

Haematological and biochemical response of burbot (*Lota lota* L.) exposed to four different anaesthetics

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ABSTRACT: The aim of this study was to compare the effect of four anaesthetics on haematological and biochemical blood profiles in burbot *Lota lota* L. Blood profiles of burbot were evaluated 10 min and 24 h after anaesthesia with tricaine methanesulfonate (MS 222) (100 mg/l), clove oil (33 mg/l), 2-phenoxyethanol (0.3 ml/l), Propiscin (1 ml/l) and compared to non-anaesthetized control. The tested anaesthetics had no effect on haematological profile of burbot. The exposure to clove oil, 2-phenoxyethanol, and Propiscin significantly ($P < 0.01$) influenced the level of ammonia and glucose. The level of lactate was significantly ($P < 0.01$) increased following anaesthesia with 2-phenoxyethanol and Propiscin. The levels of total protein, aspartate aminotransferase, and calcium were higher ($P < 0.01$) with clove oil, 2-phenoxyethanol, and Propiscin compared to control. The use of MS 222 showed the lowest effect on haematological and biochemical blood profile and is recommended as a suitable anaesthetic for burbot.

Keywords: 2-phenoxyethanol; anaesthesia; clove oil; MS 222; Propiscin

INTRODUCTION

Aquaculture practices are associated with high risk to the welfare of fishes, making them vulnerable to adverse impacts of disease and environmental conditions (Ferreira et al. 2015). Common procedures in intensive aquaculture such as handling (especially in the case of spawners), sorting, tagging, artificial reproduction, and surgery are frequent stressors, which may evoke stress-induced problems such as reduction in feeding and immune function (Ross and Ross 2008). Currently, a variety of anaesthetics are available to minimize negative effects of stressful situations during culture events. Furthermore, anaesthetizing fish is required by Institutional Animal Care and Use Committees.

Currently, there is an interest in identifying new fishes that are suitable for aquaculture and burbot *Lota lota* L. should be considered as a good candidate (Palinska-Zarska 2014). Although there have been no studies on physiological effects of anaesthetics on burbot, several options can be considered. Among the fish anaesthetics, only tricaine methanesulphonate (MS 222) has been approved for use on food fish in the USA and the UK; it is efficacious and has no reported side effects (Mercy et al. 2013). Another effective fish anaesthetic is clove oil. While clove oil is recommended as a very suitable, economically advantageous, and easily accessible anaesthetic for fish, and it is used in food products, it has not been approved for use on food fish. It has a

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high efficacy in small doses, non-specific toxicity, and low price. These are the primary reasons for its adoption in aquaculture and aquatic research (Cho and Heath 2000). The drug 2-phenoxyethanol (ethylene glycol monophenyl ether) is a colourless liquid preparation which is readily soluble in water. It is widely used in aquaculture, because of its low cost, fast effect and recovery time (Mylonas et al. 2005). Propiscin (etomidate) was developed in Poland and it is commonly used there to immobilize fish; while it is highly effective, quickly achieving anaesthesia, it has not been approved for food fish (Ross and Ross 2008).

Although anaesthetic drugs can prevent stress, fish are not free of physiological side-effects. Changes in the blood chemistry can be useful in monitoring well-being of aquatic animals. To determine and assess these changes and possible side-effects of anaesthetics, haematological, biochemical blood profile, and oxidative stress examinations are necessary (Velisek et al. 2006, 2007, 2009, 2011).

The aim of this study was to compare the effects of MS 222, clove oil, 2-phenoxyethanol, and Propiscin after exposure to anaesthetics on the basis of changes in haematological and biochemical blood profile in burbot.

MATERIAL AND METHODS

Anaesthetics. The MS 222 was obtained from Sigma-Aldrich Chemicals (St. Louis, USA). Clove oil (eugenol, concentration 76%) is marketed by the Kulich Company (Jan Kulich, Hradec Králové/Říčany, Czech Republic). The preparation 2-phenoxyethanol was obtained from Merck Schuchardt OHG (Hohenbrunn, Germany). Propiscin was supplied by the Division of Fish Pathology and Immunology at Zabieniec (Institute of Inland Fisheries in Olsztyn, Poland).

Experimental procedure. Juvenile burbot ($n = 63$) with the mean length of 245 ± 52 mm (mean \pm SD) and mean weight 110 ± 23 g were used for the experiment. The fish had been held in the recirculating aquaculture system (RAS) of the Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice. Water temperature was $15 \pm 1^\circ\text{C}$ and constant light regime of 12 h light and 12 h darkness was maintained for 5 months before and during the experiment. The methodical procedure followed

Velisek et al. (2009, 2011). Nine groups of seven fish were compared in this study:

- (1) Control group – non-anaesthetized, blood sampled prior to treating the anaesthetized groups.
- (2) Four groups (four anaesthetics) – blood was sampled immediately after a 10-min exposure and designated as: MS 222 (10 min) (100 mg/l), clove oil (10 min) (33 mg/l), 2-phenoxyethanol (10 min) (0.3 ml/l), and Propiscin (10 min) (1 ml/l). The 10 min exposure time was determined according to preliminary test, when all the tested fish reached the third phase of anaesthesia between 7 to 10 min of exposure in the mentioned anaesthetics.
- (3) Four groups (four anaesthetics) – blood was sampled 24 h after the 10-min anaesthesia treatment and designated as: MS 222 (24 h), clove oil (24 h), 2-phenoxyethanol (24 h), and Propiscin (24 h).

Blood sampling, haematological and biochemical measurement. Blood was drawn from the vena caudalis using an 18 G \times 1½" syringe which contained heparin as an anticoagulant (Heparin Léčiva inj. sol.; Zentiva, Prague, Czech Republic) at a concentration of 5000 I.U. heparin sodium salt in 1 ml. The indices used to evaluate haematological profile followed Svobodova et al. (2012); these included an erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and leukocyte count (Leuko).

Blood was centrifuged (separated) in a cooled centrifuge (4°C , 837 g). The plasma was stored at -80°C until analysis on a VETTEST 8008 analyzer (IDEXX Laboratories Inc., Westbrook, USA) and biochemical indices were assayed using the method of Kolarova and Velisek (2012). Biochemical indices determined in this study were glucose (GLU), total protein (TP), albumin (ALB), total globulins (GLOB), ammonia (NH_3), calcium (Ca^{2+}), magnesium (Mg), inorganic phosphate (PHOS), triacylglycerols (TAG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate (LACT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase (ALP), creatine (CREA), and amylase (AMYL).

Statistical analysis. Statistical analysis was carried out using STATISTICA 10 for MS Windows. Data were first tested for normality (Kolmogorov-Smirnov test) and homoscedasticity of variance

(Bartlett's test). If those conditions were satisfied, one-way analysis of variance (ANOVA) was employed to reveal significant differences in measured variables among control and experimental groups. When a difference was detected ($P < 0.05$), Tukey's multiple comparison test was applied to identify which treatments were significantly different. If the conditions for ANOVA were not satisfied, the non-parametric Kruskal-Wallis test was used.

RESULTS

Haematological profile. The effects of MS 222, clove oil, 2-phenoxyethanol, and Propiscin on haematological indices of blood plasma in burbot are shown in Table 1. The 10-min exposure in the anaesthetics had no effect on the haematological profile of burbot.

Biochemical plasma profile. The biochemical plasma profiles of anaesthetized and control group are given in Table 2. The level of GLU was significantly ($P < 0.01$) higher immediately after anaesthesia (10 min) with clove oil, 2-phenoxyethanol, and Propiscin compared to controls. GLU returned to the pre-treatment physiological level within 24 h (Figure 1). Fish treated with clove oil (10 min and 24 h), 2-phenoxyethanol (24 h), and Propiscin (10 min) had significantly ($P < 0.01$) higher levels of TP compared with the control group. The levels of ALB increased significantly ($P < 0.01$) in MS 222 (10 min) and clove oil (10 min and 24 h) anaesthesia compared to the control group. The concentration of NH_3 was significantly ($P < 0.01$) greater immediately after anaesthesia (10 min) with clove oil, 2-phenoxyethanol, and Propiscin compared with controls. NH_3 level returned to physiological levels within 24 h in all fish in the anaesthetic tested groups (Figure 2). The activity of AST showed a significant increase ($P < 0.01$) in the MS 222 (24 h) and clove oil (10 min) anaesthesia compared to the control group.

The clove oil (24 h) treatment showed significantly lower ($P < 0.01$) CK activity compared with the control group. The concentration of Ca^{2+} was significantly ($P < 0.01$) greater 24 h after anaesthesia (24 h) with clove oil, 2-phenoxyethanol, and Propiscin compared with controls.

The levels of LACT were significantly increased ($P < 0.01$) with 2-phenoxyethanol (10 min) compared with the control group. The levels of AMYL were significantly increased ($P < 0.01$) with MS 222 (10 min) compared with the control group.

Table 1. Effects of MS 222, clove oil, 2-phenoxyethanol, and Propiscin anaesthesia on haematological indices of blood in burbot juveniles. Significance levels observed are * $P < 0.05$ in comparison with the control group. All values are means \pm SD, $n = 7$; none were statistically different from the controls

Indices	Control	MS 222 (10 min)	MS 222 (24 h)	Clove oil (10 min)	Clove oil (24 h)	2-Phenoxyethanol (10 min)	2-Phenoxyethanol (24 h)	Propiscin (10 min)	Propiscin (24 h)
RBC (T/l)	1.82 \pm 0.10	1.66 \pm 0.28	2.05 \pm 0.26	1.72 \pm 0.31	1.96 \pm 0.32	2.30 \pm 0.36	1.97 \pm 0.31	2.04 \pm 0.17	2.10 \pm 0.37
Hb (g/l)	78.11 \pm 10.08	76.94 \pm 6.97	81.44 \pm 9.77	71.82 \pm 8.64	72.49 \pm 8.50	83.18 \pm 8.00	71.41 \pm 12.93	84.66 \pm 8.17	80.16 \pm 10.92
PCV (l/l)	0.44 \pm 0.02	0.49 \pm 0.04	0.43 \pm 0.02	0.44 \pm 0.04	0.43 \pm 0.03	0.50 \pm 0.05	0.46 \pm 0.04	0.48 \pm 0.05	0.42 \pm 0.05
MCV (fl)	242.09 \pm 14.87	300.14 \pm 55.05	213.08 \pm 21.54	262.56 \pm 48.83	222.49 \pm 26.86	218.58 \pm 24.64	236.83 \pm 30.65	237.45 \pm 12.05	204.91 \pm 21.02
MCH (pg)	42.79 \pm 79.00	47.68 \pm 9.70	40.02 \pm 3.79	42.95 \pm 8.76	37.47 \pm 4.79	36.60 \pm 3.96	36.10 \pm 2.37	41.78 \pm 5.03	38.83 \pm 4.85
MCHC (g/l)	177.66 \pm 23.97	158.44 \pm 7.56	188.65 \pm 15.97	163.24 \pm 7.66	168.91 \pm 14.91	167.76 \pm 8.41	154.85 \pm 21.02	176.34 \pm 23.00	190.05 \pm 18.16
Leuko (G/l)	10.20 \pm 1.18	7.07 \pm 1.86	7.84 \pm 2.43	9.11 \pm 1.63	8.03 \pm 1.88	8.10 \pm 0.78	7.60 \pm 3.27	8.93 \pm 2.84	6.24 \pm 3.32

RBC = erythrocyte count, Hb = haemoglobin, PCV = haematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, Leuko = leukocyte count

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Table 2. Effect of MS 222, clove oil, 2-phenoxyethanol, and Propiscin anaesthesia on biochemical indices of blood plasma in burbot. Significance levels observed are * $P < 0.05$, ** $P < 0.01$ in comparison with the control group. All values are means \pm SD, $n = 7$

Indices	Control	MS 222 (10 min)	MS 222 (24 h)	Clove oil (10 min)	Clove oil (24 h)	2-phenoxyethanol (10 min)	2-phenoxyethanol (24 h)	Propiscin (10 min)	Propiscin (24 h)
TP (g/l)	33.43 \pm 2.61	36.86 \pm 2.53	34.86 \pm 1.81	38.71 \pm 3.19**	40.43 \pm 2.61**	36.43 \pm 3.06	38.86 \pm 1.46**	39.71 \pm 3.37**	38.57 \pm 2.13
ALB (g/l)	5.57 \pm 1.68	8.57 \pm 1.76**	6.57 \pm 1.01	8.71 \pm 1.03**	8.14 \pm 1.25**	7.29 \pm 0.88	7.86 \pm 1.12	7.14 \pm 1.46	6.86 \pm 0.64
GLOB (g/l)	27.86 \pm 1.96	28.57 \pm 2.19	27.86 \pm 1.25	29.86 \pm 2.59	32.29 \pm 1.98	29.14 \pm 2.85	31.00 \pm 2.00	32.57 \pm 2.72	31.71 \pm 2.37
TAG (mmol/l)	1.09 \pm 0.17	1.22 \pm 0.13	1.18 \pm 0.17	1.44 \pm 0.24	1.14 \pm 0.21	1.45 \pm 0.28	1.10 \pm 0.26	1.27 \pm 0.23	1.13 \pm 0.26
AST (μ kat/l)	1.80 \pm 1.10	1.10 \pm 0.23	3.91 \pm 1.07**	3.02 \pm 0.98**	1.41 \pm 1.45	2.27 \pm 0.16	2.31 \pm 1.39	1.83 \pm 0.27	2.38 \pm 1.31
ALT (μ kat/l)	0.20 \pm 0.11	0.14 \pm 0.07	0.41 \pm 0.07	0.36 \pm 0.14	0.26 \pm 0.16	0.31 \pm 0.05	0.28 \pm 0.20	0.18 \pm 0.02	0.21 \pm 0.10
LDH (μ kat/l)	18.96 \pm 1.23	16.86 \pm 2.68	19.55 \pm 2.19	19.54 \pm 1.35	18.07 \pm 1.33	17.58 \pm 1.23	17.90 \pm 1.20	18.62 \pm 1.11	18.91 \pm 1.53
CK (μ kat/l)	14.93 \pm 0.88	15.57 \pm 0.97	15.13 \pm 1.13	15.26 \pm 0.85	8.96 \pm 3.70**	16.51 \pm 0.59	16.16 \pm 0.68	15.55 \pm 0.98	15.31 \pm 3.71
Ca ²⁺ (mmol/l)	2.00 \pm 0.13	2.36 \pm 0.20	2.29 \pm 0.35	2.32 \pm 0.14	2.74 \pm 0.23**	2.27 \pm 0.16	2.50 \pm 0.28**	2.17 \pm 0.23	2.61 \pm 0.29**
Mg (mmol/l)	0.86 \pm 0.14	0.95 \pm 0.09	1.14 \pm 0.22	1.09 \pm 0.12	1.18 \pm 0.27	0.99 \pm 0.11	1.21 \pm 0.41	1.03 \pm 0.17	1.17 \pm 0.41
PHOS (mmol/l)	2.81 \pm 0.40	3.23 \pm 0.44	2.48 \pm 0.40	3.41 \pm 0.69	2.40 \pm 0.24	2.82 \pm 0.18	2.87 \pm 0.44	2.92 \pm 0.26	2.68 \pm 0.32
ALP (μ kat/l)	0.05 \pm 0.01	0.14 \pm 0.07	0.05 \pm 0.01	0.05 \pm 0.01	0.07 \pm 0.05	0.06 \pm 0.01	0.07 \pm 0.03	0.05 \pm 0.01	0.06 \pm 0.01
LACT (mmol/l)	1.80 \pm 1.26	2.31 \pm 1.17	2.33 \pm 0.25	2.59 \pm 0.53	2.57 \pm 0.26	4.46 \pm 0.69**	2.38 \pm 0.27	4.13 \pm 1.07**	2.45 \pm 0.35
CREA (mmol/l)	6.14 \pm 8.41	13.29 \pm 9.15	16.01 \pm 6.95	8.29 \pm 10.71	1.29 \pm 1.67	18.71 \pm 13.85	10.29 \pm 10.32	3.01 \pm 3.02	9.01 \pm 8.42
AMYL (μ kat/l)	0.17 \pm 0.08	0.60 \pm 0.44**	0.04 \pm 0.06	0.01 \pm 0.01	0.03 \pm 0.04	0.28 \pm 0.36	0.05 \pm 0.05	0.08 \pm 0.10	0.28 \pm 0.20

TP = total protein, ALB = albumin, GLOB = total globulins, TAG = triacylglycerols, AST = aspartate aminotransferase, ALT = alanine aminotransferase, LDH = lactate dehydrogenase, CK = creatine kinase, Ca²⁺ = calcium, Mg = magnesium, PHOS = inorganic phosphate, ALP = alkaline phosphatase, LACT = lactate, CREA = creatine, AMYL = amylase

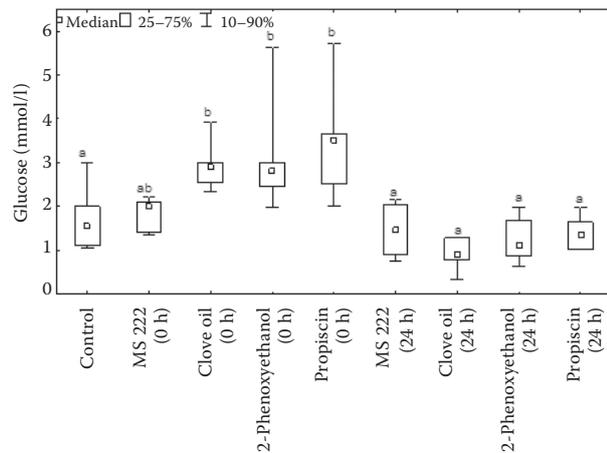


Figure 1. Effect of MS 222, clove oil, 2-phenoxyethanol, and Propiscin anaesthesia on glucose level in burbot

all values are means \pm SD, $n = 7$

different superscripts indicate significant differences ($P < 0.01$) between groups

The values for GLOB, TAG, ALT, LDH, Mg, PHOS, ALP, and CREA were similar among all groups.

DISCUSSION

The anaesthetics tested in this study are composed of different chemical substances and have different physiological effects. Different fish species may have widely disparate responses, so screening is a requisite to their use.

Both haematological and biochemical indices are valid and often used to measure fish health status and can provide information about internal environment of the organism (Velisek et al. 2005a, b, 2006, 2007, 2009, 2011). There have been no other studies on biochemical and haematological profiles in burbot anaesthetized with MS 222, clove oil, 2-phenoxyethanol or Propiscin.

The haematological characteristics can provide useful information on the physiological and pathological changes in fishes. However, these changes can vary depending on the species, age, sexual maturity, and health condition (Fazio et al. 2015). In this study, no significant differences in the haematological profile of burbot with anaesthesia (MS 222, clove oil, 2-phenoxyethanol and Propiscin) in comparison with control group were observed. These results also concur with the results of Velisek et al. (2005a, b, 2006) and Lepic et al. (2014) who also observed no changes

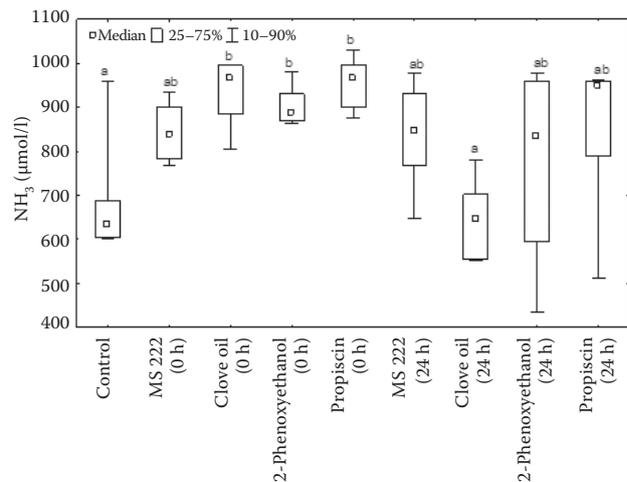


Figure 2. Effect of MS 222, clove oil, 2-phenoxyethanol, and Propiscin anaesthesia on ammonia level in burbot

all values are means \pm SD, $n = 7$

different superscripts indicate significant differences ($P < 0.01$) between groups

in haematological profile after the use of anaesthetics in rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio* L.), European catfish (*Silurus glanis*), and vimba bream (*Vimba vimba*). On the other hand, present results differ from findings of some other studies. Velisek et al. (2007) observed increase of MCV, MCHC and decrease of Leuko in European catfish with 2-phenoxyethanol. Changes as the swelling and destruction of erythrocytes, anaemia, and lymphopaenia after exposure in anaesthetics were also obtained by Gomulka et al. (2015) in Russian sturgeon (*Acipenser gueldenstaedtii*).

The values of biochemical blood plasma indices were observed after exposure in different anaesthetics. While NH_3 is a normal by-product of protein metabolism, elevated levels in blood can indicate some disturbances in NH_3 removal mechanism (Svoboda 2001). The 10-min treatment with clove oil, 2-phenoxyethanol, and Propiscin affected the concentration of NH_3 . The NH_3 levels returned to normal after 24 h. A decrease in NH_3 concentration after 24 h seems to be a result of a decreased metabolic rate (Gomulka et al. 2008). The same tendency was observed in GLU. Elevated levels of NH_3 and GLU were also observed after exposure in 2-phenoxyethanol and Propiscin in vimba bream (Lepic et al. 2014), and elevated levels of NH_3 and GLU were reported in rainbow trout after anaesthesia with clove oil (Velisek et

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al. 2011). On the other hand, we observed changes neither in GLU nor NH_3 levels after treatment with MS 222. Gomulka et al. (2008) described the same results in Siberian sturgeon and Lepic et al. (2014) in vimba bream. Physiological stress results in an increase in cortisol levels in fishes which stimulates both glycogenesis and gluconeogenesis, as well as an increase in protein catabolism and thus NH_3 production (Mommensen et al. 1999).

Physiological stress can result in increased activity which is reflected in plasma LACT particularly if oxygen availability is diminished (Thomas et al. 1999). In the present study, the elevated level of LACT after exposure in 2-phenoxyethanol and Propiscin could be caused by active swimming in anaesthesia bath or by limited respiratory movement (limiting and cessation of opercular movements). Burst swimming and limited operculum are typical initial signs of the first and third anaesthesia phases (Yoshikawa et al. 1988). In teleosts, high-speed (“burst”) swimming results in an oxygen debt and therefore, LACT is produced in anaerobic metabolism; this would be detected after exposure to some anaesthesia products.

Plasma enzymes (LDH, CK) and the transaminases (ALT, AST) are indicative of stress and can be used as indices (Ishikawa et al. 2007). They also may give specific information about organ dysfunction (Wagner and Congleton 2004). Only significantly elevated AST was detected compared to control; therefore, we did not consider higher disruption of amino acid metabolism. Higher level of AST would indicate a greater energy demand which is normally associated with synthesizing activities of the cell (Meister 1955).

Slight increases in Ca^{2+} level after exposure in MS 222 were reported by Congleton (2006) and Soivio et al. (1977), but not significantly different from the control group. Most of studies on the effects of anaesthesia in fish have described no effects on Ca^{2+} levels (Velisek et al. 2005a, b, 2009, 2011; Lepic et al. 2014). To our knowledge there are limited relevant data on Ca metabolism in fish following anaesthesia, but an increase in Ca^{2+} levels can be attributed to acute respiratory acidosis (Ghosh and Joshi 2008).

An increased level of TP in clove oil, 2-phenoxyethanol, and Propiscin would be an indicator of many different impacts of anaesthetics on metabolism. ALB represents the largest protein component of blood serum and its increase is

often caused by dehydration (relative reduction of water in blood serum) (Gammopathies 2005).

The present study was the first to describe haematological and biochemical blood profile in burbot. We suggest that blood profiles of burbot were very slightly altered by MS 222, but changed more by clove oil, 2-phenoxyethanol, and Propiscin. Furthermore, because clove oil, 2-phenoxyethanol, and Propiscin are not approved for use on food fish, we do not advocate their use even on non-food fish, until standards for Maximum Residue Limits (MRL) (Council Regulation (EEC) No. 2377/90) are set and proper licensing is acquired. On the basis of our study, it seems that MS 222 is the most suitable anaesthetic for burbot.

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REFERENCES

- Congleton J.L. (2006): Stability of some commonly measured blood-chemistry variables in juvenile salmonids exposed to a lethal dose of the anaesthetic MS-222. *Aquaculture Research*, 37, 1146–1149.
- Cho G., Heath D. (2000): Comparison of tricaine methanesulphonate (MS 222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture Research*, 31, 537–546.
- Fazio F., Saoca C., Casella S., Fortino G., Piccione G. (2015): Relationship between blood parameters and biometric indices of *Sparus aurata* and *Dicentrarchus labrax* cultured in onshore tanks. *Marine and Freshwater Behaviour and Physiology*, 48, 289–296.
- Ferreira C.I., Calisto V., Santos S.M., Cuerda-Correa E.M., Otero M., Nadais H., Esteves V.I. (2015): Application of pyrolysed agricultural biowastes as adsorbents for fish anaesthetic (MS 222) removal from water. *Journal of Analytical and Applied Pyrolysis*, 112, 313–324.
- Gammopathies M.V.P. (2005): Understanding and interpreting serum protein electrophoresis. *American Family Physician*, 71, 105–112.
- Ghosh A.K., Joshi S.R. (2008): Disorders of calcium, phosphorus and magnesium metabolism. *Journal of the Association of Physicians of India*, 56, 613–621.
- Gomulka P., Wlasow T., Velisek J., Svobodova Z., Chimelinska E. (2008): Effects of eugenol and MS 222 anaesthesia on Siberian sturgeon *Acipenser baerii* Brandt. *Acta Veterinaria Brno*, 774, 47–453.

- Gomulka P., Dagowski J., Wlasow T., Szczepkowski M., Czerniak E., Ziomek E., Szkudlarek M. (2015): Haematological and biochemical blood profile in Russian sturgeon following Propofol and Eugenol anaesthesia. *Turkish Journal of Fisheries and Aquatic Sciences*, 15, 13–17.
- Ishikawa N.M., Ranzani-Paiva M.J.T., Lombardi J.V., Ferreira C.M. (2007): Hematological parameters in Nile Tilapia, *Oreochromis niloticus* exposed to sub-lethal concentrations of mercury. *Brazilian Archives of Biology and Technology*, 50, 619–626.
- Kolarova J., Velisek J. (2012): Determination and Assessment of the Biochemical Profile of Fish Blood. Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic. (in Czech)
- Lepic P., Stara A., Turek J., Kozak P., Velisek J. (2014): The effects of four anaesthetics on haematological and blood biochemical profiles in vimba bream, *Vimba vimba*. *Veterinarni Medicina*, 59, 81–87.
- Meister A. (1955): Transamination. *Advances in Enzymology and Related Areas of Molecular Biology*, 16, 185–246.
- Mercy T.V.A., Malika V., Sajan S. (2013): Use of tricaine methanesulfonate (MS 222) to induce anaesthesia in *Puntius denisonii* (Day, 1865) (Teleostei: Cypriniformes: Cyprinidae), a threatened barb of the Western Ghats, India. *Journal of Threatened Taxa*, 5, 4414–4419.
- Mommsen T.P., Vijayan M.M., Moon T.W. (1999): Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries*, 9, 211–268.
- Mylonas C.C., Cardinaletti G., Sigelaki I., Polzonetti-Magni A. (2005): Comparative efficacy of clove oil and 2-phenoxyethanol as anaesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. *Aquaculture*, 246, 467–481.
- Palinska-Zarska K., Zarski D., Krejszef S., Nowosad J., Bilas M., Trejchel K., Kucharczyk D. (2014): The effect of age, size and digestive tract development on burbot, *Lota lota* (L.), larvae weaning effectiveness. *Aquaculture Nutrition*, 20, 281–290.
- Ross L.G., Ross B. (2008): *Anaesthetic and Sedative Techniques for Aquatic Animals*. Blackwell Publishing, Oxford, UK.
- Soivio A., Nyholm K., Huhti M. (1977): Effects of anaesthesia with MS 222, neutralized MS 222 and benzocaine on the blood constituents of rainbow trout, *Salmo gairdneri*. *Journal of Fish Biology*, 10, 91–101.
- Svoboda M. (2001): Stress in fish – a review. *Bulletin VÚRH Vodňany*, 4, 169–186. (in Czech)
- Svobodova Z., Pravda D., Modra H. (2012): Unified methods of haematological examination of fish. Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic. (in Czech)
- Thomas P.M., Pankhurst N.W., Bremner H.A. (1999): The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. *Journal of Fish Biology*, 54, 1177–1196.
- Velisek J., Svobodova Z., Piackova V. (2005a): Effects of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*). *Acta Veterinaria Brno*, 74, 139–146.
- Velisek J., Svobodova Z., Piackova V., Groch L., Nepechalova L. (2005b): Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.). *Veterinarni Medicina*, 50, 269–275.
- Velisek J., Wlasow T., Gomulka P., Svobodova Z., Novotny L., Ziomek E. (2006): Effects of clove oil anaesthesia on European catfish (*Silurus glanis* L.). *Acta Veterinaria Brno*, 75, 99–106.
- Velisek J., Wlasow T., Gomulka P., Svobodova Z., Novotny L. (2007): Effects of 2-phenoxyethanol anaesthesia on sheatfish (*Silurus glanis* L.). *Veterinarni Medicina*, 52, 103–110.
- Velisek J., Stejskal V., Kouril J., Svobodova Z. (2009): Comparison of the effects of four anaesthetics on biochemical blood profiles of perch. *Aquaculture Research*, 40, 354–361.
- Velisek J., Stara A., Li Z.H. (2011): Comparison of the effects of four anaesthetics on blood biochemical profiles and oxidative stress biomarkers in rainbow trout. *Aquaculture*, 310, 369–375.
- Wagner T., Congleton J.L. (2004): Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Onchorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61, 1066–1074.
- Yoshikawa H., Ishida Y., Ueno S., Mitsuda H. (1988): Changes in depth of anaesthesia of the carp anaesthetized with a constant level of carbon dioxide. *Bulletin of the Japanese Society of Scientific Fisheries*, 54, 457–462.

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