Original Article

DOI: 10.5582/ddt.2017.01035

Effects of *Alpinia galanga* oil on anesthesia and stress reduction in *Oreochromis niloticus*

Surachai Pikulkaew[1], Nattakanwadee Khumpirapang[2], Wasana Chaisri[1], Siriporn Okonogi[3,*]

[1] *Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand;*
[2] *Interdisciplinary Program in Nanoscience and Nanotechnology, Graduate School, Chiang Mai University, Chiang Mai, Thailand;*
[3] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.*

Summary ***Oreochromis niloticus*** **(Nile tilapia) is one widely cultured fish in Thailand. Handling processes and transportation causes high stress in Nile tilapia. This study explores anesthetic effect and stress reduction of *Alpinia galanga oil* (AGO) on Nile tilapia. The anesthetic activity was evaluated by the time for fish induction to anesthesia and full recovery. It was found that the suitable dose of AGO that caused desirable anesthesia of Nile tilapia was 700 mg/L. This dose gave induction and recovery times of approximately 257 and 438 sec, respectively. Blood glucose and plasma cortisol of the fish anesthetized with AGO showed nearly normal levels indicating that the fish stress during handling was not increased. Study on loading densities of fish mimicked general fish transportation and showed that loading density of fish was a crucial factor on fish stress. The highest water quality was found in the lowest loading density of fish. Water containing AGO at a concentration of 150 mg/L showed significantly higher potential for reducing fish activity and water improvement than without AGO. Therefore, AGO is a promising natural edible plant oil for anesthesia in Nile tilapia.**

Keywords: *Alpinia galanga*, plant oil, anesthesia, stress, *Oreochromis niloticus*

1. Introduction

The rapidly growing human population causes raising global demand for food consumption. Aquatic animals have become the fast-growing in the food-production sector globally. Several processes such as netting, weighing, sorting, vaccination, and transportation in which fish are subjected to handling and confinement can induce stress in fish (*1*). In addition, exposure to environmental stressors (aquatic pollutants) also stimulates the hypothalamic-pituitary-adrenal axis (HPA) resulting in elevated plasma cortisol levels (*2-4*). A prolonged elevation of cortisol due to stress has been considered harmful due to increased susceptibility to disease and suppression of reproductive processes (*5-7*).

**Address correspondence to:*
Dr. Siriporn Okonogi, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.
E-mail: okng2000@gmail.com

Chemical agents such as benzocaine, tricaine methansulfonate (MS-222), metomidate hydrochloride, isoeugenol, 2-phenoxyethanol and quinaldine are routinely used as anesthetics in aquaculture to minimize stress associated with handling (*8*). However, undesirable side effects such as hypersecretion of fish mucus and skin irritation have been observed when using these compounds (*9*). Clove oil is a natural anesthetic that has been used in aquaculture for a long time (*10*). The effectiveness of clove oil as anesthetic for many fish species; *Cyprinus carpio* (*11*), *Siganus guttutus* (*12*), *Siganus lineatus* (*13*) has been reported.

However, some fish species exposed to clove oil showed an elevation of blood cortisol and glucose whereas blood lymphocytes were decreased (*14-16*). Those side effects from clove oil on fish indicate that stress still occurs when clove oil is used. Moreover, eugenol, the main active compound of clove oil, has been reported that it might damage hepatic tissue in mice (*17*). It has been reported that the essential oils from *Oleum malaleucae* (*18*), *Ocimum gratissimum* (*19*), and *Lippia alba* (*20*) have anesthetic activity in fish. The

desirable properties of anesthetic agents in fish should be highly effective and safe. The extracts from inedible plants might contain some toxic compounds (*21*).

Alpinia galanga, an edible plant in family Zingiberaceae, is widely grown throughout Southeast Asian countries (*22,23*). The plant is well-known in Thai and Chinese traditional medicine and used as anti-inflammatory, antipyretic, carminative, antiflatulent, and anti-itching (*24,25*). Its rhizomes and flowers have been widely used for centuries in Asian food remedies. According to our best knowledge, no information is available in the literature about anesthetic activity of *Alpinia galanga oil* (AGO) on fish, particularly on those cultured for human food.

Oreochromis niloticus (Nile tilapia) is an important source of protein and source of human income. It is one of the most popular fish in Thai cuisine. Trends of Nile tilapia culture have increased from small to large scale production in the Americas (*26*). In addition, Nile tilapia culture also has quickly expanded due to overconsumption (*27*). Using anesthetics in fish grown for human food should be carefully done because chemical anesthetics may be harmful to humans due to their possible carcinogenic and mutagenic effects. Humans have a long history of consuming *A. galanga* without any toxicity, AGO is of interest to investigate for its effectiveness on anesthesia and stress reduction in food fish like Nile tilapia. Safety can be sure even if there is some AGO residue left in the anesthetized fish. The present study also explores the advantage of AGO for water conditions for fish transportation.

2. Materials and Methods

2.1. *Fish materials*

Juvenile and adult Nile tilapia were purchased from a local ornamental fish shop, Chiang Mai province and acclimated to laboratory conditions at Faculty of Veterinary Medicine, Chiang Mai University. The water parameters for fish maintenance are the following; temperature, 25°C; pH, 7.4; total hardness, 110 ppm; alkalinity, 90 ppm; and total ammonia and nitrite were negative. All *in vivo* experiments were conducted according to permission from the animal care and use committee of the Faculty of Veterinary Medicine, Chiang Mai University (FVM-ACUC) (process no. R3/2555).

2.2. *Plant materials*

Fresh rhizomes of *A. galanga* were collected from a local garden in Chiang Mai province of Thailand. The plant was identified and the voucher specimen (No. 009245) was deposited at the Herbarium, Northern Research Center for Medicinal Plants, Faculty of Pharmacy, Chiang Mai University.

2.3. *Chemical materials*

Absolute ethanol, anhydrous sodium sulphate, and sodium bicarbonate were of analytical grade from Merck Millipore (Darmstadt, Germany). MS-222 and Drabkin's reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents were of the highest grade available.

2.4. *Extraction and component determination of AGO*

Fresh rhizomes of *A. galanga* were washed and cut into small pieces and subjected to hydro-distillation using a Clevenger apparatus for 3 h. The AGO obtained was kept in a light-resistant container at –20°C until further use.

For component determination, gas chromatography-mass spectrometry (GC-MS) on an Agilent 6890 gas chromatograph with a fused silica capillary column (HP5-MSI; 30.0 m × 0.25 mm i.d. × 0.25 µm film thickness) was used. AGO was diluted with dichloromethane to a ratio of 1:100 (v/v) before injection. The identification of each compound was based on their retention times relative to those of authentic samples and matching spectral peaks available in the mass spectral libraries.

2.5. *Anesthetic activity study*

Stock solution of AGO was first prepared by diluting AGO with absolute ethanol to obtain an AGO ethanolic solution (AGO-EtOH) with a concentration of 100 mg/mL. Various volumes of AGO stock solution were added to the induction tank to obtain AGO final concentrations of 100-900 mg/L and the volume was adjusted using dechlorinated water. Juvenile Nile tilapia (average weight; 40.8 ± 1.2 g and total length; 13.6 ± 0.2 cm) were used in this experiment. The stages of fish anesthesia and recovery were modified from criteria outlined by Zahl *et al*. (*1*) using the following details. Stage 1; fish are disoriented and reduce swimming activity, stage 2; the fish show partial loss of equilibrium. When the fish stopped their swimming activity, presented a total loss of equilibrium and no responsiveness at all, they were in surgical anesthesia stage 3. In this study, anesthetic activity was intensively studied on anesthesia stage 3 because this stage is normally suitable for fish surgery, clinical examination, or treatment. The time that the fish reached the desired anesthetic stage was determined. Then, the fish were moved to the recovery tank containing only dechlorinated water and monitored until fully recovered. The recovery time was recorded. After that, the recovered fish were placed in another tank for one week and fed with pellet feed in order to observe mortality.

2.6. *Transportation simulation study and water quality analysis*

Juvenile Nile tilapia (average weight; 1.34 ± 0.07 g and total length; 4.25 ± 0.22 cm) at various loading density (100, 200, and 300 fish/plastic bag) were used in this study. In a control group, the plastic bag contained 4 L of dechlorinated water and 12 L of pure oxygen. In a treatment group, AGO alcoholic solution prepared as mentioned in Section 2.5 was added to the plastic bag and the volume was adjusted to 4 L with dechlorinated water to have a final AGO concentration of 150 mg/L, and then 12 L oxygen was added. The plastic bags were sealed after the fish were completely transferred inside to protect against leakage of oxygen gas. There were triplicates for each loading density. Transportation simulation was created by vertical and horizontal movement of a plastic bag every 15-20 min. Water quality parameters were analyzed before and after transportation. Dissolved oxygen (DO) and temperature were measured with a YSI oxygen meter (Model Y550A, USA). The pH was determined by pH meter (CyberScan 500, Singapore). Total ammonia nitrogen (TAN) levels were measured according to the method of Grasshoff *et al*. (*28*). Un-ionized ammonia (NH_3) levels were calculated according to the method of Emerson *et al*. (*29*). The experiment was done in triplicate. After the experiment, the fish were placed in another tank and fed with pellet feed for two weeks in order to observe mortality.

2.7. *Stress study*

The effect of AGO on fish stress was investigated by determination of hematological parameters, blood glucose, and plasma cortisol of fish after being anesthetized with AGO at 700 mg/L. MS-222 at 130 mg/L was used as a control. Adult Nile tilapia (average weight; 545.2 ± 9.2 g and length; 29.2 ± 0.5 cm) were fasted 1 day prior to use. The fish were divided into 10 groups, each group contained 10 fish and was cultured in an aquarium filled with 5 L of dechlorinated water. AGO alcoholic solution prepared as mentioned in Section 2.5 was added to five aquaria and MS-222 aqueous and solution pH was adjusted using sodium bicarbonate added to the other five aquaria. Blood was collected from anesthetized fish via a caudal vessel at time intervals of 0, 30, 60, 90, and 120 min and each fish was subjected to blood collection only at one-time point.

A portion of the blood was analyzed for hematocrit (Hct) by a standard microhematocrit method (*30,31*), hemoglobin (Hb) by a colorimetric cyanomethemoglobin method (*32*), and total red blood cells (RBCs) and white blood cells (WBCs) by counting (*33,34*), as well as glucose using a glucometer (Accu-Chek®, Roche, Australia). The remainder was centrifuged at 3,000 rpm for 10 min (Beckman Coulter Inc., USA) for plasma separation. Plasma cortisol was determined using single direct antibody competitive enzyme immunoassays previously described by Brown *et al*. (*35*).

2.8. *Statistical analysis*

The data presented as mean ± S.E.M. were analyzed by independent *t*-test or a one-way ANOVA followed by Tukey's post hoc test. Kolmogorov-Smirnov's test was used as normality of data evaluation. The statistical significance was considered as p-value < 0.05.

3. Results

3.1. *Extraction and component determination of AGO*

A. galanga rhizomes yielded 0.11% AGO. The obtained AGO appeared as a clear pale yellowish liquid. The GC chromatogram of AGO as shown in Figure 1 demonstrated that AGO contained many components. It was found that 1,8-cineole (retention time at 6.6 min) and 4-allylphenyl acetate (retention time at 18.9 min) were the major compounds whereas eugenol (retention time at 19.2 min) and its derivatives *i.e*., methyl eugenol (retention time at 21.4 min) and eugenyl acetate (retention time at 26.0 min) were the minor compounds in AGO.

3.2. *Anesthetic activity study*

AGO at a concentration of 100-600 mg/L showed sedative and anesthetic effects but did not induce Nile tilapia to anesthesia stage 3 during a 20 min evaluation period whereas 700-900 mg/L AGO was capable of inducing fish to anesthesia stage 1, 2, and 3 within 83-26, 163-126, and 257-151 sec, respectively (Figure 2). The induction and recovery times for fish anesthetized to all stages showed significant differences among concentrations (p < 0.05), but there was no significant difference of stage 1, 2, and recovery time between 800-900 mg/L AGO. An elevation of concentration caused an induction time decrease. In terms of recovery, high concentration of AGO caused longer recovery times than low concentrations. No mortality was observed after one week of monitoring.

3.3. *Transportation simulation study*

Transported fish in the AGO group showed slow movement whereas the fish in the control group showed

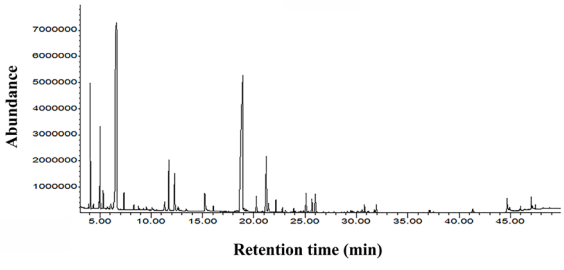

Figure 1. GC chromatogram of AGO

normal activity. After transport, an elevation of loading density significantly affected water parameters. TAN and NH_3 levels were found to be increased but DO level and the pH value were decreased ($p < 0.05$) (Table 1). The content in the plastic bags of the groups with the highest loading density exhibited the lowest DO and pH but highest TAN and NH_3. Mortality of 4.2 ± 1.2% was found in the highest density in the control group while no mortality was found in the AGO group. DO levels in the control group were significantly higher than those in the AGO group in the high loading density (200 and 300 fish/4L). In the AGO group, the pH values of all loading densities was similar. In addition, the TAN and NH_3 levels were significantly higher in the control group than in AGO groups at all loading densities except NH_3 in the groups with the highest loading density ($p < 0.05$).

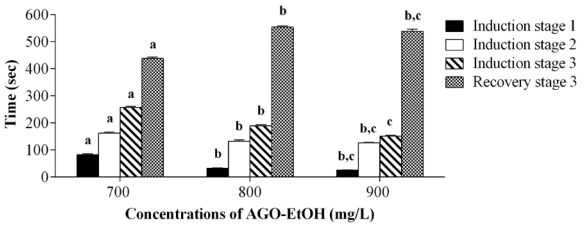

Figure 2. Induction and recovery times for Nile tilapia (n = 20) exposed to AGO-EtOH in dose-dependent manner. Different lowercase letters indicate significant differences of induction time and recovery time between concentrations based on one-way ANOVA and Tukey's test ($p < 0.05$).

3.4. *Stress study*

Blood analysis of fish exposed to AGO showed 13 g/dL Hb and 33% Hct whereas the concentrations of RBC and WBC were 1×10^6 and 19×10^3 cells/µL, respectively. The blood of those exposed to MS-222 exhibited approximately 13 g/dL Hb, 35% Hct, 2×10^6 cells/µL RBC, and 20×10^3 cells/µL WBC (Figure 3). For the anesthesia study, the results showed a significant difference among the treatments over the period of study except at two time points (30 and 60 min) of exposure (Figure 4a). The blood cortisol level of Nile tilapia anesthetized with MS-222 was

Table 1. Effect of AGO on water parameters before and after 4-h transportation in plastic bags

Water parameters	Before transport	End of transport simulation					
		n = 100		n = 200		n = 300	
		Control	AGO	Control	AGO	Control	AGO
DO (mg/L)	8.51 ± 0.24	5.92 ± 0.16	6.14 ± 0.27	4.49 ± 0.20	4.82 ± 0.10*	3.91 ± 0.39	4.41 ± 0.27*
pH	7.41 ± 0.03	6.47 ± 0.20	6.61 ± 0.10	6.67 ± 0.20	6.72 ± 0.26	6.40 ± 0.09	6.57 ± 0.35
TAN (mg/L)	0.01 ± 0.01	0.96 ± 0.08	0.75 ± 0.08*	1.46 ± 0.13	1.30 ± 0.07*	2.22 ± 0.20	1.94 ± 0.20*
NH_3 (mg/L)	0.00 ± 0.01	0.002 ± 0.01	0.001 ± 0.01*	0.003 ± 0.01	0.002 ± 0.01*	0.004 ± 0.01	0.004 ± 0.01

*significant differences between group in the rows using AGO and control after transportation ($p < 0.05$).

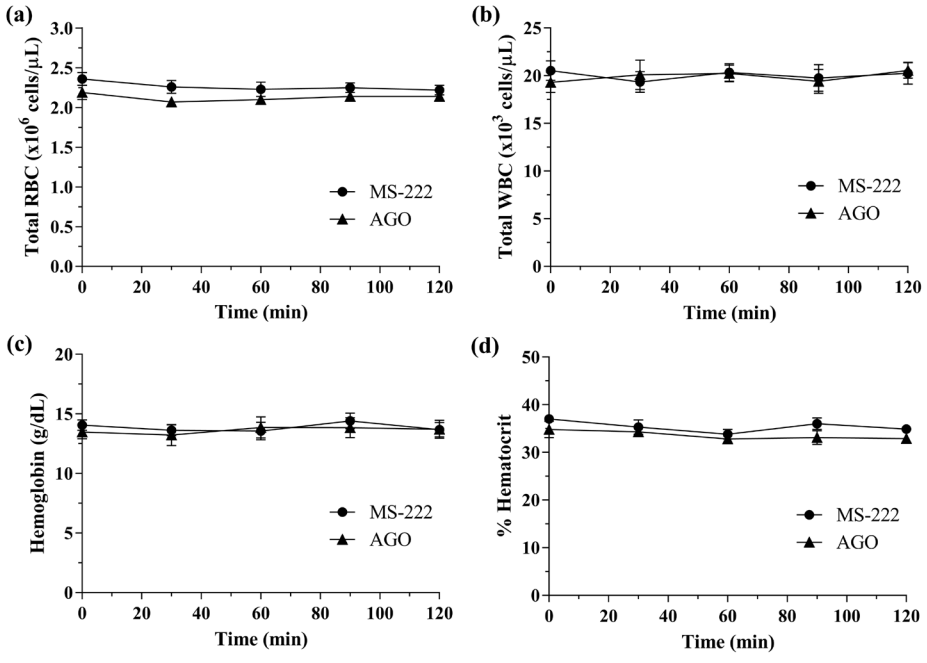

Figure 3. Comparison of total RBC (a), WBC (b), Hb (c) and Hct (d) in Nile tilapia after exposure to AGO and MS-222.

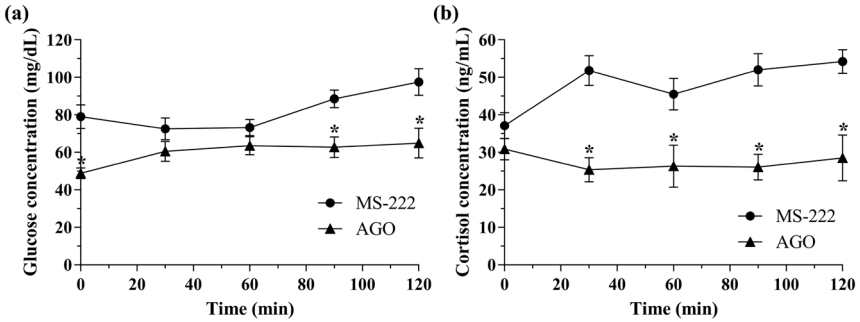

Figure 4. Comparison of glucose (a) and cortisol (b) levels in fish after exposure to 700 mg/L AGO and 130 mg/L MS-222. Asterisks (*) indicate significant differences between treatments at a given time point ($p < 0.05$).

significantly higher than those anesthetized with AGO after 30 min of exposure (Figure 4b). It was found that the cortisol level of the fish anesthetized with MS-222 increased rapidly up to 52 ng/mL within 30 min and did not return to normal levels within 120 min.

4. Discussion

Eugenol is known as a bioactive compound that can induce fish anesthesia. Plant oils composed of eugenol are expected to have potential for anesthesia of fish. However, analysis of AGO in the present study shows that this oil contains not more than 6% of eugenol and eugenol derivatives whereas 1,8-cineole and 4-allylphenyl acetate are found in the oil at a very high concentration. Therefore, these two compounds are considered to also have anesthetic activity on the fish.

According to the criteria of the ideal fish anesthetic, an anesthetic activity is determined by its ability to allow fish to reach surgical anesthesia stage 3 with an induction time of 3 or 5 min with a recovery time that should not be more than 2 fold of the induction time (*36*). In this study, AGO at a concentration of 700 mg/L was an appropriate concentration for Nile tilapia because of its induction and recovery time. We previously reported that a concentration of 300 mg/L of AGO was most suitable to anesthetize *Cyprinus carpio*. These dose differences were considered to be mainly due to species difference of the fish. Different fish species have different characteristics such as surface area of the body especially the surface area of the gill, which is the most important organ. The gill is the main entrance for anesthetics to the vascular system and to the target receptors. Therefore, the gill surface and the thickness of gill epithelium plays an important role for anesthesia (*37*). Generally the gill surface area decreases when the body weight increases (*38*). Moreover, body design, enzymes, lipid content or metabolic rate are different according to fish species difference (*39*). Therefore, the suitable dose for different fish species is different.

Fish transportation is a common procedure for fish farms and is considered to be a stressor that could negatively affect fish health (*3*). Stress during fish transport is due to handling and aquatic pollutants such as low oxygen and acidified water (*40*). In the present study, the transportation simulation was built to reproduce vertical and horizontal movement of a vehicle on a road, thus mimicking a commercial operation. Usually the farmers decrease the cost of fish transportation by increasing fish loading density. However, the results in the current study show that when fish are transported at high loading density, particularly without anesthetics, it can cause more stress than those transported at low loading density. Fish stress can be indicated by increased levels of DO, TAN, and NH_3. Stress also affects the respiratory system by an increase in oxygen uptake rate, ventilation rate, oxygen transport capacity of the blood (*41*), and results in an increase of Hct, Hb, RBCs and causes variation of WBCs (*42*). The results of the present study demonstrate that TAN and NH_3 levels in the water of the AGO group were significantly lower than the control group. These results indicate that AGO has a significant effect on reducing activity in fish. It was reported that low ammonia excretion indicated a decreased metabolic rate for fish (*43*). Therefore, it is considered that AGO may decrease the metabolic rate of Nile tilapia. This was the reason explained why DO level consumed by the fish was higher in the AGO group than in the control group.

Stress response of the fish after exposure to AGO was intensively studied compared to those using MS-222. The recommended dose of MS-222 on Nile tilapia was 130 mg/L (*4*) while the suitable dose of AGO was 700 mg/L. Generally, the hematological parameters; Hb, Hct, RBC, and WBC of resting fish are approximately 6 g/dL, 26%, 1×10^6 cells/μL, and 15×10^3 cells/μL, respectively (*44,45*). In the present study, the hematological parameters of anesthetized Nile tilapia with AGO and MS-222 were higher than the normal level. In addition, blood glucose and plasma cortisol levels are important stress indicators (*46*). The blood glucose and plasma cortisol levels of resting Nile tilapia are approximately 50-70 mg/dL (*47*) and 20-30 ng/mL (*48*). Surprisingly, the glucose and cortisol

levels in Nile tilapia exposed to AGO were quite constant at normal levels while the fish exposed to MS-222 showed higher levels of blood glucose and plasma cortisol than the resting fish at all-time points. These results suggested that AGO has potential for reducing stress in fish, which showed blood glucose and plasma cortisol levels of Nile tilapia to be close to normal. In conclusion, the current study indicates that AGO is a promising natural agent suitable for anesthesia in Nile tilapia.

Acknowledgements

The authors are grateful for the financial support received from the Thailand Research Fund (TRF) and the Research and Researchers for Industries (RRI) grant no. PHD56I0040. We also thank for the support from National Research Council of Thailand (NRCT) and Research Center of Pharmaceutical Nanotechnology, Chiang Mai University, Thailand.

References

1. Zahl IH, Samuelsen O, Kiessling A. Anaesthesia of farmed fish: Implications for welfare. Fish Physiol Biochem. 2012; 38:201-218.
2. Barton B, Schreck C, Ewing R, Hemmingsen A, Patino R. Changes in plasma cortisol during stress and smoltification in coho salmon, *Oncorhynchus kisutch*. Gen Comp Endocrinol. 1985; 59:468-471.
3. Barton BA, Iwama GK. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis. 1991; 1:3-26.
4. Coyle S, Durborow R, Tidwell J. Anesthetics in aquaculture, Publication No. 3900. Southern Regional Aquaculture Center (SRAC), Stoneville, Mississippi, USA, 2004; pp. 1-6.
5. Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology. 2000; 25:1-35.
6. Pickering A, Pottinger T, Carragher J, Sumpter J. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. Gen Comp Endocrinol. 1987; 68:249-259.
7. Carragher J, Sumpter J, Pottinger T, Pickering A. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and Salmo gairdneri Richardson. Gen Comp Endocrinol. 1989; 76:310-321.
8. Woody CA, Nelson J, Ramstad K. Clove oil as an anaesthetic for adult *sockeye salmon*: Field trials. J Fish Biol. 2002; 60:340-347.
9. Guenette SA, Uhland FC, Helie P, Beaudry F, Vachon P. Pharmacokinetics of eugenol in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2007; 266:262-265.
10. Endo T, Ohshima S, Ogishima K, Tanaka H. Studies on anesthetic effect of eugenol in some fresh water fishes. Bull Jap Soc Sci Fish. 1972; 38:761-767.
11. Hikasa Y, Takase K, Ogasawara T, Ogasawara S. Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*. Jpn J Vet Sci. 1986; 48:341-351.
12. Burhanuddin S, Wikanta T. Minyak cengkeh sebagai obat bius untuk ikan beronang (*Siganus guttatus*). J Penel Budidaya Pantai. 1989; 5:61-65.
13. Soto CG. Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). Aquaculture. 1995; 136:149-152.
14. Hoseini SM, Nodeh AJ. Changes in blood biochemistry of common carp *Cyprinus carpio* (Linnaeus), following exposure to different concentrations of clove solution. Comp Clin Path. 2013; 22:9-13.
15. Velisek J, Svobodova Z, Piackova V, Groch L, Nepejchalova L. Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.). Vet Med. 2005; 50:269-275.
16. Abrishamifar A, Bahonar A, Yousefi P. Anesthetic effect of tricaine methanesulfonate, clove oil and electroanesthesia on lysozyme activity of *Oncorhynchus mykiss*. Iran J Fish Sci. 2011; 10:3.
17. Mizutani T, Satoh K, Nomura H. Hepatotoxicity of eugenol and related compounds in mice depleted of glutathione: Structural requirements for toxic potency. Res Commun Chem Pathol Pharmacol. 1991; 73:87-95.
18. Hajek GJ. The anaesthetic-like effect of tea tree oil in common carp *Cyprinus carpio* L. Aquacult Res. 2011; 42:296-300.
19. de Lima Silva L, Parodi TV, Reckziegel P, de Oliveira Garcia V, Bürger ME, Baldisserotto B, Malmann CA, Pereira AMS, Heinzmann BM. Essential oil of *Ocimum gratissimum* L.: Anesthetic effects, mechanism of action and tolerance in silver catfish, *Rhamdia quelen.* Aquaculture. 2012; 350:91-97.
20. da Cunha MA, de Barros FMC, de Oliveira Garcia L, de Lima Veeck AP, Heinzmann BM, Loro VL, Emanuelli T, Baldisserotto B. Essential oil of *Lippia alba*: A new anesthetic for silver catfish, *Rhamdia quelen*. Aquaculture. 2010; 306:403-406.
21. Chaudhury RR, Rafei UM. Traditional medicine in Asia. World Health Organization, Regional Office for South-East Asia, New Delhi, India, 2001; pp. 1-309.
22. Matsuda H, Morikawa T, Managi H, Yoshikawa M. Antiallergic principles from *Alpinia galanga*: Structural requirements of phenylpropanoids for inhibition of degranulation and release of TNF-α and IL-4 in RBL-2H3 cells. Bioorg Med Chem Lett. 2003; 13:3197-3202.
23. De Pooter HL, Omar MN, Coolsaet BA, Schamp NM. The essential oil of greater galanga (*Alpinia galanga*) from Malaysia. Phytochemistry. 1985; 24:93-96.
24. Chudiwal A, Jain D, Somani R. *Alpinia galanga* Willd. – An overview on phyto-pharmacological properties. Indian J Nat Prod Resour. 2010; 1:143-149.
25. Arambewela LS, Arawwawala M, Owen NL, Jarvis B. Volatile oil of *Alpinia galanga* Willd. of Sri Lanka. J Essent Oil Res. 2007; 19:455-456.
26. Fitzsimmons K. Future trends of tilapia aquaculture in the Americas. In: Tilapia aquaculture in the Americas (Costa-Pierce BA, Rakocy JE, eds.). World Aquaculture Society and the American Tilapia Assoication, Baton Rouge, Louisiana, USA, 2000; pp. 252-264..
27. Charoendat U, Areechon N, Srisapoome P, Chantasart D. Efficacy of synthetic eugenol as an anesthetic for Nile tilapia (*Oreochromis niloticus* Linn.). Kasetsart J Nat Sci.

2009; 43:132-140.
28. Grasshoff K, Kremling K, Ehrhardt M. Methods of seawater analysis. Verlag Chemie GmbH, Weinheim, Germany, 1983; pp. 1-317.
29. Emerson K, Russo RC, Lund RE, Thurston RV. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. J Fish Res Board Can. 1975; 32:2379-2383.
30. Dorafshan S, Kalbassi MR, Pourkazemi M, Amiri BM, Karimi SS. Effects of triploidy on the *Caspian salmon Salmo* trutta caspius haematology. Fish Physiol Biochem. 2008; 34:195-200.
31. McGovern JJ, Jones AR, Steinberg AG. The hematocrit of capillary blood. New Engl J Med. 1955; 253:308-312.
32. Drabkin DL, Austin JH. Spectrophotometric studies I. Spectrophotometric constants for common hemoglobin derivatives in human, dog, and rabbit blood. J Biol Chem. 1932; 98:719-733.
33. Natt MP, Herrick CA. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poult Sci. 1952; 31:735-738.
34. Hrubec TC, Smith AS. Hematology of fish. In: Schalm's veterinary hematology (Feldman BF, Zink JG, Jain NC, eds.). Lippincott Williams & Wilkins, Philadelphia, USA, 2000; pp. 1120-1125.
35. Brown J, Walker S, Steinman K. Endocrine manual for the reproductive assessment of domestic and non-domestic species. Smithsonian's National Zoological Park, Virginia, USA, 2004; pp. 44-46.
36. Marking L, Meyer F. Are better anesthetics needed in fisheries? Fisheries. 1985; 10:2-5.
37. Bridger CJ, Booth RK. The effects of biotelemetry transmitter presence and attachment procedures on fish physiology and behavior. Rev Fish Sci. 2003; 11:13-34.
38. Muir B. Gill dimensions as a function of fish size. J Fish Res Board Can. 1969; 26:165-170.
39. Ross LG, Ross B. Anaesthetic and sedative techniques for aquatic animals. John Wiley & Sons, Scotland, 2009; pp. 65-68.
40. Xu J, Liu Y, Cui S, Miao X. Behavioral responses of tilapia (*Oreochromis niloticus*) to acute fluctuations in dissolved oxygen levels as monitored by computer vision. Aquacult Eng. 2006; 35:207-217.
41. Randall D, Ferry S. 4 Catecholamines. Fish Physiol. 1992; 12:255-300.
42. Soldatov A. The effect of hypoxia on red blood cells of flounder: A morphologic and autoradiographic study. J Fish Biol. 1996; 48:321-328.
43. Randall DJ, Wright PA. Ammonia distribution and excretion in fish. Fish Physiol Biochem. 1987; 3:107-120.
44. Ishikawa NM, Ranzani-Paiva MJT, Lombardi JV, Ferreira CM. Hematological parameters in Nile Tilápia, *Oreochromis niloticus* exposed to sub-letal concentrations of mercury. Braz Arch Biol Technol. 2007; 50:619-626.
45. Martins M, Mouriño J, Amaral G, Vieira F, Dotta G, Jatobá A, Pedrotti F, Jerônimo G, Buglione-Neto C. Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp. Braz J Biol. 2008; 68:657-661.
46. Carey JB, McCormick SD. Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. Aquaculture. 1998; 168:237-253.
47. Biswas A, Maita M, Yoshizaki G, Takeuchi T. Physiological responses in Nile tilapia exposed to different photoperiod regimes. J Fish Biol. 2004; 65:811-821.
48. Barcellos L, Nicolaiewsky S, De Souza S, Lulhier F. Plasmatic levels of cortisol in the response to acute stress in Nile tilapia, *Oreochromis niloticus* (L.), previously exposed to chronic stress. Aquacult Res. 1999; 30:437-444.

(Received June 28, 2017; Revised August 8, 2017; Accepted August 11, 2017)