


Use of essential oils for *Nile tilapia* breeders during breeding season

Uso de óleos essenciais para tilápia do Nilo durante a estação reprodutiva

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Abstract

The present study aimed to evaluate the dietary supplementation of a commercial Saluto® compound containing essential oils extract of cinnamon (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*) and pepper (*Capsicum* sp.) on breeding performance of female and male Nile tilapia (*Oreochromis niloticus*). The experiment had a completely randomized design with five treatments (0, 50, 100, 150, and 200 ppm of essential oil supplementation) and four repetitions during the breeding season of Nile tilapia. The apparent feed conversion, daily weight gain, condition factor, hepatosomatic index, gonadosomatic index,

egg diameter, relative fecundity, egg weight, larvae yield, hatching rate, blood calcium and blood glucose levels were analyzed. In the correlations of variables at the female reproductive peak no statistical differences were found for the reproductive parameters and quality of the larvae. The nutritional additive inclusion did not impair reproductive parameters during the 120 days of evaluation, and inclusions above 50 ppm promoted an increase in the hepatosomatic index of Nile tilapia females during the reproductive season.

Keywords: Reproduction. Nutrition. Additive. Liver. Larvae.

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Resumo

O objetivo do presente estudo foi avaliar a suplementação alimentar de um composto comercial Saluto® contendo extrato de óleos essenciais de canela (*Cinnamomum zeylanicum*), orégano (*Origanum vulgare*), alecrim (*Rosmarinus officinalis*) e pimenta (*Capsicum sp.*) no desempenho reprodutivo de fêmeas e machos de tilápias do Nilo (*Oreochromis niloticus*). O experimento teve um delineamento inteiramente casualizado, com cinco tratamentos (0, 50, 100, 150 e 200 ppm de suplementação de óleo essencial) e quatro repetições durante o período de reprodução da tilápia do Nilo. Foram analisados a conversão alimentar aparente, ganho de peso diário, fator de condição, índice hepatossomático, índice gonadossomático, diâmetro do ovo, fecundidade relativa, peso do ovo, rendimento de larvas, taxa de eclosão, níveis séricos de cálcio e glicemia. Nas correlações das variáveis no pico reprodutivo das fêmeas não foram encontradas diferenças estatísticas para os parâmetros zootécnicos e a qualidade das larvas. A inclusão do aditivo nutricional não prejudicou os parâmetros reprodutivos durante os 120 dias de avaliação, e inclusões acima de 50 ppm promoveram aumento do índice hepatossomático de fêmeas de tilápia do Nilo durante o estação reprodutiva.

Palavras-chave: Reprodução. Nutrição. Aditivo. Fígado. Larvas.

Introduction

Nutrition may affect fish reproduction and gonadal development (Navarro et al., 2009), the number and quality of oocytes and sperm (Navarro et al., 2010), as well as the animal's health. High feed costs, no appropriate nutritional management, and difficult access to innovative technologies are setbacks that put fish farming in Brazil in disadvantage compared to worldwide development of the activity (Resende, 2009).

Antibiotics are commonly used to ensure farmed animal health, which in turn may promote the selection of antibiotic-resistant pathogenic bacteria, change the microbiota of farming environments, and generate the potential transfer

of antibiotic-resistant pathogenic bacteria to humans (Hölmstrom et al., 2003). In this regard, nutritional and health strategies should be developed and implemented in aquaculture, to provide good quality offsprings sanitarly safe for subsequent use.

The search for growth promoters that do not lead to bacterial resistance or result in residues in fish meat has led research on the use of natural products (Meurer et al., 2009a). In this sense, the use of essential oils may support the healthy development of Nile tilapia breeders, as the intensification of the farming systems enhances fish exposure to opportunistic organisms and subsequent incidence of pathogens.

Essential oils (*Cinnamomum zeylanicum*, *Origanum vulgare*, *Rosmarinus Officinalis* and *Capsicum sp.*) can reduce bacterial growth by changing intestinal microbiota like probiotics (Suzuki et al., 2008) that alter intestinal microbiota, positively affecting the immune and enzymatic system (Toledo et al., 2010) by improving its intestinal morphology (Heidarieh et al., 2012). The use of essential oils as an alternative to antibiotics and as a prophylactic additive is an interesting option against such pathogenic microorganisms (Traesel et al., 2011).

In this context, this study aimed to investigate the effect of the dietary supplementation with a commercial additive containing essential oils of cinnamon (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), and pepper (*Capsicum sp.*) extracts on female and male Nile tilapia breeders on various growth and reproductive performance parameters.

Material and methods

The experiment was conducted in the Laboratory of Reproduction Technology of Aquacultured Animals (Latraac); at the Institute for Research in Environmental Aquaculture (InPAA), Universidade do Oeste do Paraná (Unioeste), Toledo campus; and in the Blood Parameters Laboratory, Unioeste, Marechal Cândido Rondon campus. The experiment began in September 2010 and lasted for 120 days. Thai Nile tilapia (*Oreochromis niloticus*) (420

females and 140 males) were housed separately by sex into 40 hapas and randomly distributed in two earthen ponds (20 m x 10 m).

Female individuals (90.85 ± 2.53 g) were stocked in 20 hapas of 3 m x 2 m at a density of 3.5 animals m⁻², totaling 21 females per hapa. Male individuals (81.05 ± 0.75 g) were stocked in 20 hapas of 2 m x 1 m at a density of 3.5 animals m⁻², totaling seven males per hapa. The sex ratio was one male to three females (Little and Hulata, 2000). The breeders were separated by sex and after the rest period were placed to mate. We considered an experimental unit a hapa of 3 m x 2 m containing 21 females and seven males at mating. The experiment was conducted in accordance with the ethical standards and approved by the Ethics Committee on Animal Experiments and Practical Classes of Unioeste, protocol 06/10, no. 012012. Fish were distributed in a completely randomized design with five treatments and four replications.

The treatments consisted of five formulated diets prepared at the Laboratory of Nutrition of Aquatic Organisms, Universidade Federal do Paraná, Palotina sector, containing 32% of digestible protein (DP), 3,200 kcal of digestible energy (DE) kg⁻¹ diet and increasing levels of a compound of essential oils (0; 50; 100; 150 and 200 ppm) (Table 1). The additive was a commercial product Saluto® consisting of extracts of cinnamon (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*) and pepper (*Capsicum* sp.). In order to prepare the diets, ingredients were ground in a hammer mill with 0.5 mm sieve, mixed and then pelleted through a 3-mm die, as suggested by Siddiqui et al. (1998) and adapted by Meurer et al. (2003). Fish were fed twice a day (adapted from Bombardelli et al., 2009) at 10 am and 4 pm at a feeding rate of 1.5% biomass per day, adjusted every 17 days after fish weighing.

During the experiment, water temperature in the hapas was measured daily, in the morning and afternoon; dissolved oxygen (oximeter YSI® 550A) and pH (pH meter Tecnal®) fortnightly, at 6 am and 4 pm. Breeders were subjected to reproductive management, in mating for four days and isolated in reproductive rest for 10 days (Bombardelli et al., 2009). At the end of each mating, fertilized eggs of all females in each hapa were collected.

Eggs were collected in a 500-L tank with two nets and an overlapped braided net (10 mm mesh size) to hold the breeders and prevent mechanical shock between fish and eggs. During this procedure, all hapas were washed (Huchette and Beveridge, 2003), fish of both sexes were measured and weighed on a digital scale accurate to 0.01 g (Marte® AS2000C) and ichthyometer accurate to 0.1 cm. Then, breeders were separated again in their respective hapas for a new reproductive rest. This procedure was repeated for 120 days.

Total egg volume was measured in each hapa and 1.0 mL samples of eggs were fixed in 4% formalin (El-Sayed et al., 2003, 2005). Fixed eggs were weighed ($n > 100$) on an analytical balance (± 0.0001 g; Marte® AY 220), mean diameter ($n = 20$) measured in stereoscopic microscope equipped with ocular micrometer (Ballestrazzi et al., 2003) and counted for estimation of fecundity parameters. The remaining eggs were subjected to artificial incubation in 3.5-L polypropylene round-bottom incubators, connected to a recirculation system with a total volume of 3.5 m³. Water temperature was controlled by electrical heating and maintained at 26 ± 1 °C.

Approximately two days after the start of incubation, with the hatching of eggs, 1.0 mL sample was taken from each incubator to measure the weight of the offspring at hatching (El-Sayed et al., 2003). Three days after hatching, when the animals started surface swimming (Macintosh and Little, 1995), the offspring of each experimental unit was counted.

One thousand surface swimming post larvae were used to evaluate the effects of the treatments on the fasting survival time, according to the methodology described by Bombardelli et al. (2009).

At the end of the experiment, five females from each treatment were anesthetized by immersion in benzocaine solution (250mg L⁻¹), slaughtered (CFMV, 2008), weighed and dissected to weigh viscera, liver and gonads. Five other females from each treatment were fasted for 24 h, anesthetized as described above and subjected to blood collection according to Tavares-Dias and Moraes (2004) procedures. Blood samples were placed in test tubes with EDTA, properly identified for calcium

plasma and glucose analysis. Blood samples were centrifuged (3,500 rpm) for 10 minutes and plasma samples separated. Analyses were performed by enzymatic colorimetric method (Analisa®) in a spectrophotometer.

At the end of the experiment, the following growth performance parameters were evaluated: feed intake, weight gain, standard length, apparent feed conversion, survival, specific growth

rate (Bomfim et al., 2005), and condition factor (Vazzoler, 1996). Furthermore, viscerosomatic, hepatosomatic and gonadosomatic indices were calculated (Bombardelli et al., 2009, 2010; Ng and Wang, 2011). Reproduction and offspring quality parameters evaluated were average egg diameter (mm), relative fecundity (number of eggs g mated female⁻¹), egg weight (mg), larvae yield, hatching rate (%), calcium (mg dL⁻¹) and glucose (mg dL⁻¹).

Table 1 - Ingredient and proximate composition of the experimental diets with different levels of inclusion of Saluto® essential oils extract of cinnamon (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), and pepper (*Capsicum* sp.), on breeding performance of Nile tilapia (*Oreochromis niloticus*) females

Ingredients (%)	Inclusion of the Essential Oil (ppm)				
	0	50	100	150	200
Soybean meal	51.23	51.23	51.23	51.23	51.23
Corn	34.90	34.90	34.90	34.90	34.90
Fish waste meal	10.00	10.00	10.00	10.00	10.00
Mineral-vitamin suppl. ¹	2.00	2.00	2.00	2.00	2.00
Common salt	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	0.52	0.52	0.52	0.52	0.52
Calcitic limestone	0.80	0.80	0.80	0.80	0.80
Antifungal agent ²	0.01	0.01	0.01	0.01	0.01
Nutrients					
Linoleic Acid (%)	1.04	1.04	1.04	1.04	1.04
Starch (%)	28.66	28.66	28.66	28.66	28.66
Ash (%)	7.60	7.60	7.60	7.60	7.60
Gross energy (kcal. kg ⁻¹)	3,999.26	3,999.26	3,999.26	3,999.26	3,999.26
Digestible energy (kcal.kg ⁻¹)	3,200.00	3,200.00	3,200.00	3,200.00	3,200.00
Crude fiber (%)	2.90	2.90	2.90	2.90	2.90
Total phosphorus (%)	1.00	1.00	1.00	1.00	1.00
Fat (%)	3.28	3.28	3.28	3.28	3.28
Crude protein (%)	33.40	33.40	33.40	33.40	33.40
Digestible protein (%)	32.00	32.00	32.00	32.00	32.00
Total lysine (%)	1.73	1.73	1.73	1.73	1.73
Methionine + total cystine	0.95	0.95	0.95	0.95	0.95

Note: ¹ Mineral-vitamin supplement: 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 UI; 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 - 20,000UI; vitamin K3 - 500 mg; zinc - 25,000 mg. ² Propionic acid.

Data were subjected to analysis of variance (one way ANOVA) at a 5% significance level. Differences between treatments were detected using Fisher-LSD test, also at a 5% significance level. The variables for this analysis were: apparent feed conversion, average daily weight gain, condition factor, gonadosomatic index, hepatosomatic index, viscerosomatic index, mean egg diameter, relative fecundity, egg weight (mg), calcium (mg dL⁻¹) and glucose (mg dL⁻¹). The software used to run the statistical analyses were Statistica 7.0®, Statsoft, 2004 and Xlstat2010 (Addinsoft, 2010).

Results

Water temperature showed mean values of 22.29 ± 2.37 °C in the morning and 24.25 ± 2.76 °C in the afternoon. The mean concentration of dissolved oxygen in the water during the experimental period was 21.4 ± 1.00 mg L⁻¹ in the morning and 5.83 ± 1.25 mg L⁻¹ in the afternoon (Reidel et al., 2010; Tessaro et al., 2012). These parameters are suitable for reproduction (Al

Hafedh et al., 1999; Bhujel, 2000; Hussain, 2004) and early embryonic development of tilapia (Rana, 1988, 1990).

The mean pH values of water were 6.94 ± 0.17 in the morning and 8.06 ± 0.23 in the afternoon, suitable values for the growth of the species (Little and Hulata, 2000; Ross, 2000).

Table 2 presents feed conversion, daily weight gain, condition factor, gonadosomatic index, hepatosomatic index and viscerosomatic index of female Nile tilapia breeders fed increasing levels of essential oils. Among the growth parameters and body indices only the hepatosomatic index was significantly different ($p < 0.05$) between treatments. The best index was observed in females fed diet containing 200 ppm of essential oils for both apparent feed conversion and hepatosomatic index (Table 2, Figure 1).

Table 3 shows egg diameter, relative fecundity, egg weight, larvae yield, hatching rate, blood calcium and blood glucose of female Nile tilapia breeders fed increasing levels of essential oils. The reproductive parameters, quality of eggs and post larvae were not affected by the treatments ($p > 0.05$).

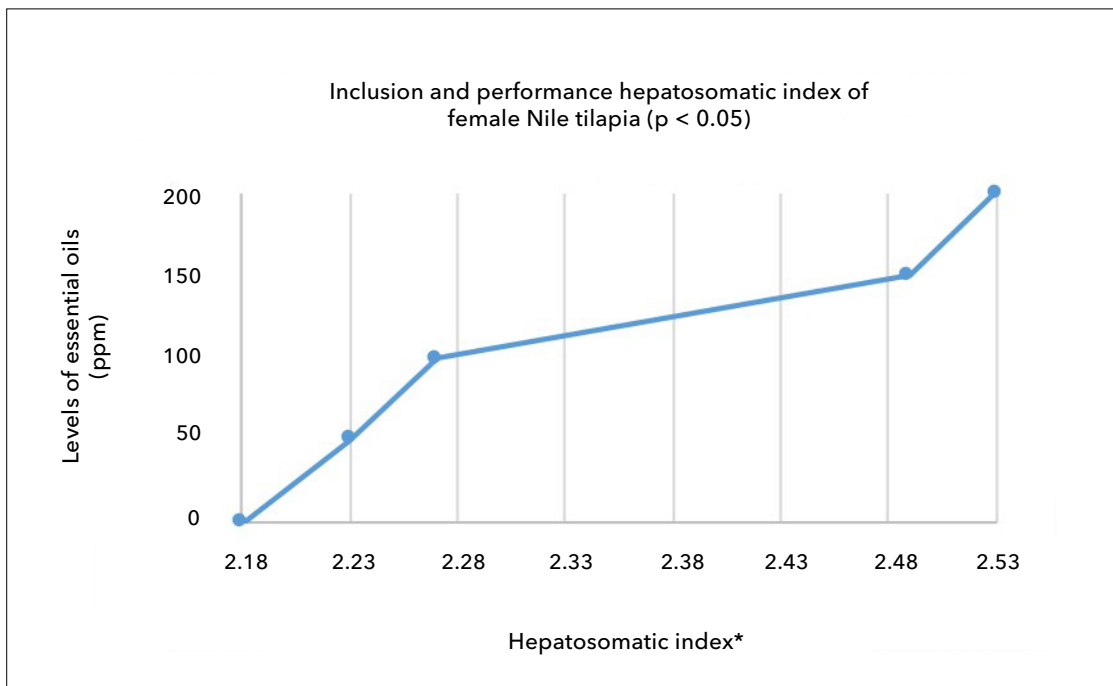


Figure 1 - Performance hepatosomatic index of female Nile tilapia (*Oreochromis niloticus*) breeders fed diets supplemented with different levels of essential oils. * Absolute value = total liver weight/fish weight x 100.

Table 2 - Performance parameters of female Nile tilapia (*Oreochromis niloticus*) breeders fed diets supplemented with different levels of essential oils

	Inclusion of the Additive (ppm)					P	CV
	0	50	100	150	200		
Apparent feed conversion*	1.60	1.92	1.30	1.24	1.10	0.069	28%
Daily weight gain (mg)	2.10	1.74	2.06	2.33	2.55	0.165	20%
Condition factor*	3.45	3.59	3.51	3.72	3.67	0.561	7%
Gonadosomatic index*	3.74	3.94	3.65	4.08	4.14	0.929	24%
Hepatosomatic index*	2.18 ^c	2.23 ^{bc}	2.27 ^{abc}	2.49 ^{ab}	2.53 ^a	0.041	8%
Viscerosomatic index*	11.95	11.59	12.10	12.24	12.45	0.852	9%

Note: * Absolute value. Different letters in the same row indicate significant differences by Fischer-LSD test ($p < 0.05$).

Table 3 - Reproductive performance parameters and post-larvae quality of female Nile tilapia (*Oreochromis niloticus*) fed different levels of essential oils

	Inclusion of the Additive (ppm)					P	CV
	0	50	100	150	200		
Mean egg diameter (mm)	32.16	32.13	32.51	33.59	32.09	0.167	3%
Relative fecundity*	1.56	2.55	2.13	2.19	1.90	0.873	65%
Egg weight (mg)	4.69	4.66	4.91	4.95	4.95	0.856	11%
Larvae yield (%)	3,300	4,530	4,711	5,293	4,640	0.876	60%
Hatching rate (%)	49.80	58.21	61.20	69.41	70.09	0.656	35%
Calcium (mg dL ⁻¹)	7.61	8.71	9.54	10.61	8.69	0.829	40%
Glucose (mg dL ⁻¹)	176.17	195.89	172.15	186.22	196.56	0.954	30%

Note: *Relative fecundity = absolute fertility/body weight unit).

Discussion

For female Nile tilapia breeders during the fingerling stage Freccia et al. (2014) reported a similar response for the hepatosomatic index with inclusions above 50 ppm of the same additive. Accordingly, Caballero et al. (1999) mentioned that liver indicators may be used to determine physiological and reproductive status of fish.

In fish, liver plays a key role during reproduction, mobilizing nutrient reserves to produce vitellogenin (El-Sayed and Kawanna, 2007; Tsadik and Bart, 2007; Andrade et al., 2010). For feed conversion, no differences were observed (Table 2), in agreement with Ahmad et al. (2011), who found improved feed conversion at different levels of cinnamon (*C. zeylanicum*) in diets for Nile tilapia.

There was no significant difference between the treatments on female Nile tilapia growth performance, which may indicate that diet and reproductive management were adequate for the species. No effect of additives on fish performance when subjected to adequate rearing conditions have been reported (Meurer et al., 2006, 2007, 2008, 2009a,b; Campagnolo et al., 2013). This also indicates that at the levels and period tested, the additive did not cause any toxicity.

Gonadal growth and maturation in female fish are related to nutrient availability (Burton, 1994; Gunasekera et al., 1996) under feed restriction (Cerdà et al., 1995). However, Freccia et al. (2014) reported linear increase in liver crude protein content in female Nile tilapia during the initial phase using the same additive. This could have been due

to increased peripheral liver blood flow by the action of substances such as capsaicin (Robbins et al., 1998), effect not observed in the gonads.

Has been reported no influence of additive dietary supplementation on reproductive performance of female Nile tilapia (Abdelhamid et al., 2009; Navarro et al., 2009). Although fecundity was not affected ($p > 0.05$) (Table 3), these results are lower than suggested by MacIntosh and Little (1995), Little and Hulata (2000), and El-Sayed et al. (2005).

The variation in egg production could be related to the origin of the breeder, as intraspecific variation existed (Coward et al., 2002). Furthermore, water temperature may also have affected results by influencing fish metabolism and egg production (Navarro et al., 2009). Mean egg weight and diameter, and survival rate during the incubation period were also not affected by the essential oils in the feed ($p > 0.05$) (Table 3). El-Sayed et al. (2005) also reported the lack of effect of different sources of oil for Nile tilapia breeders in freshwater, as regards mean egg diameter and survival rate during the incubation period.

The hypothesis that essential oils have no effect on vitellogenesis can be supported by the results of plasma calcium levels, which is an indirect metabolic indicator of vitellogenic activity in fish (Srivastav and Srivastav, 1998; Gillespie and Peyster, 2004). Plasma calcium levels observed in this study were not influenced by the level of inclusion of essential oils in feed ($p > 0.05$). Similarly to calcium, plasma glucose levels (Table 3) were not affected ($p > 0.05$) by the treatments, in agreement with Ahmad et al. (2011), who supplemented different levels of cinnamon in diets for Nile tilapia.

The mean values of plasma glucose found in this study are higher than those determined in Nile tilapia during reproductive activity (Thrall et al., 2007; Silva et al., 2012). According to Ranzani-Paiva (1991), hematological oscillations in fish are influenced by temperature, dissolved oxygen, salinity and pH, as well as stress, seasonal cycle and endogenous factors such as sex, gonadal maturation stage, nutritional status, and diseases.

However, such discrepancies between tilapia of the same sex are probably due to the choice of anticoagulant used in the collection of samples, in

which samples from heparinized plasma are larger when compared to samples obtained using EDTA (Tavares-Dias et al., 1998).

In the present experiment heparinized plasma samples differed from the study by Silva et al. (2012), who used samples with EDTA obtaining mean glucose values 82.39 mg dL^{-1} under stress by air exposure, whereas the present experiment obtained mean glucose values $185.40 \text{ mg dL}^{-1}$. The lack of measurement standardization regarding fish blood samples can raise controversial discussions, since farming systems and blood sampling methods may affect results.

No significant variations in parameters of reproductive performance of female Nile tilapia between treatments with essential oils were detected. In the literature, the effect of different essential oils on fish production is tested separately, and the synergy between different essential oils can bring zootechnical benefits (Ahmad and Abdel-Tawwab, 2011; Ahmad et al., 2011; Campagnolo et al., 2013).

Lower feed conversion may suggest better absorption, which in turn may indicate improved quality of the intestinal mucosa, due to higher surface irrigation promoted by the presence of pepper oil. In addition, rosemary and oregano have antimicrobial effect (Bakkali et al., 2008), mainly carminative effect, and reduce the production of gases, and thus increasing the intestinal absorption (Bakkali et al., 2008; Picoli et al., 2019). In this regard, better intestinal quality promoted by pepper and oregano oils, as well as improved overall health state, directly reflected on mean hepatosomatic index.

In general, dietary supplementation of the essential oils compound did not affect the parameters of reproductive performance and growth performance of female and male Nile tilapia. This may be supported by two hypotheses: a) the concentrations tested were low to induce any metabolic changes that would affect growth and reproduction, b) fish were kept in adequate rearing conditions without suffering any stress challenge. However, effects could be not observed at the time of reproductive peak, i.e. with increasing concentration of essential oils, and there was no increase in the values relative to growth.

Therefore, further studies are necessary on the use of compound essential oils in different inclusion levels and with health and/or microbial challenges.

Conclusion

The supplementation above 50 ppm of a compound commercial containing essential oils of cinnamon (*Cinnamomum zeylanicum*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*) and pepper (*Capsicum* sp.) (Saluto®) in diets for Nile tilapia increased the hepatosomatic during the reproductive season.

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