Anesthetic effect of tricaine methanesulfonate, clove oil and electroanesthesia on lysozyme activity of *Oncorhynchus mykiss*

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Abstract

There is a few available information about the effect of anesthetics, specially electroanesthesia on immune parameters in fish. In the present work, two anesthetics, MS222 (50 ppm), clove oil (25 ppm), and electroanesthesia were tested in rainbow trout (*Oncorhynchus mykiss*) in narcosis stage. The results showed, clove oil and electroanesthesia increase the lysozyme activity 24 h after anesthesia($p<0.05$) and it induces neutrophilia and lymphopenia in the same group 24h after anesthesia($p<0.05$), but fish specimens anesthized with MS222 didn't show any difference 1h and 24h after exposure in lysoyme level and differential white blood cell count ($p>0.05$). It seems use of MS222 during aquacultural practices may induce lesser effect in rainbow trout.

Keyword: Clove oil, Electroanesthesia, MS222, Rainbow trout

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Introduction

Rapid increase of intensive aquaculture as a result of high demand for fish as a food source and employment of the technological advances applied, has made a new demand on the necessary chemicals. Nowadays, there are strict control in chemicals used in aquaculture, particularly with regard to their safety and efficacy (Taylor and Roberts, 1999). Anesthetics are among important and broadly used veterinary medicines.

Anesthesia is a biological state with the partial or complete loss of sensation or loss of voluntary neuromotor control induced by chemical or nonchemical means (Summerfelt and Smith, 1990).Practices that require fish handling are a common source of stress in aquaculture operations and research activities (kakoolaki et al., 2009). To solve this problem, fish handlers have employed the use of anesthetics, added to water, to immobilize fish, reduce stress levels, and prevent mortality. The use of anesthetics facilitates work with fish at the research level and is required for invasive studies such as surgical preparations for physiological investigations, where the fish must be held immobile for extended periods of time. Anesthetics reduce stress responsiveness by causing a depression in nervous function (Iwama and Ackerman, 1994). However the type of anesthetic, the dosage and the time it can affect the physiology of the fish are the important points (Ortuno et al., 2002). There are various types of anesthetics; the one most commonly used are tricaine methanesulfonate (MS-222, TMS) and it's the only anesthetic drug currently approved by the FDA for use on food fish. Clove oil is a dark-brown liquid, a distillate of flowers, stalks and leaves of the clove tree *Eugenia aromatica* (Soto and Burhanuddin, 1995) having a mild anesthetic effect in human since antiquity (Nagababu and Lakshmaiah 1992; Taylor and Roberts, 1999;) and fish (Ross and Ross, 2008). Eugenol, the active ingredient of clove oil, inhibits the synthesis of prostaglandin H (PHS), which accounts for the analgesic effect of clove oil (Thompson and Eling, 1989; Pongprayoon et al., 1991).

Keene et al. (1998) showed clove oil is much less expensive than other chemicals including MS222. Most chemical anesthetics leave residues in fish tissues that require a certain withdrawal time before the animal could be released into the environment. Therefore, alternatives are needed in cases when fish must be released immediately, for example, during spawning, biopsy, surgery or after implanting elastomer tags (Mark et al., 2001).

Electroanesthesia has primarily been used to immobilize adult fish for tagging or hatchery broodstock. Three types of electric current have been used to immobilize fish: alternating current (AC), direct current (DC), and pulsating forms of AC and DC. The main advantages of electroanesthesia are that no chemicals are used, it has no residual, its eco-friendly, induction of anesthesia and recovery are rapid, and operating costs are low. Lysozyme is secreted into the blood by lysosomes of neutrophils and macrophages (Mock et al., 1990) and can function in the blood or intercellular spaces. Lysozyme level or activity is an important index of innate immunity of fish and is ubiquitous in its distribution among living organisms. In fish, lysozyme, an enzyme with antibiotic properties that is released by leucocytes, has a broader activity than mammalian lysozyme (Demers and Bayne,

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1997) and has been frequently used as an indicator of nonspecific immune functions with bacteriolytic effects. It has also been shown to alter blood concentrations in fish following stressful stimuli (Mock and Peters, 1990; Røed et al., 1993). However it was suggested that under certain circumstances, lysozyme activity may be a more stable indicator of stress in fish than cortisol. (Fevolden and Røed, 1993)

Anesthesia generally involves a cessation of breathing which, in turn, reduces gas transfer leading to hypoxia and respiratory acidosis due to the reduction of blood oxygen (O_2) tension and a concomitant rise in blood CO_2 (Thomas and Robertson, 1991). As a result of the lack of respiration, it has been reported that anesthetics may lead to stress response in fish.

The present study aimed to compare the affect of MS 222, clove oil and electroanesthesia (relative to non-anaesthetized controls) on lysozyme activity of rainbow trout *Oncorhynchus mykiss*, 1 and 24 h post anesthesia, to recognize any difference among these groups in narcosis stage.

Materials and methods

Experimental design

One hundred fifty rainbow trout weighting (Mean weight 100±10g, mean length 25±2cm) were held in a recirculation system of the aquatic animal health department of University of Tehran, in 1000 liter fiber glass tanks at water temperature 19-20 ^{0} C. After one week acclimatization, all fish were starved for 24 h before the experiments. Water quality parameters consisting of pH = 8.2, electrical conductivity=918 µs /cm, dissolved oxygen = 6.8 mg/ L, hardness ($CaCO_3$) = 300 mg/ L, TDS=588 mg/ L) were used during the experiment.

A preliminary pilot study was performed to achieve proper dosage for induction a deep narcosis stage in fish showing clinical signs of low to normal respiratory rate, total loss of equilibrium, no effort to right itself, decrease muscle tone, reactivity to strong tactile and vibratory stimuli . The required time to recovery stage was recorded. (Ross et al., 2008).

Four experimental groups of fish were established, each containing five fish. One group was maintained without anesthesia as a control, while the other 3 groups were anesthetized with 50 ppm MS222 (Finquel®; Argent TR2905, Redmond, WA, USA). 25ppm clove oil (Zardband Co., Tehran, Iran) and with Electroanesthesia: a prototype of an alternating current (AC) electroanesthesia apparatus was designed and constructed based on Chiba et al. (2006) study and Ross et al. (2008). This unit was equipped by microcontroller system to allow an automatic voltage-time adjustment of the anesthesia steps (Sattari et al., 2009), instead of manual adjustments (Chiba et al., 2006). The sinusoidal pulse width modulation (PWM) electrical waves were found to have the least effect on behavior in rainbow trout allowing the authors to devise a pattern of voltage-time application. In order to adjust and calibrate the apparatus, for anesthizing fish in narcosis stage, the pilot experiments were performed on rainbow trout to modify voltage and time. The PWM voltage waves form was applied to electrodes for 121 sec with some modification in pattern of voltage-time (Sattari et al., 2009). Groups of three fish were transported to the anesthesia tank (19×20×33 cm) and placed

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between two submersible stainless steel plate electrodes (19×20 cm) which inserted in a distance 33 cm from each other.

Fish were anestethized for 10 min, the fish were immediately transported to recovery tanks and time of recovery was noted. These groups were blood sampled through peduncle, 1 and 24 h post anesthesia. The experiment was triply repeated for each treatment and control (no anesthetic).

Blood sample and lysozyme assay

Blood was collected from the caudal vein with heparinized and unheparinized syringes. Serum sample were obtained from blood by non-heparinized syringes guage 21 after centrifugation of blood at 3000 g. and stored at -70°C until analyzed for lysozyme activity. The smears obtained from heparanized sample were first air dried, fixed in 96% ethanol for 30 min and stained in Gimesa for leukocyte differential count under light microscope. (Ghiasi et al., 2010)

Lysozyme activity was measured with the turbidimetric method described by Kumari (2006). Suspention of 150 µL lyophilized *Micrococcus lysodeikticus* (Sigma M 3770) (0.2 mg/ml as the substrate in M sodium acetate buffer adjusted to pH 5. 5) was added to previously dispensed test serum (15 ml of each fish) in a 96-well U-bottom microtitre plate and initial OD was taken at 450 nm immediately. The final OD was taken 1 h after incubation (Sahoo et al., 2005) at 24°C. A standard curve was prepared using lyophilized hen egg white lysozyme (HEWL; Sigma, USA). Serum lysozyme values were expressed as µg/ml equivalent to HEWL activity.

Statistical analyses

One way analysis of variance (ANOVA; SYSTAT 16.0 software, SPSS) was used to determine whether significant variation between the treatments existed. Bonferroni multiple range test was used as post-test to indicate differences for each group. Statistical differences between groups at each particular time point (1h &24h) were tested by one way analysis. Data are presented as Mean±SE. The confidence level of 95%, ($p < 0.05$) was used for data analysis.

Results

Time to anesthesia and recovery are shown in table 1. Fish were anesthetized up to deep narcosis stage after induction with MS222, clove oil and electroanesthesia respectively after 114±11 sec, 125±23 sec, 120±33 sec (Mean ±SE, $n = 30$).Recovery times for anesthetics were 150±20 sec for MS-222, 180±12 sec for clove oil and 140±24 sec for electroanesthesia. No Significant differences were observed between anesthetics in induction and recovery times ($p>0.05$, Table 1).

The results of lysozyme content in experimental groups are shown in table 2. Fish exposed to clove oil showed a significant increase in level of serum lysozyme, 24 hour post anesthesia when compared to control ($p < 0.05$). However, serum lysozyme level was not affected 1 hour post anesthetisia with clove oil ($p>0.05$). No statistically significant difference was noted between serum lysozyme activity, in fish anesthetized with MS222 both 1 and 24 hour post anesthesia ($p>0.05$). lysozyme activity showed significant increase 24 hour post anesthesia with electroanesthesia, but it was insignificant 1 hour after anesthesia (Table 2). Only in electroanaesthesia group, lysozyme level was significantly higher 24h after anesthesia in comparison with 1h after anesthesia (Table 2).

The effects of different anesthetic methods on the level of immunocompetent cells are shown in table 3. The levels of lymphocytes in fish anesthetized with

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clove oil and electroanesthesia were significantly lower ($p<0.05$) than control group, 24h post anesthesia. Also a neutrophilia was seen in these group 24h post anesthesia ($p<0.05$).

Table 1: Duration of induction and recovery times for electroanesthesia (*n* = 30), clove oil and MS222 (Mean±SE) , (*n* = 30)

Anesthetic	Induction time(sec)	Recovery time(sec)
electroanesthesia	120±33	140±24
Clove oil	125±23	180±12
MS222	114±11	150±20

Table 2: lysozyme activity measured in anesthetized and control rainbow trout specimens 1h and 24h after deep narcosis. (Mean ±SE, *n*=15). *p < 0.05.

Lysozyme(µg/ml)	Time post sampling	
	1h	24h
Control	77.4±6.43	84±3.91
Clove oil	113.8±14.00	135±18.92*
MS222	114.9±13.64	106±13.08
Electroanesthesia	90.7±8.73	153±5.58*

Table 3: Leukocyte profile of rainbow trout following 1h and 24h after deep narcosis. : (Mean±SE, *n*=15) *p < 0.05

	Time post sampling	
	1h	24h
Lymphocyte(%)		
Control	87.43±1.3	89.00±1.0
Clove oil	86.93±1.2	78.53±1.7*
MS222	83.80±1.3	86.67±1.8
Electroanesthesia	83.07±1.8	82.40±1.5*
Neutrophil(%)		
Control	13.73±1.2	11.00±1.0
Clove oil	13.00±1.2	21.47±1.7*
MS222	16.13±1.2	13.33±1.8
Electroanesthesia	16.93±1.8	17.60±1.5*
Monocyte(%)		
Control	0.0	0.0
Clove oil	.007±.06	0.0
MS222	.007±.06	0.0
Electroanesthesia	0.0	0.0

Discussion

Anesthesia may have immunosuppressive effects in mammals (Bardosi et al., 1992), but a few attempts have been made to detect anesthesia-induced immunodepression in fish particularly electroanesthesia (Ourtano et al., 2002). The present paper provides further information based on the in vivo

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experiments in an attempt to find whether anesthesia in deep narcosis stage may has any effect on lysozyme and immunocompetent cell level in rainbow trout. The objective was to provide a comparative effect on these nonspecific immune variables using chemical, herbal and electroanesthesia procedures.

In present study, lymphopenia and neutrophilia in rainbow trout anesthetized with clove oil and electroanesthesia were seen 24h post anesthesia, while no effect was seen on leukogram after exposing fish to MS222($p>0.05$). Variable results have been reported after anesthizing different species to clove oil and MS222. In a study by Velisek et al. (2005a) no effect was seen when rainbow trout was exposed to clove oil at 30 mg/1 for 10-min, that was in accordance with our results.

In common carp 10-min exposure to clove oil on concentration of 30 mg/1 had no effect on Leukogram, immediately after anesthesia. (Velisek et al., 2005b)
Also, lymphopenia and neutropenia were seen in Siberian sturgeon after exposing to 0.075 ml l^{-1} eugenol and 125 mgl^{-1} MS222, immediately and 24 h after anesthesia were observed (Gomulka et al., 2008).

Sudgar et al. (2009) observed that the anesthetic effect of clove powder on roach (*Rutilus rutilus*) after 7min anesthesia, at the concentration of 175, 225, 275 and 350 mgl^{-1} immediately and 24h post anesthesia had no effect on hematological parameters. Therefore, it is difficult to compare such results, because of difference in water quality parameters and species of fish used.Leukocyte response to stress and to exogenous glucocorticoid treatment (neutrophilia and lymphopenia) is readily quantifiable variable in fish (Ellsaesser and Clem, 1986; Bly et al., 1990). In general, acute stress induces both neutrophilia and lymphopenia in fish (Pulsford et al., 1994), and these stress-induced changes have been shown repeatedly to be related to elevated glucocorticoids. Neutrophilia (increase in Neutrophil), lymphopenia(decrease in lymphocytes) and increased (Neutrophil: Lymphocyte) ratios are apparent after treatment with either cortisol or hydrocortisone and ACTH (Wojtaszek et al., 2002). Therefore the occurrence of neutrophilia and lymphopenia following anesthesia with clove oil and electroanesthesia may be in part due to the stress induction in rainbow trout.

In our experiment, lysozyme activity was increased 24 h post anesthesia with clove oil and electroanesthesia. While, it was insignificant in group anesthetized with MS222 both 1 and 24 hour after anesthesia. Cho and Heath (2000) found that lysozyme concentration in juvenile chinook salmon was increased both 1 and 24 h post anesthesia with clove oil and MS222, such increase in lysozyme content is due to the occurrence of neutrophilia in clove oil group as indicated in this study.

Stress is inherently involved in aquaculture, research, clinical examination, and all handling procedures in fish. These stresses in combination with poor water quality in intensive aquaculture operations, results in poor performance, disease outbreaks and, in severe cases, increased mortality (Palic et al., 2006 and Pickering, 1998). Rainbow trout is one of the most often used fish, and as such, often exposed to long transport and poor

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environmental conditions in temporary holding facilities . Therefore use of ideal anesthetic with a proper dose for handling procedures of fish is the critical point; this is because the anesthetic drug may induce stress in case of mammals (Oyama, 1973) and fish (Strange and Schreck, 1978; Robertson et al., 1988; Molinero and Gonzalez, 1995).

In conclusion, this study showed that anesthesia may affect fish stress responses and therefore might be unsuitable for some research purposes, it seems that clove oil and electroanesthesia can induce stress in rainbow trout due to an increase in neutrophil and also causing a lymphopenia. Also an increase in lysozyme level was seen in fish anesthized with clove oil and electroanesthesia. While, use of MS222 during aquacultural practices may induce lesser stress in rainbow trout. However, more works are required to assess other immune parameters of fish anestethized with these chemical, herbal and electrical tools.

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References

Bardosi, L., Bardosi, A. and Gabius, H. J., 1992. Changes of expression of endogenous sugar receptors by polymorphonuclear leukocytes after prolonged anaesthesia surgery. *Canadian Journal of Anaesthesia*, 39, 143–150.

Bly, J. E., Miller, N. W. and Clem, L. W., 1990. A monoclonal antibody specific for neutrophils in normal and stressed catfish. *Developmental and Comparative Immunology*, 14, 211–221.

Chiba, H., Hattori, T., Yamada, H. and Iwata, M., 2006. Comparison of the effect of chemical anesthesia and electroanesthesia on plasma cortisol level in the Japanese eel anguilla japonica. *Journal of Fisheries Science*, 72, 693-695.

Cho, G. K. and Heath, D. D., 2000. Comparison of tricaien methan sulphonate(MS222) and clove oil anesthesia effect on the physiology of juvenile Chinook salmon *oncorhynchus tshawytsha* .*Aquaculture research*, 31, 537-546.

Demers, N. E., and Bayne C. J., 1997. The immediate e!ects of stress on hormones and plasma lysozyme in rainbowtrout. *Developmental and Comparative Immunology*, 21, 363–373.

Ellsaesser, C. F. and Clem, L.W., 1986. Hematological and immunological changes in channel catfish stressed by handling and transport. *Journal of Fish Biology*, 28, 511–521.

Fevolden, S. E., and Røed, K. H., 1993. Cortisol and immune characteristics in rainbow trout (Oncorhynchus mykiss) selected for high or low tolerance to stress. *Journal of Fish Biology*. 43, 919– 930.

Ghiasi, F., Mirzargar, S. S., Badakhsh, H., and Shamsi, S., 2010. Effects of low concentration of cadmium on the

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[DOR: 20.1001.1.15622916.2011.10.3.3.6]

level of lysozyme in serum, leukocyte count and phagocytic index in Cyprinus carpio under the wintering conditions. *Journal of fisheries and Aquatic Science*. 5(**2**), 113-119.

Gomulka, P., Wlasow1, T., Velíšek, J., Svobodová, Z. and chmielinska, E. 2008. Effects of Eugenol and MS-222 Anaesthesia on Siberian Sturgeon (*Acipenser baerii* Brandt). *ACTA VET. BRNO*, 77, 447–453.

Iwama, G. K., and Ackerman. P. A., 1994. Anaesthetics in Biochemistry and molecular biology of fishes: *Analytical techniques*, Eds. Hochachka and T.P. Mommsen. Elsevier Science, Amsterdam. Pages 1-16.

Kakoolaki, S., Sharifpour, I., Soltani, M., Ebrahimzadeh Mousavi, H. A., Mirzargar, S. Rostami M., 2010. Selected morpho-chemical features of hemocytes in farmed shrimp, Fenneropenaeus indicus in Iran . *Iranian Journal of Fisheries Sciences*, 9(**2**), 219-232.

Kumari, J., 2006. Seasonal variation in the innate immune parameters of the asian catfish *Clarias batrachus*, *Aquaculture*, 252, 121-127.

Keene, J. L., Noakes, D. L. G., Moccia, R. D. and Soto, G. G., 1998. The efficacy of clove oil as an anesthetic for rainbow trout, *Oncorhynchus Mykiss* (Walbaum). *Aquaculture. Research.*, 29, 89-101.

Mark, P., Gaikowski, W., Gingerich, H., and Gutreuter, S., 2001. Short-Duration Electrical Immobilization of Lake Trout, *North American Journal of Fisheries Management*; 21, 381-392.

Moeck, A., and Peters, G., 1990. Lysozyme activity in rainbow trout, *Oncorhynchus mykiss* (Walbaum), stressed by handling, transport and water pollution. *Journal of Fish Biology*, 37, 873– 885.

Molinero A., and Gonzalez J., 1995. Comparative effects of MS 222 and 2-phenoxyethanol on gilthead sea bream (*Sparus aurata L.*) during confinement. *Comparative Biochemistry & Physiology A-Comparative Physiology*, 111 (**3**), 405-414.

Nagababu E., and Lakshmaiah , N., 1992. Inhibitory effects of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. *Journal of Biochemistry Pharmacology*, 43, 2393–2400.

Ortuño, J., Esteban, M. A. and Meseguer J. 2002. Effects of four anaesthetics on the innate immune response of gilthead seabream (Sparus aurata L.). *Fish & Shellfish Immunology*, 12(**1**), 49-59.

Oyama T., 1973. Endocrine responses to anaesthetic agents. *Br. J. Anaesth*, 45, 276-281.

Palić, D., Herolt, D. M., Andreasen, C.B., Menzel, B. W. and Roth, J. A., 2006. Anesthetic efficacy of tricaine methanesulfonate metomidate and eugenol effects on plasma cortisol concentration and neutrophil function in fathead minnows (Pimephales promelas Rafinesque 1820). *Aquaculture*, 254, 675-685.

Pongprayoon, U., Baekstorm, P., Jacobsso, U., Linstorm, M., Bohlin,

[Downloaded from jifro.ir on 2026-11-07]

[DOR: 20.1001.1.15622916.2011.10.3.3.6]

L., 1991. Compounds inhibiting prostaglandin synthesis isolated from Ipomoea pes-caprae. *Planta Medicine*, 57, 515-518.

Pulsford, A. L., Lemairegony, S., Tomlinson, M., Collingwood, N. and Glynn, P. J., 1994. Effects of acute stress on the immune system of the Dab, Limanda limanda. *Comparative Biochemistry and Physiology C – Pharmacology Toxicology and Endocrinology*, 109, 129–139.

Robertson, L., Thomas, P. and Arnold, C. R., 1988. Plasma cortisol and secondary stress responses of cultured red drum (Sciuenopsocellarus) to several transportation procedures. *Aquaculrure*, 68, 115-130.

Røed, K. H., Larsen, H. J., Linder, R. D., and Refstie, T., 1993. Genetic variation in lysozyme activity in rainbow trout (Oncorhynchus mykiss). Aquaculture 109, 237– 244.

Ross, L. G., and Ross B., 2008. Anaesthetic and Sedative Techniques for Aquatic Animals. 3rd Edn., Blackwell, Oxford. ISBN:978-1-4051-4938-9

Sahoo, P. K., Kumari, J. and Mishra, B. K., 2005. Non-specific immune responses in juveniles of Indian major carps. *Journal of Applied Ichthyology*. 21, 151–155.

Sattari, A., Mirzargar, S. S., Abrishamifar, A., Lourakzadegan, R., Bahonar, A., Mousavi, H. E. and Niasari, A., 2009. Comparison of electroanesthesia with chemical anesthesia (MS222 and Clove Oil) in rainbow trout (Oncorhynchus mykiss) using plasma cortisol and glucose responses as physiological stress indicators. *Asian Journal of Animal Veterinary Advances*, 4, 306-313.

Soto, C. G., and Burhanuddin, S., 1995. Clove oil as a fishanaesthetic for measuring length and weight of rabbitfish(*Siganus lineatus*). *Aquaculture*, 136, 149–152.

Strange R. J. and Schreck C. B., 1978. Anaesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (Oncorhynchus /shawyrscha). *Journal of Fisheries Research*, 35, 345-349.

Sudagran, M., Mohammadizarejabada, A., Mazandarania, R and Pooralimotlagh. 2009. The effect of clove powder as an anesthetic and its effects on hematological parameters on roach (*Rutilus rutilus*). *Journal of aquaculture feed science and nutrition*, 1, 1-5.

Summerfelt R. C., and Smith, L. S., 1990. Anesthesia, surgery and related techniques. pp. 213-272. In: C.B. Schreck and P.B. Moyle(eds.). Method for Fish Biology. Am. Fish. Soc., Bethesda, Maryland.

Taylor P. W., and Roberts S. D., 1999. Clove oil: An alternative anaesthetic for aquaculture. *North American Journal of Aquaculture,* 61, 150–155.

Thomas, P. and Robertson, L., 1991. Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow water stressors and anesthesia with MS-222, quinaldine sulphate and metomidate. *Aquaculture* , 96, 69–86.

Thopson, D, and Eling, T., 1989. Mechanism of inhibition of prostaglandin H synthase by eugenol

[Downloaded from jifro.ir on 2026-11-07]

[DOR: 20.1001.1.15622916.2011.10.3.3.6]

and other phenolic peroxidase substrates. *Molecular Pharmacology*, 36, 809-817.

Velisek, J., Svoboda, Z., and Plackova, V. 2005a. Effects of Clove Oil Anaesthesia on Rainbow Trout (Oncorhynchus mykiss) .*ACTA VET. BRNO* , 74, 139-146.

Velisek, J., Svoboda, Z., Plackova, V., Groch, L., and Nepejchalova, L. 2005b. Effects of clove oil anaesthesia on commoncarp (*Cyprinus carpio L.*) .*Veterinary Medicine. – Czech*, 6, 269–275.

Wojtaszek, J., Dziewulska-Szwajkowska, D., Lozinska-Gabska, M., Adamowicz, A. and Dzugaj, A., 2002. Hematological effects of high dose of cortisol on the carp (Cyprinus carpio L.): cortisol effect on the carp blood.*General and Comparative Endocrinology*, 125, 176–183.

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[DOR: 20.1001.1.15622916.2011.10.3.3.6]