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Full Length Research Paper

Performance of Nile tilapia (*Oreochromis niloticus*) fed with diets containing caffeine

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The objective of this study was to investigate the possible effects of caffeine on the performance of juveniles of Nile tilapia. One hundred and forty four (144) juveniles of Nile Tilapia with mass, total length and initial mean height of 2.94 ± 0.16 g, 5.19 ± 0.14 cm and 1.49 ± 0.04 cm, respectively, were used. The fishes were housed in 24 plastic boxes in a closed water recirculation system, where each box contained six fishes. A completely randomized design with six treatments and four replicates was used. The treatments tested were 0.0; 0.5; 1.0; 1.5; 2.0 and 2.5 g of caffeine/3 kg of feed. The experiment lasted for 40 days. Caffeine levels, up to and including 1 g/3 kg of diet, positively affected weight, total length, standard length, height, weight gain, feed intake and specific growth rate. Dosages higher than 1 g/3 kg resulted in negative results. Caffeine can be added in up to 1 g/3 kg of feed without negatively affecting the productive performance of juvenile Nile Tilapia fingerlings.

Key words: Production index, metabolism, fish, trimethylxanthine.

INTRODUCTION

Intensive fish farming has provided the potential for raising fishes in captivity, and plans needed to promote their management, without adversely affecting the socioeconomic conditions of the activity. Ostia et al. (2018) reported that good feeding management of the animals is of great importance, since the expenses of feeding are usually high. Carvalho et al. (2012) and Souza et al. (2013) perceived that the feeding management of fish is the highest production costs, reaching 70%. With this, the use of alternative foods has gained prominence, being able to meet the nutritional needs of the animals without affecting the quality of the diet and the final product. According to Radhakrishnan et al. (2016), it is necessary to develop alternative diets that are more economical, and consequently more feasible. Veras et al. (2016) pointed out that failures in food quality

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can lead to undesired consequences, such as: heterogeneous batch size, weight loss and nutrient absorption inefficiency, which compromise fish performance. With this, the use of alternative foods has gained prominence, and is able to meet the nutritional needs of fish without affecting the quality of the diet and the final product.

Caffeine is a bioactive substance found in many food items. Its exaggerated consumption can cause a series of disorders to the human, as well as, beneficial effects when ingested in suitable dosages (Salinas-Rios et al., 2014). Due to its diverse physiological and behavioral actions, as well as, ample availability in the market, caffeine and its various effects have been increasingly studied (Cazarim and Ueta, 2014). Based on these results, new researches on this substance as well as the dosages indicated for species according to the purpose of the study have been developed in animals (Mahboob, 2014; Silva et al., 2016; Radhakrishnan et al., 2016), with an increase in fish production that is able to meet the demand of a growing population.

Research on caffeine and its possible effects on animals, as well as on fish, are still sparse. Due to this difficulty of access to such information, studies needed to meet this need, triggering new food alternatives. The objective of this study was to analyze the possible effects of caffeine on the performance of juveniles of Nile Tilapia.

MATERIALS AND METHODS

The experiment was conducted at the Federal Institute of Education, Science and Technology of Espírito Santo (IFES)–Alegre Campus, at the Laboratory of Nutrition and Production in Ornamental Species (LNPEO), in August and September 2015.

The feed was initially ground in a hammer mill with a 0.5 mm sieve, and subsequently the caffeine dosages were added, and subsequently homogenized. After homogenization, the feed was submitted to the pelletizer with granulometry (5 mm) and then taken to the forced ventilation oven at 60°C for 24 h.

The caffeine used in the experiment was the anhydrous U.S.P. ($C_8H_{10}N_40_2$) manufactured by Jilin and Labsynth Products for Laboratory LTDA, certified by Synth (ISO14001) belonging to lot 147983, with 99% purity. The animals used came from the Reproduction and Larviculture Laboratory of IFES- Alegre Campus. The experiment was carried out in a completely randomized design, with six treatments and 4 replicates, for 40 days.

Nile Tilapia juveniles used had mass, total length and initial mean height of 2.94 ± 0.16 g, 5.19 ± 0.14 cm and 1.49 ± 0.04 cm, respectively. In order to allocate the animals, a water recirculation system was used, containing 24 plastic boxes with 56 L of total volume and 45 L of useful volume each, where each one had 6 animals, totaling 144 animals used in the experiment.

Approximately 3 kg of feed was used for each treatment. The treatments used were: T0 = control; T1 = 0.5 g; T2 = 1.0 g; T3 = 1.5 g; T4 = 2.0 g and T5 = 2.5 g caffeine/3 kg. The animals were fed three times a day (07:00, 12:00 and 17:00), offered *ad libitum* with omnivorous ration, containing 36% crude protein.

The water quality measurements were performed every day in the afternoon period and determined by the following parameters: dissolved oxygen, temperature, conductivity, ammonia and pH. The rooms were cleaned on alternate days, counting at least three times a week.

The enclosures were wrapped with black tarpaulin to mitigate the stress of the animals (Merighe et al., 2004), as well as shelters and structures were added in each box to minimize fights and any other abnormal behavior. In addition, eight 300W heaters were also added for temperature maintenance.

The following performance indexes were evaluated: weight (W), total growth (TG), partial growth (PG), height (H), weight gain (WG), apparent feed intake (AFI), protein efficiency rate (PER), specific growth rate (SGR), and survival (S).

For the animal body measurements, they were sedated with 10% eugenol in a dosage of 2 mL/L with the aid of a pipette, and the container used for the animals contained a volume of 16 L. After sedation and biometry, the animals were placed in a second vessel, also with 16 L, with constant aeration to recover the anesthetic procedure.

Biometry was performed three times during the experimental period: at the beginning of the experiment, after 20 days and at 40 days. To perform the biometry, the animals had fasted for 24 h. The animals were weighed on the analytical balance and measured by means of an analog caliper.

All the variables of the analyses were initially submitted to the homoscedasticity test and later to the analysis of variance and polynomial regression. The statistical program used was SAEG 9.1 (2007).

RESULTS

The values obtained by the physico-chemical variables of the water of the experiments are within the stipulated for tilapias (Table 1) (Salaro et al., 2006), not interfering with the obtained results. Table 2 shows the averages of the variables analyzed for the performance of Nile Tilapia fingerlings fed diets containing different levels of caffeine. The dispersion plots of the means of the treatments and the polynomial regression equations for the variables are presented in Figures 1, 2 and 3.

DISCUSSION

It can be seen that the inclusion of caffeine up to the dosage of 1 g had a positive effect on the variables analyzed. However, dosages above 1 g of caffeine had a negative effect (Lin et al., 2010; Pimenta et al., 2011). According to Gatlin et al. (2007), there is interference in feed consumption through the presence of antinutritional factors inherent in the food, as well as, caffeine (Sotelo and Alvarez, 1991), according to the dosage used. Although, the caffeine used in this research is purified, the information described corroborates with the results from inclusions above 1 g of the substance.

Braga et al. (2010), by studying Nile Tilapia (40 g), described the worst means as those derived from the diet containing cocoa meal, the latter fruit, which presents caffeine in its composition. Carvalho et al. (2012) reported lower mean values for weight gain, specific growth and protein efficiency when animals (100 g) were fed with 150 g of the cocoa meal. Pezzato et al. (1996) observed no difference ($p > 0.05$) for weight gain in Nile Tilapia (7g) with the inclusion of up to 20% of cocoa meal in the diet.

Table 1. Mean values, standard deviation and coefficient of variation of water quality parameters.

Parameter	Mean	Standard deviation (±)	Coefficient of variation (%)
Total ammonia (μg.L^{-1})	0.43	0.25	0.55
pH	6.64	0.30	0.05
Electric conductivity (μS.cm^{-1})	98.94	13.37	0.13
Dissolved oxygen (mg.L^{-1})	4.73	0.50	0.10
Temperature (°C)	26.87	1.19	0.45

Table 2. Performance of Nile Tilapia fry fed diets containing caffeine.

Variable	Treatments (g of caffeine/3 kg of feed)					
	0	0.5	1	1.5	2	2.5
**W (g)	17.9^{b}	23.11^{a}	23.36^{a}	17.3^{b}	18.79^{b}	22.00^{a}
**TL (cm)	4^{c}	5.13^{a}	5.22^{a}	3.54^{c}	4.66^{b}	4.83^{ab}
**PL (cm)	3.43^{b}	4.08^{a}	4.16^{a}	3.33^{b}	3.87^{b}	3.94^{ab}
*H (cm)	1.34^{c}	1.61^{ab}	1.67^{a}	1.29^{c}	1.43^{b}	1.58^{ab}
**WG(g)	14.95^{c}	20.05^{b}	20.41^{a}	14.42^{c}	15.89^{c}	19.04^{ab}
**FI (g)	95.33^{a}	92.94^{b}	92.73^{b}	91^{bc}	97.61^{a}	97.08^{a}
***AFC	6.65^{a}	4.91^{a}	4.82^{a}	6.45^{a}	6.17^{a}	5.57^{a}
***PER (%)	0.42^{a}	0.6^{a}	0.69^{a}	0.44^{a}	0.46^{a}	0.55^{a}
**SGR (%)	5.46^{c}	6.65^{a}	6.86^{a}	4.97^{c}	6.21^{b}	5.27^{c}
** S (%)	79.17^{a}	66.67^{b}	54.16^{c}	58.33^{c}	79.16^{a}	75.00^{ab}

W - Weight; TL- total length; PL- partial Length; H- height; WG- weight gain; FI- Feed Intake; AFC- apparent food conversion; PER- protein efficiency rate; SGR- specific growth rate; S- survival. Averages followed by the same capital letter in the column and lowercase in the row do not differ: * ($p<0.05$); ** ($p<0.01$); *** (ns).

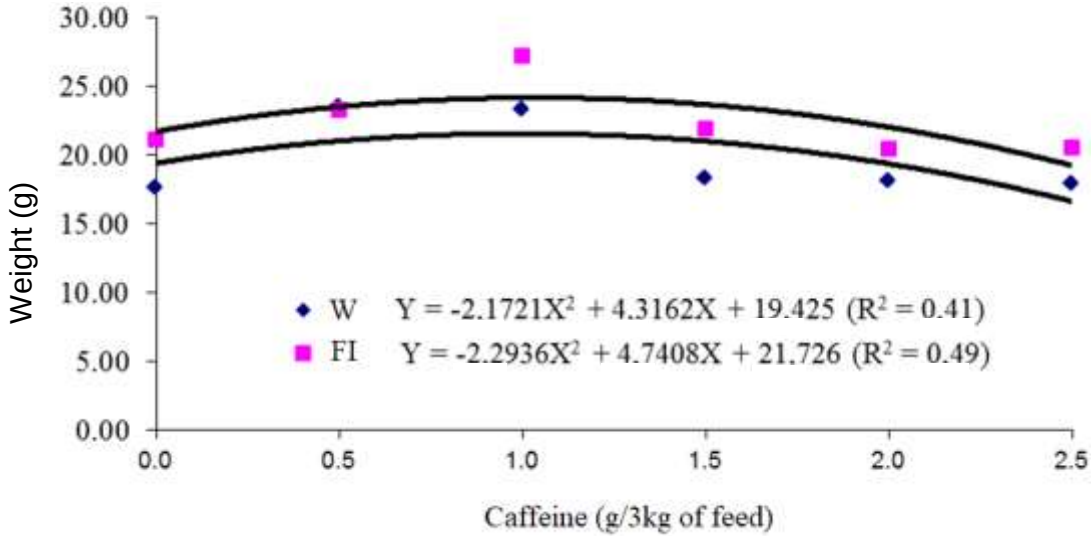

Figure 1. Second-degree polynomial regressions of weight (W) and feed intake (FI) in relation to the different caffeine dosages for juveniles of Nile Tilapia.

Other species of fish, such as catfish (*Clarias mossambicus*), were investigated and it was concluded that the inclusion of the substance in the diets negatively affected the growth and efficiency of feed conversion (Christensen, 1981), as well as carp (*Cyprinus carpio* L) (Moreau et al., 2003) and tilapia (*Oreochromis aureus*)

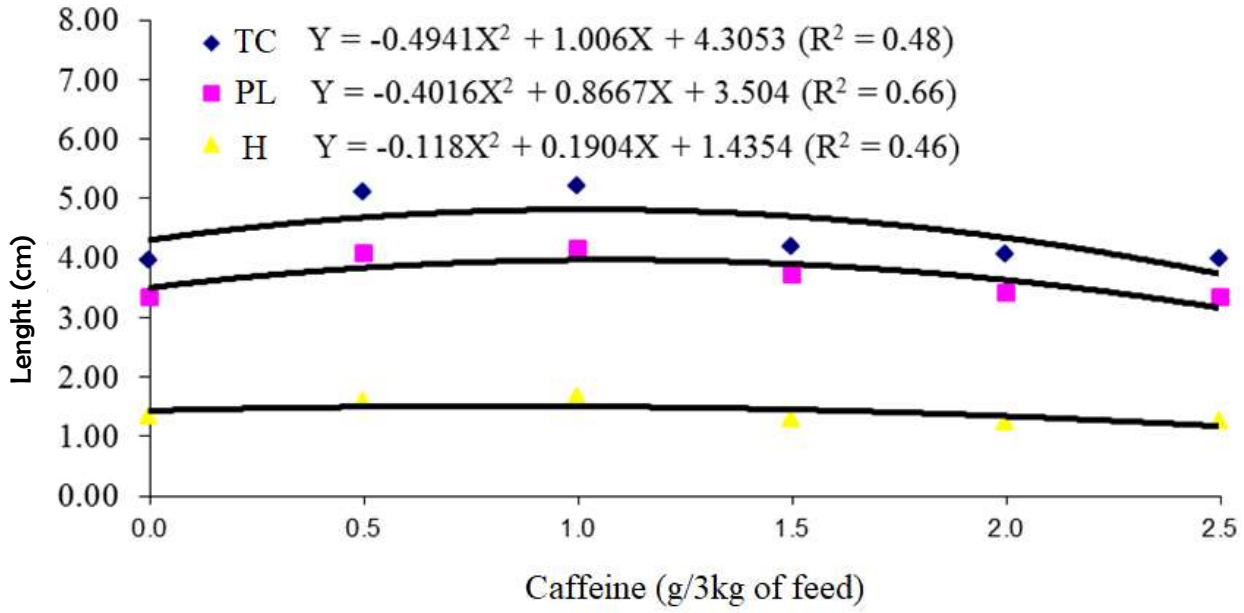

Figure 2. Total length (TL), partial Length (PL) and height (H) second-degree polynomial regressions for different caffeine dosages for juveniles of Nile Tilapia.

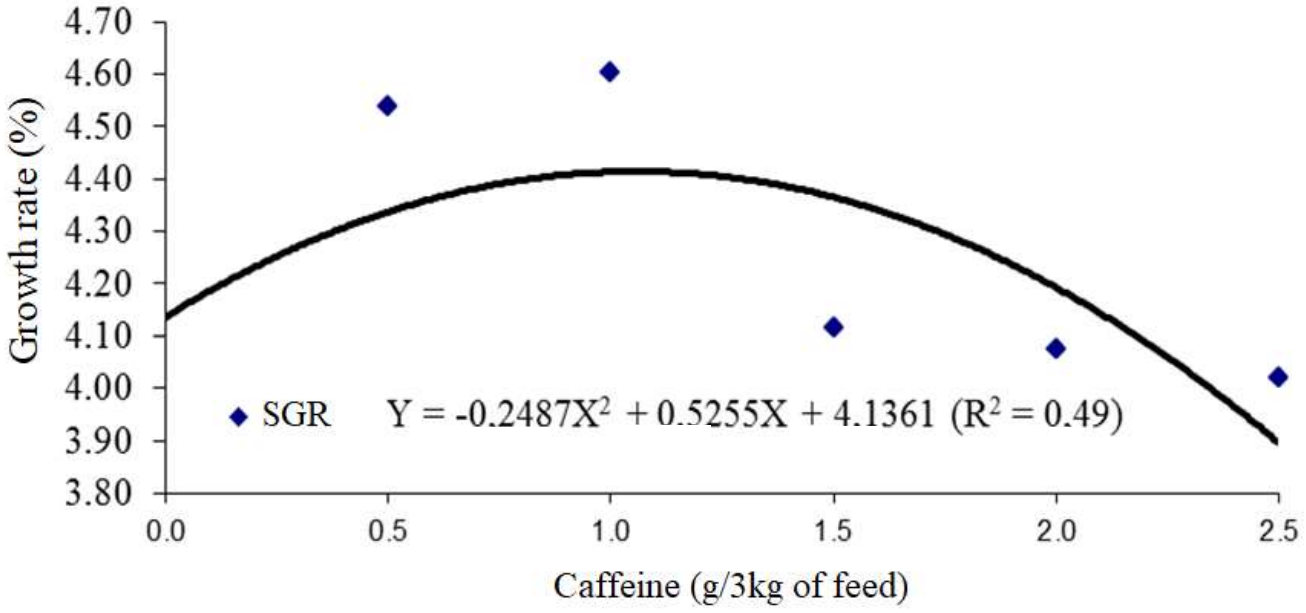

Figure 3. Second-degree polynomial regression between specific growth rate (SGR) and different caffeine dosages for juveniles of Nile Tilapia.

(Rojas and Verreth, 2002). Fagbenro (1992) showed a decline in growth rate by replacing maize with cocoa meal by up to 45%, however, without changes in carcass quality, digestibility and feed conversion ratio of *Clarias isiheriensis.*

In the present study, the survival variable did not present difference ($p > 0.05$). It is suggested that the use of black tarpaulin and shelters added in the animal enclosures contributed positively to their survival, and it is possible that the effect of stress mitigation had a positive influence on this variable. Merighe et al. (2004) also observed a decrease in stress in tilapias by means of the

black staining of the enclosures. Although not significant, the same together with the others that were significant may also have been imposed, due to the situations of agonistic confrontations, which demand energy reserves, which may have been diverted from growth and focused on the metabolic demands imposed by such confrontations. However, all these factors, when associated with the possible metabolic and lipolytic action of caffeine, triggered different responses according to the dosages used.

The results described by Bayne et al. (1976) presented no difference ($p > 0.05$) for weight gain. Although, the mean values of all the variables analyzed were satisfactory until the inclusion of 1 g of caffeine, it is emphasized that the substance used in the present study was 99% pure, unlike that used by Bayne et al. (1976) where caffeine was from the brown pulp. According to Caielli (1984), the chemical compositions of the pulp and the coffee husk are very similar. According to Souza et al. (2001), fiber, tannins, crude protein, among others, are components that may interfere negatively with animal performance, as already observed in ruminants (Bernardino et al., 2009; Carvalho Júnior et al., 2010). Pimenta et al. (2011) reported a high digestibility coefficient and better performance of Nile Tilapia (3 g) fed with the ration containing coffee husk, molasses, whey and formic acid, with different drying methodologies. Costa et al. (2017) studying waste of coconut biscuit residues for Nile tilapia observed that, the inclusion of biscuit residue affected significantly, the variability that can replace maize bran by up to 11.62%, improving fish performance because it affects fish metabolism and performance.

It is important to note the marked behavioral change of the individuals who received caffeine in their diets, characterizing agonistic behaviors, such as confrontations, changes in disposition in the room and high excitability. These manifestations were also in the present study as high frequency of biting, especially in the treatments with higher inclusion rates. Just as excess caffeine consumption can cause behavioral disturbances (Santos and Sant'ana, 2014), excitement, euphoria and increased motor activity in humans (Valenzuela, 2004), the same was identified in this research using juveniles of Nile Tilapia.

Behavioral changes due to substance use have also been described in other animals, such as mares (Santana, 2009) and rats (Marin et al., 2011), with excitation and increased motor activity being observed. This can be explained by the stimulating effect of the central nervous system caused by caffeine (Mello et al., 2007), where inadequate dosages may trigger psychomotor changes in animals (Holtzman et al., 1991).

The effect of caffeine on aggressiveness may vary among species, according to Nehling et al. (1992). Ingestion of psychoactive substances, such as caffeine, according to Fernández-Serrano et al. (2010) led to functional and structural changes throughout the body, mainly through interference in neurotransmitters. Caffeine is able to bind to metabotropic receptors for adenosine, further blocking the gamma-amino-butyric acid neurotransmitter ($GABA_A$) (Meyer and Quenzer, 2005), and adenosine receptors co-located with dopamine receptors (Garett and Griffiths, 1997).

These neurotransmitters are closely linked to the central nervous system, where the binding of caffeine to its active sites can trigger various behavioral actions, as well as act on mood and food intake (Terry et al., 1995), which may have influenced consumption of animals. Changes in dopamine through caffeine may also alter the level of stress, aggressiveness (Montoya et al., 2012) and competitiveness (Arias-Carrion et al., 2010). The neurotransmitter GABA, when in interference, can also cause disturbances in food intake (Tsuji and Bray, 1991). It is suggested that caffeine dosages greater than 1 g may have triggered the described situations and caused the negative effect of the substance as the variables analyzed.

The energy balance of the animals should be considered. Vazzoler (1996) reported that fish can distribute their energy through several alternatives, directing it towards reproduction and growth. Such patterns of allocation, according to Encina and Granado-Lorencio (1997) and Huntingford et al. (2001) may vary according to habitat and food conditions, leading to changes in their physiological state. The energetic balance of the organism may undergo modifications during the life cycle of the species, as well as express the proportion of energy consumed, directed to the vital processes (Phan et al., 1993).

According to Benedito-Cecilio et al. (2005), seasonal changes in the reproductive and fish growth cycle, cause changes in the caloric content of the tissues, showing a close relationship between physiological status and body composition. Folkvord and Ottera (1993), Neu et al. (2012) and Carvalho et al. (2012) reported that the stage of development of the animals is one of the main factors that determine their feeding frequency, since young fish have a higher metabolic activity and need a greater food supply than adult animals. In addition, the intense metabolic activity of younger animals triggers less accumulation of fat, unlike the individuals in the termination phase (Lima et al., 2012).

It is inferred that higher feed consumption estimated in the present study, when compared with others, may be derived from the life stage of the animals of the present research, juveniles, which according to Kubitza (1999), direct a large part of their energy to growth. The positive effect of caffeine with the inclusion of up to 1 g in the diet can be inferred by the direct action of the substance in the central nervous system, causing an increase in metabolism (Silva et al., 2014). Thus, making the individual to present greater energy expenditure and need to feed on the higher frequency and/or breed

quantity, resulting in greater food consumption, weight gain and body growth.

However, it is evidenced that even in a phase of intense metabolism and need for greater food consumption, caffeine dosages above 1 g interfered negatively with the consumption of the same, consequently causing a decrease in weight and body growth, demonstrating that caffeine can act satisfactory or not, according to the dosage used. In dosages higher than 1 g, it is assumed that the lipolytic effect of caffeine may have occurred negatively, reducing the weight gain of the animals and interfering with their body development. One of the biggest arguments involving caffeine would be its lipolytic potential. Mello et al. (2007) described that this substance triggers an increase in the mobilization of free fatty acids in the tissue with less oxidation of carbohydrates and greater oxidation of muscle fat, once, according to Altimari et al. (2006) acts on the enzyme lipase. In addition, caffeine increases the synthesis of catecholamines, the neurotransmitters that stimulate lipolysis (Saldanha, 2012).

In humans, it is known that dosages of 3 to 9 mg caffeine/kg stimulate lipolysis; above 9 mg/kg, it no longer produce such an effect and doses of 10-15 mg/kg has toxic effects on the body (Graham et al., 2001). Studies on pigs showed positive effects when added. Chorilli et al. (2005) found that caffeine applied in the dosage of 2 mL of a solution (2%) for piglets, caused the reduction of adipose tissue thickness of the hypoderm in 55.3%. Parra et al. (2008) using coffee residue in pig feed, observed a decrease in fat in the carcass, and it is possible that this result is related to the lipolytic action of caffeine.

For other animals, as well as fish, the lipolytic effect of caffeine and its adequate dosages have not yet been stipulated; such effect is achieved in a positive manner as the decrease of fat without causing negative changes in the productive performance of the animal. It is of great economic interest that such an effect be achieved once; and excess fat in carcasses and fillets is an undesirable feature and should be maintained at levels that do not adversely affect the organoleptic characteristics of the final product, fillet yield and commercial value.

However, Spriet (1995) has described that there is no single mechanism explaining the action of caffeine, once it is able to overcome the blood-brain barrier, and the cell membranes of all tissues, making it difficult to determine specifically its action. Therefore, more studies on their potentialities in all species including fish, are needed, so that adequate dosages can be stipulated and economic, production and mainly animal welfare improvement can be provided.

Conclusions

The inclusion of caffeine in feed at doses up to 1 g/3 kg of feed improves the productive performance of juveniles of Nile Tilapia (*Oreochromis niloticus*).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Anaesthetic effects and haematological responses of *heterobranchus bidorsalis* juveniles exposed to clove oil

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This study aims to investigate the use of clove oil as an anaesthetic agent for *Heterobranchus bidorsalis* juveniles and assess the effects on the test fish, using some hematological parameters. Different concentrations of clove oil (0.8 to 1.2 ppm) were used in static water to determine the induction time and recovery time of the test organisms. Behavioral changes of the test fish were observed, the time the fish became immobilized and recovery from anaesthesia was recorded. The results showed erratic swimming, loss of equilibrium, loss of body movement with continued operculum movements, and loss of reflex of the test organisms during the period of exposure at higher concentrations (1.0 to 1.2 ppm) of clove oil. The physiological stress responses of *H. bidorsalis* juveniles increase when compared with fish in the control experiment. The induction time (119 s) significantly decreased with increasing concentrations of clove oil (1.2 ppm), while the recovery times ranged between 180 to 906 s from lower concentration (0.8 ppm) of clove oil to the highest concentrations (1.2 ppm).There was a positive linear effect (R^2=0.95, P<0.05) between the concentration of clove oil and the induction time. The values obtained from hematological responses of the test organisms exposed to different concentrations of clove oil showed that the clove oil caused a concentration dependent changes in the blood of *H. bidorsalis* juveniles with significant reduction (P<0.05) in the values of packed cell volume (PCV) (26.00 to 16.00), haemoglobin (Hb) (8.70 to 5.90), mean corpuscular volume (MCV) (130.00 to 70.70) and the values of mean corpuscular haemoglobin (MCH) (38.61 to 32.41), and white blood cell (WBC) (29.50 to 73.50) increased significantly (P<0.05) with increasing concentrations of clove oil, while the values of red blood cell (RBC) and mean cell haemoglobin concentration (MCHC) varies with no definite pattern. Clove oil could be used as anaesthetic agent in fisheries because the induction and recovery times were within the recommendation range used in fishery management and does not pose any environmental hazard.

Key words: *Heterobranchus bidorsalis*, clove oil, anaesthetic effect, haematology examination, induction time, recovery time.

INTRODUCTION

Anaesthetics are often used in aquaculture, fisheries and biological researches as a way to minimizes fish hyper-motility which is a considerable source of injuries during handling procedures (Cho and Heath, 2000; Rose and Rose, 2008; Olufayo and Ola, 2010). The consequent damages from such accidents succumbed fish to increase the susceptibility to pathogens and infection diseases (Rose and Rose, 2008). Therefore, reducing

fish motility by anaethetics may decrease the undesirable handling consequences (Rose and Rose, 2008). There are many researches on the physiological effects of different anaesthetics on fishes and clove oil has received favourable reviews as an alternative anesthetic for different fish species (Okey et al., 2013; Hitoki et al., 2011) due to the antifungal and antibacterial properties (Keene et al., 1998; Cho and Heath, 2000). Plant naturally possess substances which are used by man in production of medicine especially antibiotics (Okwu and Josiah, 2006), thus, their importance in aquaculture and fishery management is very important.

Clove oil is an organic natural product, a dark brown liquid resulting from distillation of flowers, flower stalks and leaves of Eugenia aromatica (Nagababu and Lakshmaiah, 1992), its contains 70 to 90% eugenol, more than 17% eugenol acetate and 12% kariofilen. It is derived from the stem, leaves and buds of Eugenia caryophylatta tree and its active ingredients are eugenol (a-methoxy-4-2 (2-propenyl)-Phenol and isoeugenol (4-propenyl-2-methoxy phenol).

The use of clove oil is more potent than synthetic anaesthetics used in fish and its efficacy as an anaesthetic for various fish has been demonstrated in various fish species (Olufayo and Ola, 2010, Dang- Won Seol et al., 2007) and the U.S Food and Drug administration has considered it as safe compound (Summerfeit and Smith, 1990) for use in biological research.

Some research works have been carried out using clove oil as anaesthetics on different fish species: Alok et al. (2014) and Hajek (2011) worked on common carp (Cyprinus carpio); Cho and Heath (2000) worked on Chinook salmon (Oncorhynchus tshawytscha); Veliek et al. (2006) and Olufayo and Ola (2010) worked on African catfish; Keene et al. (1998) and Prince and Powell (2000) worked on rainbow trout (Oncorhynchus mykiss); Anderson et al. (1997) and Prince and Powell (2000) investigated the effects of clove oil rainbow trout; while Woody et al. (2002) worked on adult sockeye salmon. The main objective of this study was to investigate the effects of clove oil on H. bidorsalis juveniles using haematological parameters to assess the changes in the test fish induced by the anaesthetic.

MATERIALS AND METHODS

Experimental fish

One hundred and fifty healthy juveniles of H. bidorsalis (weight 25.8 g) were obtained from Ayoola Commercial Fish Farm, Nigeria and were transported in pond water at 23°C temperature to the Department of Fisheries and Aqua culture Technology Laboratory at Federal University of Technology Akure in a plastic container containing 50 L of fresh water to reduce stress. The fish were acclimated in the laboratory in a well aerated large fibre glass holding tank (1500 L volume) for one week. The fish were fed during the acclimation period with Coppen feed according to their body weight, but were unfed for 24 hours prior to the test in order to minimize the production of waste, thereby reducing ammonia production from the wastes.

Preparation of anaesthetic solution

Clove oil manufactured by B.D.H Chemical- Limited (Poole England), Boisar 401501 with Batch No: 0392-459-232422 and Product No: 46063 were obtained from Pascal Scientific Pharmaceutical Stores, Akure, Nigeria. The stock solution (100 ml) of clove oil was dissolved in 95% ethanol at a ratio of 1:10 (Cho and Health, 2000) before adding to fresh water, because it is insoluble at water temperature below 15°C. Different concentrations clove oil of 0.8, 0.9, 1.0, 1.1 and 1.2 ppm was applied to the tanks in duplicates.

Experimental procedure

Ten fish were distributed each into prepared anesthetic baths of different concentrations of clove oil in duplicates with a control with 10 L of water in twelve plastic tanks each (n=10 fish/tank) and the hyper-activities of the test fish, changes in the body color, loss of sensitivity to stimuli, motionless (induction time) and other behavioural activities were observed. Individual test fish were transfer to a recovery tank (50L) as they reach anesthesia and recovery time was established at the point when the animal recovered normal activities and regular breathing. Blood was collected into a syringes containing 0.5 mg disodium salt of Ethylene Diamine Tetra-acetic acid (EDTA) during anaesthesia state. This anticoagulant prevented the blood from clotting. The following haematological parameters were measured from the blood samples collected before, during and after recovery from anaesthesia: haematocrit (RBC),haemoglobin (Hb), erythrocytes (RBC), leucocytes counts (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration. All these parameters were determined using standard methods.

Water quality parameters

Dissolved oxygen (DO), pH, temperature and conductivity in each aquarium were measured and observed before the experiment, during the 96 h exposure and after the experiment using the methods described by APHA (1989).

Statistical analysis

Linear regression and graphs were used to determine the relationship between concentration, induction and recovery time while experimental data were analyzed using one way analysis of variance (ANOVA) to test for significant differences. Standard deviation (SD) and Pearson correlation coefficient were calculated. Significance was set at $P < 0.05$. All analyses were performed

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Table 1. Induction and recovery time and mortality in *H. bidorsalis* exposed to various concentration of clove oil.

Clove oil concentration(ppm)	Induction time (s)	Recovery time (s)	Mortality (%)
0	0	0	0
0.8	477.50±3.54^{e}	180.00±0.00^{a}	0
0.9	443.00±11.31^{d}	305.00±7.07^{b}	0
1.0	360.00±0.00^{c}	514.00±48.08^{c}	10
1.1	186.00±8.49^{b}	675.00±21.21^{d}	20
1.2	119.00±1.41^{a}	906.50±9.19^{e}	20

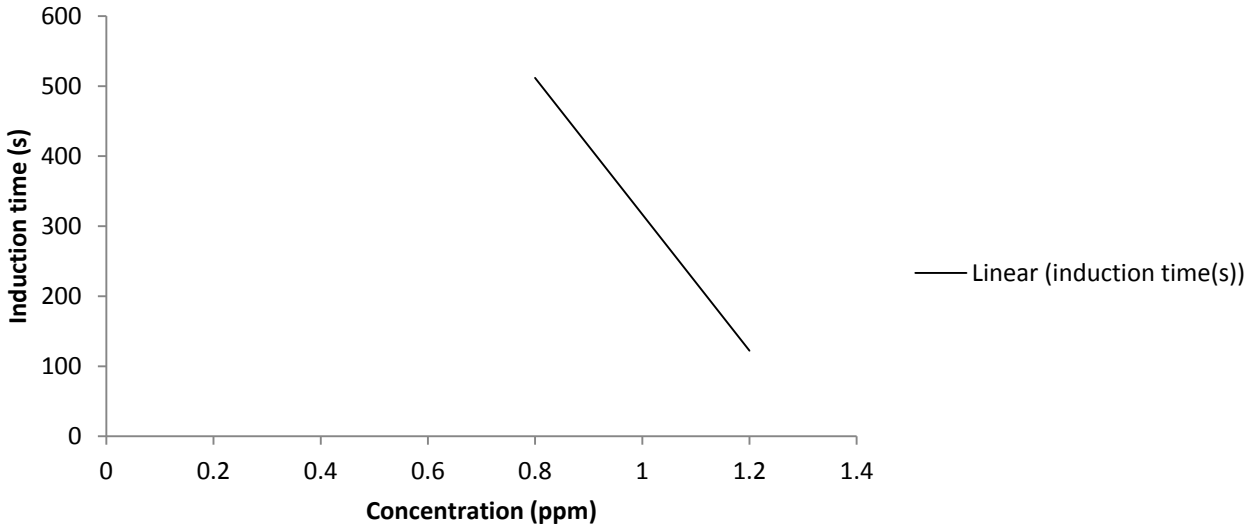

Figure 1. Relationship between concentration and induction time of *H. bidorsalis* exposed to different concentrations of clove oil: (P<0.05).

using statistical package for social sciences (SPSS) software (version 17.0, 2015).

RESULTS AND DISCUSSION

All the test fish reached anaesthesia within 2 min in the highest concentration (1.2 ppm) and 8 min in the lowest concentration (0.8 ppm). Results from the experiment revealed that there was relationship between concentration of clove oil, induction time, recovery time and mortality of *H. bidorsalis* juveniles exposed to various concentration of clove oil (Table 1).

Induction time

The induction time decreases with increasing concentration of clove oil (y= -974x + 1291, Figure 1). There was a positive linear effect (P<0.05) R^2=0.950 between the concentration of clove oil and the induction time. In this experiment, the induction time in highest concentration (1.2 ppm) is 119 s and took longer time to recover (906 s) (Table 1). The rate of mortality recorded at the highest concentrations (1.1 ppm and 1.2 ppm) of clove oil agreed with the study of Sladky et al. (2002); who reported that at higher concentration, there was high mortality rate

Recovery time

The recovery time increases with increase in concentration of clove oil (y=1822x – 1306, Figure 2). There was a positive linear effect (P<0.05) R^2=0.991 between the concentration of clove oil and the recovery time. The result clearly showed that, the higher the concentration of anaesthetic, the shorter the induction time and the longer the recovery time. The longer recovery time observed in fish anaesthetized with clove oil in higher concentrations could be as a result of stress incurred from the anaesthetic influence, metabolic rate, oxygen consumption, branchial respiration and the blood pressure of the test fish. Effects of dissolved oxygen were

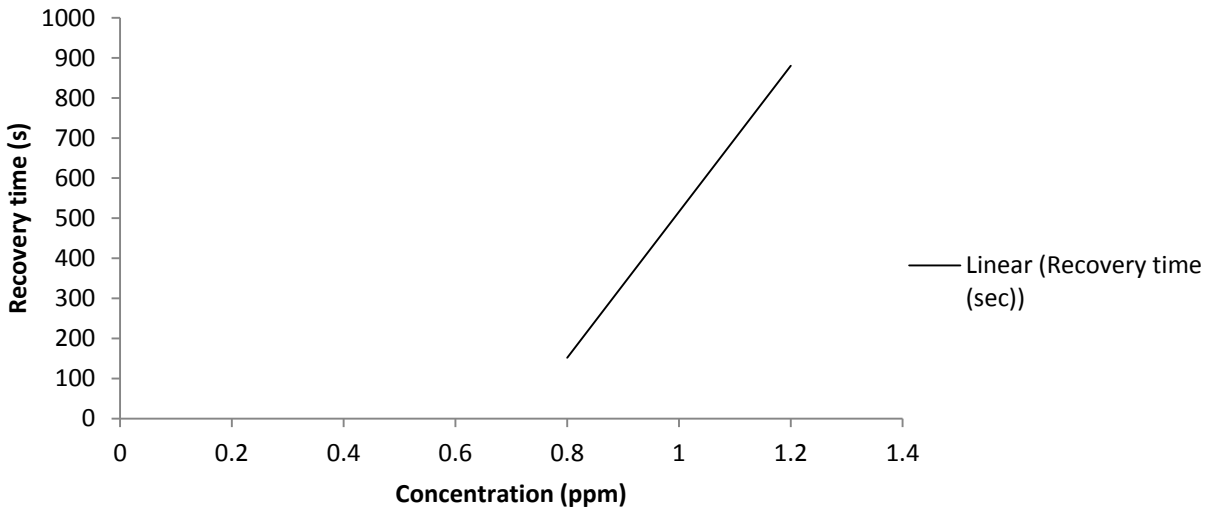

Figure 2. Relationship between concentration and recovery time of *H. bidorsalis* exposed to different concentration of clove oil (P<0.05).

Table 2. Physico-chemical analysis of water after subjecting to various concentration of clove.

Parameter	Concentrations (ppm)					
	0	0.8	0.9	1.0	1.1	1.2
Conductivity	2201.00±1.41^{a}	206.57±0.76^{b}	206.53±0.11^{b}	208.71±0.83^{b}	210.93±0.38^{c}	213.25±1.20^{d}
D.O (mgl^{-1})	6.57±0.21^{c}	2.95±0.35^{b}	2.54±0.03^{b}	2.47±.08^{b}	1.40±0.28^{a}	1.50±0.71^{a}
Temperature (°C)	27.28±0.75^{a}	26.65±0.92^{a}	25.70±0.08^{a}	26.15±0.80^{a}	26.36±0.52^{a}	26.37±1.03^{a}
$_{P}$H	7.30±0.08^{a}	7.36±1.11^{a}	7.48±0.12^{a}	8.15±0.21^{b}	8.28±0.18^{b}	8.40±0.28^{b}

Values on the same row with different superscript letters are significantly different (P<0.05).

obvious in all of the concentrations shows significant differences in both induction and recovery time.

Physiochemical parameters

Conductivity was observed to increase with increasing concentration of clove oil. The dissolved oxygen in the control was significantly higher than other varying concentrations of clove oil (0.8 to 1.2 ppm) and decreases drastically with the increase of concentration of clove oil. Temperature is observed to be fluctuating while pH increases with increasing concentration (Table 2).

Haematological parameters

Table 3 shows that the haematological parameters measured were significantly affected by clove oil. Treatment with clove oil caused a progressive decrease (P < 0.05) in the PCV values (26.00±2.83 to 16.00±1.41), HB (8.70±0.14 to 5.90±0.42), MCV (130.00±14.14 to 70.70±14.14) and increase in MCH values (32.41±5.43 to 38.61±6.12) and WBC (29.50±6.36 to 73.50±49.50) when compare with the treatment in the control tanks. The RBC and MCHC values vary within different concentrations of clove oil (1.80±0.14 to 2.85±0.07 and 26.30±0.14 to 41.75±2.47) respectively. Exposure of *H. bidorsalis* juveniles to clove oil caused significant decrease in PCV, HB, RBC and MCV. This significant reduction may be an indication of severe anaemia caused by clove oil on the exposed fish. This finding agreed with Olufayo and Adeyanju (2012) who worked on haematological effect of neem leaves (*Azadirachta indica*) on *H. bidorsalis* and reported that the toxicant caused a significant decrease in PCV, Hb, RBC, MCHC and MCV.of *H. bidorsalis* Otherwise, increase in the values of MCH and WBC of the fish, may be due to the physiological reaction of the fish to the effects of stress induced by the anaesthetics used. This was attributed to a generalized stress response resulting from increased pituitary inter-renal activity which agreed with Thompson and Eling (1989).

Behavioral changes

The behaviors exhibited by the test fish ranged from

Table 3. Hematology of *H. bidorsalis* juveniles exposed to different concentrations of clove oil.

Parameter	Concentrations (ppm)					
	0	0.8	0.9	1.0	1.1	1.2
PCV (%)	26.00±2.83^{c}	24.00±0.41^{bc}	22.00±1.41^{abc}	19.00±2.83^{ab}	16.00±1.41^{a}	17.00±4.24^{a}
RBC(mm)	2.00±0.00^{ab}	2.20±0.14^{b}	2.85±0.07^{c}	2.70±0.14^{c}	2.11±1.01^{b}	1.80±0.14^{a}
WBC(mm)	29.50±6.36^{a}	41.50±3.53^{ab}	56.50±9.19^{ab}	59.50±12.02^{ab}	64.50±12.02^{c}	73.50±49.50^{c}
HB(g 100 ml^{-1})	8.70±0.14^{c}	8.15±0.35^{b}	7.55±0. .21^{b}	7.20±0.28^{b}	5.90±0.42^{a}	6.10±0.14^{a}
MCH(pg cell^{-1})	32.41±5.43^{c}	32.83±3.94^{bc}	34.14±1.55^{a}	38.61±6.12^{a}	35.55±5.35^{a}	37.39±9.74^{b}
MCHC(g 100 ml)	41.75±2.47^{a}	35.80±4.53^{a}	26.30±0.14^{a}	26.80±1.13^{a}	26.85±1.63^{a}	34.25±3.04^{a}
MCV(µm-1)	130.00±14.14^{c}	109.05±0.64^{bc}	77.15±3.04^{a}	70.70±14.14^{a}	76.15±6.72^{a}	73.75±16.19^{a}

Values on the same row with different superscript letters are significantly different (P<0.05).

slight loss of reactivity to external stimuli at lower concentrations toincreased opercula rate; erratic swimming; partial and total loss of equilibrium and loss of reflexes in fish exposed to higher concentrations of clove oil. Results from this experiment agreed with previous works, which reported that *H. bidorsalis* juveniles exposed to toxicants or anaesthesia usually exhibits changes in opercula rate, erratic movement and different behavioral activities (Olufayo and David, 2009; Akinbulumo, 2004). The observation of fish responses in this experiment also agreed with Pascuel el al. (1994), which says fish settled at the bottom of experimental tanks indicates stress or weakness.

Conclusion

Clove oil is effective, relatively safe and economically sustainable anaesthetic agent. It is environmental friendly at low concentration and less persistence in aquatic environment. The results of this study provide baseline information on the responses of *H. bidorsalis* to various concentrations of clove oil and show that active anaesthetic components of clove oil could be used as potential anaesthesia at concentrations 0.8 to 1.0 pmm when associated with a reasonable recovery time and mortality. It does not cause irreversible damage in *H. bidorsalis*, therefore, the use of clove oil in fisheries should be encouraged in preference to synthetic anaesthetic such as MS22 when searching for medicinal plants with anasthetic active compound.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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