and gonadal and hepatic parameters were analyzed. Quantitative parameters of growth, ovary fat and crude protein were not affected by supplementation of the additive. However, an increase in crude protein in the female liver was observed in correlation with increased essential oil levels. The female hepatosomatic index was also correlated with supplementation of essential oils. Feeding Nile tilapia broodstock diets containing an essential oil compound (SALUTO®) affects only the females, promoting an increase in liver protein inclusion and hepatosomatic index without impairing performance.

Key Words: additive, fish nutrition, growth promoter, hepatosomatic index

Introduction

The accelerated growth of Brazilian aquaculture (MPA, 2010), associated with the intensification of fish farming, has resulted in animal health problems. The market pressure to reduce the use of antibiotics in aquaculture has been growing in recent years (Ardó et al., 2008). To reduce antibiotic resistance and avoid the presence of residues in food (Bruun et al., 2003), nutritional additives have been used in animal diets (Burt, 2004; Oncu et al., 2005).

The prohibition of growth promoters in Europe has stimulated the search for alternatives such as prebiotics (Silva and Nörnberg, 2003), probiotics (Santin et al., 2001; Meurer et al., 2006; Meurer et al., 2007; Meurer et al., 2008; Pozza et al., 2010), enzymes (Furuya et al., 2001; Surek et al., 2008), organic acids (Maiorka et al., 2004), and plant extracts (Çabuk et al., 2006; Santurio et al., 2007; Windisch et al., 2008; Applegate et al., 2010, Soltani et al., 2010). For example, specifically in fish, the use of garlic extract for silver catfish (*Rhamdia quelen*) (Fernandes et al., 2007) and propolis for the Nile Tilapia (Meurer et al., 2009a) has yielded satisfactory results.

The use of essential oils in the diet can promote improvement in the animal digestive tract (Chrubasik et al., 2005) by enhancing performance (Muhl and Liebert, 2007; Santurio et al., 2007). These substances act by stimulating the secretion of digestive enzymes (Ramakrishnarao et al., 2003; Platel and Srinivasan, 2004), delaying gastric emptying (Manzanilla et al., 2004), accelerating the uptake of glucose by the intestine (Kreydiyyeh et al., 2003), or increasing the difficulty of pathogen adhesion to intestinal mucosa (Jamroz et al., 2006).

The initial phase of fish farming is important due to its influence on the other stages (Hayashi et al., 2002); appropriate growth is directly reflected in broodstock formation (Navarro et al., 2010). In the initial phase of broodstock formation, the use of additives that promote maximum growth (Traesel et al., 2011) can be an important management strategy.

There are many studies about administering essential oil blends to farm animals with contradictory results (Jesus, 2010; Barroca, 2011). However, a beneficial effect is observed when essential oils are used in situations of high yield production. Chilante et al. (2012) observed a reduction in the rate of cumulative mortality and increased egg production in broiler breeders using the same additive as that used in the present study.

In consideration of these observations, the use of essential oils could be an adequate and even strategic tool because it is a natural component without residual effects on

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the meat, especially in Nile tilapia, the most important fish in the Brazilian aquaculture. However, no study regarding the use of essential oils in Nile tilapia was found.

The objective of this study was to evaluate the effects of increasing levels of a compound made from essential oils in reproductive and growth parameters of Nile tilapia (*Oreochromis niloticus*) broodstock diets during the initial phase of fish farming.

Material and Methods

The experiment was conducted from January to June 2010 (180 days) in the Laboratory of Reproductive Technology of Cultivable Aquatic Animals (LATRAAC), located at Instituto de Pesquisa em Aquicultura Ambiental (InPAA), Universidade Estadual do Oeste do Paraná (UNIOESTE), Toledo Campus, Brazil. One thousand three hundred Nile tilapia (*Oreochromis niloticus*) from Thai lineage (13.66±0.16 g) stocked in 20 fine-mesh net cages called “hapas” (3 m × 2 m; 1 mm mesh) were used. Males and females were stocked at a density of 65 fish per hapa. The experiment was conducted in accordance with the ethical standards and approval of Colégio Brasileiro de Experimentação Animal (COBEA) and was approved by Comitê de Ética na Experimentação Animal e Aulas Práticas (CEEAAP/UNIOESTE) as protocol 06/10 minutes number 012012.

The hapas were installed in two earth ponds (20 × 10 m) and distributed in a completely randomized experimental design, with five treatments and four replicates. Treatments consisted of the provision of feed containing increasing levels of SALUTO®, a compound produced from various doses of essential oils (0, 50, 100, 150 and 200 mg/kg) as a microencapsulated blend of carvacrol, cinnamaldehyde, 1,8-cineol, and pepper oleoresin. One hapa containing 65 fish was considered as an experimental unit. Experimental diets containing 300 g/kg digestible protein (DP) and 3,000 kcal of digestible energy (DE)/kg of ration (Table 1) were formulated at the Laboratory of Aquatic Organisms Nutrition from the Technology in Aquaculture Graduate at Universidade Federal do Paraná, Palotina Sector, Brazil. To manufacture rations, crushed feed was pelleted in appropriate sizes according to the mouth of the fish (adapted from a protocol described by Bombardelli et al., 2009).

Fish count and measurements for each experimental parameter were performed every 15 days for the evaluation of growth and correction of feed rate. During this procedure, hapas were cleaned.

Fish were fed four times a day (8.00 h, 10.00 h, 14.00 h and 16.00 h) at a rate of 5.5% biomass. The feeding rate was adjusted according to water temperature (adapted from Ostrensky and Boeger, 1998).

Water temperature was measured daily, in the morning and afternoon, with a mercury thermometer. Every two weeks, the levels of dissolved oxygen (YSI® 550A) and pH (TEC® TECNAL 5) of the water were measured at 6.00 h and 16.00 h (Reidel et al., 2010; Tessaro et al., 2012).

During the experimental period (January-June 2010), the mean water temperature was 21.63±3.67 °C (16.5 to 30 °C). The mean concentration of dissolved oxygen in the water during the experimental period was 4.28±1.55 mg/L in the morning and 5.54±1.10 mg/L in the afternoon. The mean values of water pH ranged from 7.02±0.36 in the morning to 7.03±0.22 in the afternoon. The recorded temperature, pH, and dissolved oxygen were suitable for the species, according to Hussain (2004) and Barbosa et al. (2007).

At the end of the experiment, the weight, standard length, average weight gain, daily weight gain, specific growth rate, apparent feed conversion ratio, survival rate (Bombardelli et al., 2009) and the condition factor (Vazzoler, 1996) were measured.

Afterwards, 80 fish (four females per experimental unit) were anesthetized and euthanized (CFMV, 2008). Liver, gonads, and viscera were weighed for the calculation of hepatosomatic, viscerosomatic (Bombardelli, 2007), and gonadosomatic indexes (Ng and Wang, 2011).

Table 1 - Composition of experimental diets with different levels of the compound made from essential oils for Nile tilapia broodstock diets

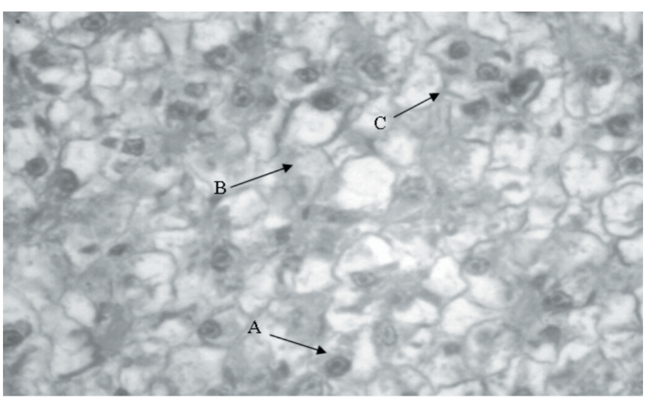
Ingredients (g/kg)	Treatments (mg/kg)				
	0	50	100	150	200
Soybean meal	553.100	553.100	553.100	553.100	553.100
Corn	317.300	317.300	317.300	317.300	317.300
Fishmeal	100.000	100.000	100.000	100.000	100.000
Supplement vit. + min.[1]	20.000	20.000	20.000	20.000	20.000
Salt (NaCl)	5.000	4.950	4.900	4.850	4.800
Dicalcium phosphate	4.300	4.300	4.300	4.300	4.300
Calcitic limestone	0.180	0.180	0.180	0.180	0.180
Propionic acid	0.100	0.100	0.100	0.100	0.100
Essential oil	0	50	100	150	200
Nutrients					
Linoleic acid (g/kg)	10.100	10.100	10.100	10.100	10.100
Starch (g/kg)	272.300	272.300	272.300	272.300	272.300
Ash (g/kg)	71.100	71.100	71.100	71.100	71.100
Fat (g/kg)	37.600	37.600	37.600	37.600	37.600
Digestible energy (kcal/kg)	3.000	3.000	3.000	3.000	3.000
Digestible protein (g/kg)	3.000	3.000	3.000	3.000	3.000
Crude fiber (g/kg)	28.300	28.300	28.300	28.300	28.300
Total lysine (g/kg)	18.400	18.400	18.400	18.400	18.400
Total phosphorus (g/kg)	10.000	10.000	10.000	10.000	10.000

[1] Supplement vitamin + mineral: composition of product in milligram per kilogram (Folic acid - 200 mg; pantothenic acid - 4,000 mg; biotin - 40 mg; copper - 2,000 mg; iron - 12,500 mg; iodine - 200 mg; manganese - 7,500 mg; niacin - 5,000 mg; selenium - 70 mg; vitamin A - 1,000,000 IU; vitamin B1 - 1,900 mg; vitamin B12 - 3,500 mg; vitamin B2 - 2,000 mg; vitamin B6 - 2,400 mg; vitamin C - 50,000 mg; vitamin D3 - 500,000 UI; vitamin E - 20,000 IU; vitamin K3 - 500 mg; zinc - 25,000 mg).

The livers from 40 females were fixed in Bouin aqueous solution for a period of 8 hours and transferred to a 70% alcohol solution. Then, the material was dehydrated by passage through a series of increasing concentrations of alcohol, diaphonized in xylene, and embedded in paraffin for obtaining semi-serial transverse sections with a thickness of 5 µm. From each liver, two microscope slides were prepared containing five histological sections, which were stained with hematoxylin-eosin.

After histological processing, images were captured using a biological microscope at 40x magnification (Figure 1). In each histological section, five images were captured, totaling 25 images per liver. Measurements of average areas (Nikon microscope, model Eclipse 200, coupled to a Basler 602fc digital camera) of hepatocytes were performed according to Tessaro et al. (2012), using the software Image Pro-Plus 4.0.

Liver and gonads from the other 40 females were used for the analysis of crude protein from the gonads and livers and of the ether extract from the gonads. These analyses were performed in the Nutrition Laboratory at UNIOESTE – Marechal Cândido Rondon Campus, Brazil, according to Silva and Queiroz (2002).



A - hepatocyte; B - hepatocyte core; C - sinusoidal capillaries.
Supplemented with essential oils at a dose of 200 mg/kg.
Captured in biological microscope under 40x magnification.

Figure 1 - Liver tissue of female Nile tilapia.

The studied parameters were assessed by analysis of variance (ANOVA), and when differences were significant ($P<0.05$), the data were subjected regression analysis. All evaluations were performed using the software SAEG (Sistema para Análises Estatísticas e Genéticas, version 9.1).

Results and Discussion

The parameters final weight, standard length, weight gain, daily weight gain, specific growth rate, apparent feed conversion, condition factor, and survival were not affected ($P>0.05$) by administration of the compound made from essential oils in the diets of the fish (Table 2).

Discussion of similar additives fed to fish is challenging because of the lack of experiments. However, comparisons with results from experiments with other species of farm animals, usually with monogastrics such as pigs and poultry (Chilante et al., 2012), are important. According to the results presented herein, additives fed to fish and other farming animals may not lead to increased performance parameters and may not provide different results in different species.

Using probiotics in the diet of Nile tilapia, Meurer et al. (2006, 2007, 2008, 2009b) found no effect on growth performance and survival. Meurer et al. (2009a), using brown propolis extract fed to Nile tilapia fingerlings, observed effects on growth and on feed conversion ratio. Barroca (2011) did not find effective results with SALUTO® fed to weaned piglets.

However, Jesus (2010) showed that SALUTO® improved weight gain, feed intake, feed conversion and production efficiency of broilers in the growth phase. Positive results were observed by Chilante et al. (2012), who fed a similar compound to broiler breeders using the same blend of essential oils.

Probiotics used in broilers showed a final weight and weight gain similar to observations in animals supplemented with antibiotic growth promoters (Çabuk et al., 2006, Traesel et al., 2011). Muhl and Liebert (2007) found that

Table 2 - Performance in the initial phase of broodstock rearing of Nile tilapia subjected to experimental diets with different levels of the compound composed of essential oils

Parameters	Treatments (mg/kg)					CV (%)
	0	50	100	150	200	
Average final weight (g)	80.73	80.05	82.05	80.97	81.48	8.26
Standard length (cm)	14.02	14.20	14.24	14.26	14.35	3.62
Average weight gain (g)	12.51	12.38	12.40	12.18	12.84	14.51
Average daily weight gain (g)	0.83	0.82	0.83	0.81	0.86	14.51
Specific growth rate (%)	0.47	0.48	0.48	0.47	0.49	7.09
Apparent feed conversion	1.54	1.55	1.59	1.58	1.51	8.54
Survival (%)	100.0	98.08	96.15	98.85	96.92	2.70

CV - coefficient of variation (%).

inclusion of a phytogenic additive in pig diets did not improve the performance or intestinal microflora of piglets. Hashemi and Davoodi (2010) and Hernandez et al. (2004) attributed the positive effects of plant extracts on growth performance and nutrient digestibility to the appetite and digestion-stimulating and antimicrobial properties of these compounds.

The addition of the essential oil compounds to the diet at levels above 50 to 200 mg/kg influenced ($P<0.05$) the female hepatosomatic index (Figure 2; Table 3) and increased crude protein inclusion in the female liver ($P<0.06$) (Figure 3; Table 5). On the other hand, the treatments did not affect ($P>0.05$) female gonadosomatic index, female viscerosomatic index, average area of female hepatocytes, female condition factor, male condition factor, male gonadosomatic index, male hepatosomatic index and male viscerosomatic index (Tables 3 and 4). The morphometry indexes of hepatocytes from female broodstocks were not affected by treatments ($P>0.05$) (Table 3).

In vertebrates, the liver is a key organ that controls many vital functions (Guyton and Hall, 2002). In fish, the liver plays an important role in females because during reproduction, this organ mobilizes food reserves for the production of vitellogenin (El-Sayed and Kawanna, 2007; Tsadik and Bart, 2007; Andrade et al., 2010). In this sense, the liver can be used as a body indicator of the nutritional or reproductive physiological status of fish (Caballero et al., 1999).

The increase in the female hepatosomatic index and liver crude protein might be a result of hyperplasia or hypertrophy (Caballero et al., 2004) due to greater liver overload derived from the diet. Ibrahim et al. (2011) hypothesized that pancreatic protein enzymes trypsin and chymotrypsin produce hydrolyses and break down proteins into the basic amino acids.

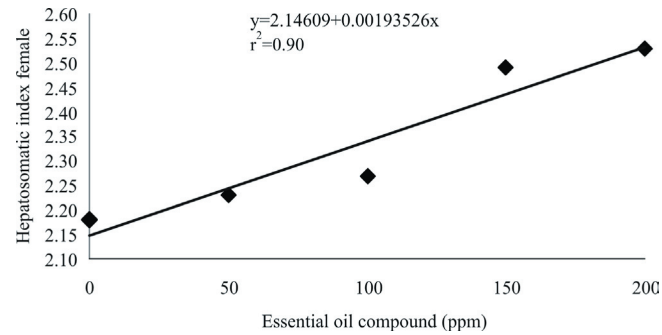


Figure 2 - Effect of increasing levels of essential oils (mg/kg) in the diet of Nile tilapia broodstock on the female hepatosomatic index ($P<0.05$).

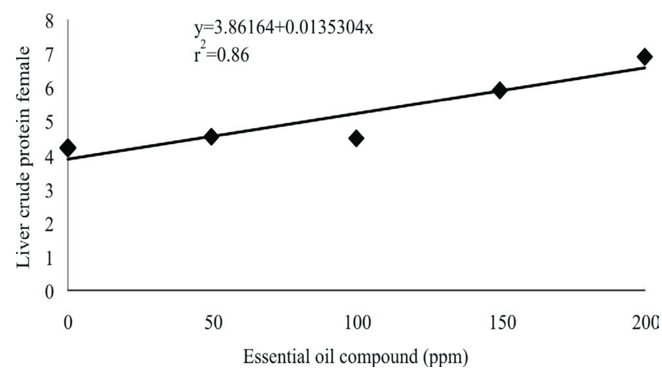


Figure 3 - Effect of increasing levels of essential oils (mg/kg) in the diet of Nile tilapia broodstock on the liver crude protein of females ($P<0.06$).

Table 3 - Somatic parameters and the area of hepatocytes in the initial phase of life of female Nile tilapia broodstock subjected to experimental diets with increasing levels of the essential oils compound

Parameters	Treatments (mg/kg)					CV (%)
	0	50	100	150	200	
Average area of female hepatocytes (μm^2)	127.24	135.58	135.27	124.76	126.03	20.26
Female gonadosomatic index (%)	3.73	3.94	3.65	4.08	4.14	23.71
Female viscerosomatic index (%)	11.95	11.59	12.10	12.23	12.40	9.27
Female condition factor	3.45	3.59	3.50	3.72	3.67	7.14

CV - coefficient of variation (%).

Table 4 - Somatic parameters in the initial phase of life of male Nile tilapia broodstock subjected to experimental diets with increasing levels of the essential oils compound

Parameters	Treatments (mg/kg)					CV (%)
	0	50	100	150	200	
Male gonadosomatic index (%)	0.76	0.76	1.15	0.96	0.82	32.88
Male hepatosomatic index (%)	2.16	2.35	2.71	2.34	2.44	16.12
Male viscerosomatic index (%)	9.17	9.74	10.71	10.02	10.12	13.95
Male condition factor	3.78	3.87	4.05	3.72	3.87	6.50

CV - coefficient of variation (%).

Table 5 - Chemical composition in the initial phase of life of female Nile tilapia subjected to experimental diets with different levels of the essential oil compound

Parameters	Treatments (mg/kg)					CV (%)
	0	50	100	150	200	
Crude protein in the female gonad (%)	42.85	31.65	41.91	32.51	26.20	30.69
Female gonadal fat (%)	7.99	6.33	8.71	4.13	8.17	59.27
Crude protein in the female liver[1] (%)	4.19	4.55	4.52	5.92	6.89	34.32

CV - coefficient of variation (%).
[1] Linear effect, $y = 3.86164 + 0.0135304x$, $r^2 = 0.86$.

Some plants and bacteria stimulate trypsin secretion in the upper portion of the small intestine (Platel and Srinivasan, 2000), allowing a microflora modulating effect that might aid digestion and absorption of nutrients, thereby providing a greater array of amino acids for protein synthesis and thus increasing the body protein content.

As there were no changes in liver protein levels and hepatocyte area, the changes in hepatosomatic index are possibly related to the development of structures unrelated to the hepatocytes. Such structures might be related to the exocrine pancreatic tissue, or hepatopancreas, commonly present with diffuse distribution among the hepatic tissue of teleosts (Epple and Brinn, 1986), which is responsible for producing digestive enzymes that act in the intestine (Hinton and Pool, 1976; González et al., 1993).

In sex-reversed Nile tilapia fingerlings, the reduction of hepatosomatic index was observed when feed was supplemented with probiotic additives (Meurer et al., 2009a). Meurer et al. (2009b) suggested that this decrease in hepatosomatic index could be related to the nutritional aspect of fish and their growth rate, indicating that their metabolic reserves (glycogen/lipid) are used to enhance immunity against opportunistic organisms.

Because there was no health challenge in the experimental conditions, the fish used in this study might have directed their hepatic metabolism to produce vitellogenin and to promote the reproductive process. This process is most likely reflected in the liver metabolism and hepatosomatic index (Navarro et al., 2006). At the levels used in the present study, the essential oils compound did not elicit any toxicity problems. This is a positive factor because according to Traesel et al. (2011), when incorporated in excess, toxic effects of essential oils are observed. The most important mechanism of phytogenic feed additives arises from beneficially affecting the ecosystem of gut microflora through controlling potential pathogens (Hashemi and Davoodi, 2011).

The mode of absorption is a limiting factor for the use of feed additives for animals (Juven et al., 1994). The site of intestinal absorption of essential oils in monogastrics might influence its effect on the animal. Kohlert et al. (2000) observed that the absorption of thymol in humans occurs in the upper intestine. In pigs, essential oils can be absorbed in the proximal portion of the intestine, and therefore, the antimicrobial activity in the distal portion of the intestine is limited (Muhl and Liebert, 2007). Thus, these factors may also influence the use of active principles present in the additive under study in this experiment because little information about the absorption modes of these substances through the digestive tract of tilapia is available.

Future experiments are needed to assess aspects of feeding essential oil compounds such as possible toxicity levels and the effects on microbiota and immunology at the various stages of development of farmed Nile tilapia.

Conclusions

Feeding Nile tilapia broodstock diets containing an essential oil compound (SALUTO®) affects only the females, promoting increased liver protein inclusion and hepatosomatic index without diminishing performance.

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