ORIGINAL ARTICLE

Comparison of the effects of four anaesthetics on haematological and blood biochemical profiles in pikeperch (*Sander lucioperca* L.)

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Abstract

OBJECTIVES: The objectives of the study were to compare the effects of Propiscin, 2-phenoxyethanol, clove oil and tricaine methane sulphonate (MS 222), anaesthetics frequently used in aquaculture.

DESIGN: The haematological and biochemical blood profiles of pikeperch (*Sander lucioperca* L.) anesthetized with Propiscin (1.5 ml L^{-1}), 2-phenoxyethanol (0.3 ml L^{-1}), clove oil (33 mg L^{-1}), MS 222 (150 mg L^{-1}) and non-anesthetized control group were tested. Each tested group was divided into two subgroups, the first subgroup was sampled in anaesthesia 10 min after application of the anaesthetic and the second one live on 24h.

RESULTS: The erythrocyte count and haematocrit was significantly decreased in 2-phenoxyethanol (24 h) compared with control group (CG). The mean corpuscular haemoglobin concentration was significantly increased in 2-phenoxyethanol (10 min), Propiscin (10 min and 24 h) compared to CG. The 2-phenoxyethanol (10 min and 24 h), MS 222 (24 h), clove oil (24 h), and Propiscin (10 min and 24 h) showed significantly lower leukocyte count compared with CG. The level of glucose was significantly ($p<0.05$) elevated with MS 222 (10 min) and clove oil (10 min) compared with CG. The 2-phenoxyethanol (10 min and 24 h), MS 222 (24 h), clove oil (24 h), and Propiscin (24 h) showed significantly lower ($p<0.01$) ammonia levels compared with CG. The triacylglycerols was significantly decreased ($p<0.01$) with Propiscin (10 min and 24 h), MS 222 (24 h), clove oil (24 h) and with 2-phenoxyethanol (24 h) compared with CG. After 24 hours MS 222 (24 h) and Propiscin (24 h) anaesthesia, fish showed significantly lower ($p<0.01$) concentration of inorganic phosphate compared with CG.

CONCLUSIONS: On the basis of this experiment, it appears that clove oil was associated with the lowest effects in pikeperch and therefore would be recommended as an alternative to MS 222, while Propiscin and 2-phenoxyethanol are not suitable for manipulation with pikeperch in aquaculture.

Abbreviations:

CG	- control group
RBC	- erythrocyte count
Hb	- haemoglobin
PCV	- haematocrit
MCV	- mean corpuscular volume
MCH	- mean corpuscular haemoglobin
MCHC	- mean corpuscular haemoglobin concentration
GLU	- glucose
TP	- total protein
ALB	- albumin
GLOB	- total globulins
NH_3	- ammonia
AST	- aspartate aminotransferase
ALT	- alanine aminotransferase
LDH	- lactate dehydrogenase
CK	- creatine kinase
PHOS	- inorganic phosphate
ALP	- alkaline phosphatase
LACT	- lactate
ANOVA	- analysis of variance

INTRODUCTION

Anaesthetic are commonly used to reduce handling stress and are routinely administered in cases of blood sampling, measuring, tagging, sorting, photographing and the artificial reproduction procedures of fish (Cooke *et al.* 2004; King *et al.* 2005; Macova *et al.* 2008; Park *et al.* 2008; Gullian & Villanueva 2009; Kiessling *et al.* 2009; Velisek *et al.* 2009a, 2011). A variety of anti-stress agents with different properties have been used to anesthetise in aquaculture (Cho & Heath 2000; Kazun & Siwicki 2001; Velisek & Svobodova 2004; Velisek *et al.* 2005).

Currently, the commonly used anaesthetics in pikeperch (*Sander lucioperca*) are Propiscin (Zakes & Demska-Zakes 2005), 2-phenoxyethanol (Demska-Zakes *et al.* 2005), clove oil (Kristan *et al.* 2012) and tricaine methane sulphonate (Zarski *et al.* 2012).

Propiscin was developed at the Inland Fisheries Institute in Poland and is routinely used for immobilization of fish in Poland fisheries (Szkudlarek and Zakes 1996). The active substance of Propiscin is etomidate [etomidate (1)-ethyl 1-(a-methylbenzyl) imidazole-5-carboxylate] (Kazun & Siwicki 2001). The used and recommended concentration for anaesthesia of pikeperch is 1.5-2 $mL.L^{-1}$ water (Szkudlarek & Zakes 1996; Kazun & Siwicki 2001). Ethylene glycol monophenyl ether (2-phenoxyethanol) is used for short-term immobilization of fish before artificial spawning and the recommended concentration for use during artificial propagation of pikeperch is 0.1–0.4 $mL.L^{-1}$ water (Kaminnski *et al.* 2002). Clove oil is a dark brown liquid and is derived from stems, leaves and buds of the clove trees *Eugenia aromatica* and *Eugenia caryophyllata* (Sato & Burhanuddin 1995; Keene *et al.* 1998). The active ingredient of clove oil is eugenol (4-allyl-2-methoxyphenol), which makes up about 70–90% of the oil weight. It is generally used as a disinfectant and analgesic in dentistry (Curtis 1990) and as an additive in perfumes (Maura *et al.* 1989). The used and recommended concentration for percid fish is 33 $mg.L^{-1}$ (Hamackova *et al.* 2001; Kristan *et al.* 2012).

Tricaine methane sulphonate is an isomer of benzocaine with an additional sulphonate radical, making it more soluble but also more acidic in solution (Congleton 2006). It is the most commonly used anaesthetic for fish (Marking & Meyer 1985). The used concentration for anaesthesia in pikeperch is 150 $mg.L^{-1}$ water (Zarski *et al.* 2012).

Therefore, the purpose of this study was to compare the affects Propiscin, 2-phenoxyethanol, clove oil and MS 222 (relative to non-anesthetized controls) on haematological and blood plasma biochemical indices in pikeperch (*Sander lucioperca*) immediately following 10-min anaesthesia and 24 h after 10-min anaesthesia. These anaesthetics are commonly used for anaesthesia of pikeperch.

MATERIALS AND METHODS

Anaesthetics

Propiscin was supplied by the Division of Fish Pathology and Immunology at Zabieniec (Inland Fisheries Institute in Olsztyn, Poland). Clove oil (eugenol concentration 78%) was from the Kulich Company (Jan Kulich, Hradec Kralove/Ricany, Czech Republic), and 2-phenoxyethanol from MERCK – Schucherd, 85 662 Hohenbrunn, Germany. MS 222 was purchased from Sigma-Aldrich Chemicals Ltd.

Experimental procedure

For assessment of the haematological and blood biochemical profile, 72 fish (71.48±15 g body weight and 228±14.59 mm total body length) were used. To maintain growth, the fish were fed with food (Inicio Plus, BioMar) of the appropriate size at the appropriate rate during a 10-day acclimatization period. All fish were starved for 24 h before the experiments. Fish were maintained at water temperature 20.5–20.7 °C and 12:12 light: dark times during both experimental and acclimatization period. In this study, nine groups were compared, of 8 fish eachControl group – no anaesthetic, blood immediately sampled, prior to the treatment of anesthetized groups.

Four groups with blood sampled immediately after 10 min anaesthesia and designated as: Propiscin (10 min) (1.5 $ml.L^{-1}$), 2-phenoxyethanol (10 min), (0.3 $ml.L^{-1}$), Clove Oil (10 min) (33 $mg.L^{-1}$) and MS 222 (10 min) (150 $mg.L^{-1}$).

Four groups with blood sampled 24 h after 10 min anaesthesia and designated as: Propiscin (24 h), 2-phenoxyethanol (24 h), Clove Oil (24 h) and MS 222 (24 h).

The conditions were duplicated for all groups, each held in cylindrical plastic tank (volume 185 L) containing freshwater with the anaesthetic. There were no mortalities in the study.

Blood was drawn from the *caudal vessels* with heparin as an anticoagulant (Heparin inj., Leciva, Czech Republic) at a concentration of 5000 I.U. heparin sodium salt in 1 ml. Erythrocyte count (RBC), haematocrit (PCV), leucocrit (Bc), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) and leukocyte count (Leuko), were determined by Svobodova *et al.* (1991).

For biochemical analysis, blood was centrifuged in a cooled centrifuge (4 °C, 837 × g). The plasma was stored at –80 °C until analysis. Biochemical indices in plasma included 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 dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase (ALP) and lactate (LACT) were analysed. For the biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX Laboratories Inc. USA) manufactured by Medisoft was used. Sample analysis was carried out on selective testing discs (Multi-layer film slides, Kodak). QA/QC (quality assurance/quality control) measures were consistently applied within the experiment. The measurements were carried out according to validated standard operation procedures.

Statistical analysis was carried out using Statistica software 9.0 for Windows (StatSoft, Czech Republic). 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 AND DISCUSSION

The biochemical, haematological and histopathological profile can give important information about the internal environment of the organism and these parameters are commonly used in toxicology studies (Velisek *et al.* 2009b; Haluzova *et al.* 2010; Plhalova *et al.* 2011). For evaluation of the effect of anaesthetics are frequently used the haematological and biochemical profiles (Iwama *et al.* 1989; Velisek & Svobodova 2004; Velisek *et al.* 2005, 2006, 2007, 2009c, 2011).

In our study, we evaluated both profiles. To our knowledge, no other data on biochemical and haematological profiles in pikeperch (*Sander lucioperca*) anesthetized with Propiscin, 2-phenoxyethanol, clove oil and MS 222 are available in the literature. The biochemical profiles of control and anesthetized pikeperch are given in Table 2. The level of glucose was significantly ($p<0.05$) higher with MS 222 (10 min) and clove oil (10 min) compared to controls. On the one hand, our results corresponded to the results published by Velisek *et al.* (2009) who reported an increased glucose level with MS 222 and clove oil in perch (*Perca fluviatilis*). On the other hand, these authors also found an increase of glucose in 2-phenoxyethanol. Increase of glucose concentration after 2-phenoxyethanol was also detected by Ortuno *et al.* (2002) in gilthead sea bream (*Sparus aurata*) and Park *et al.* (2008) in kelp grouper (*Epinephelus bruneus*). However, Velisek *et al.* (2007) found no changes with 2-phenoxyethanol in European catfish (*Silurus glanis*). The increase in blood glucose concentration demonstrated the response of exposed fish to metabolic stress (Simon *et al.* 1983).

The 2-phenoxyethanol (10 min and 24 h), MS 222 (24 h), clove oil (24 h), and Propiscin (24 h) showed significantly lower ($p<0.01$) ammonia levels compared with the control group (Table 2). The concentration of ammonia do not correspond with those of Velisek *et al.* (2004, 2005, 2007, 2009a) who detected no change in level of ammonia in rainbow trout, common carp, sheat-fish and perch. Nevertheless, Gomulka *et al.*

Tab. 1. Effects of MS222, clove oil, 2-phenoxyethanol (2-PE) and Propiscin anaesthesia on haematological indices in pikeperch.

Indices	Control	MS222 (10 min)	MS222 (24h)	Clove oil (10 min)	Clove oil (24h)	2-PE (10 min)	2-PE (24h)	Propiscin (10 min)	Propiscin (24h)
RBC ($T.L^{-1}$)	2.30±0.48	2.17±0.41	1.84±0.31	1.67±0.53	1.93±0.31	2.21±0.25	1.65±0.25*	2.21±0.35	1.87±0.36
Hb ($g.L^{-1}$)	40.11±6.21	32.45±5.09	45.39±12.47	37.51±9.76	44.13±4.46	50.58±3.70	39.93±6.55	47.58±6.99	46.03±8.30
PCV ($L.L^{-1}$)	0.46±0.07	0.43±0.05	0.37±0.05	0.36±0.05	0.41±0.05	0.39±0.06	0.33±0.06**	0.37±0.06	0.36±0.08
MCV (fl)	204.07±22.88	209.70±59.29	203.45±32.45	246.68±83.35	215.97±27.81	175.61±32.03	197.83±26.42	166.99±23.16	199.95±54.27
MCH (pg)	17.74±2.22	15.17±1.80	24.96±6.92	23.75±9.49	23.23±3.46	21.70±2.34	24.33±2.92	21.70±2.34	24.74±1.51
MCHC ($g.L^{-1}$)	87.55±11.43	76.42±16.79	125.98±40.21	108.00±32.49	107.47±15.79	131.79±15.46*	123.56±19.20	130.87±9.18*	131.71±32.23*

Significance levels observed are *$p<0.05$, **$p<0.01$ in comparison to the control group. All values are mean ± SD, n=8. See text for description of experimental procedure.

Tab. 2. Effects of MS222, clove oil, 2-phenoxyethanol (2-PE) and Propiscin anaesthesia on biochemical indices of blood plasma in pikeperch.

Indices	Control	MS222 (10 min)	MS222 (24h)	Clove oil (10 min)	Clove oil (24h)	2-PE (10 min)	2-PE (24h)	Propiscin (10 min)	Propiscin (24h)
GLU (mmol.L^{-1})	10.56±3.63	15.00±3.26*	7.83±1.69	15.35±2.96*	8.77±0.98	13.79±3.05	8.28±0.71	11.75±3.58	7.51±2.68
TP (g.L^{-1})	42.25±2.63	44.25±3.03	37.25±2.44	41.63±3.28	42.88±4.11	43.25±3.73	41.13±1.62	37.50±6.95	41.25±1.20
ALB (g.L^{-1})	2.88±1.62	4.13±1.27	2.50±0.86	3.25±1.85	3.50±1.66	2.88±2.03	3.88±1.05	2.00±0.87	3.38±0.99
GLOB (g.L^{-1})	39.25±2.11	40.65±2.23	34.88±1.76	38.38±2.50	39.63±3.64	40.25±2.63	37.00±1.32	36.13±6.73	37.63±1.32
NH_3 (µmol.L^{-1})	921.1±151.8	857.3±147.0	488.9±40.8**	715.3±58.8	528.1±60.7**	689.9±151.2**	506.3±69.4**	734.0±203.2	343.4±91.6**
TAG (mmol.L^{-1})	3.51±0.16	3.16±0.57	1.57±0.44**	2.81±0.60	1.74±0.60**	3.01±0.65	2.43±0.54**	1.98±0.58**	2.25±0.71**
AST (µkat.L^{-1})	2.40±0.91	2.39±0.85	1.12±0.49	1.59±0.77	1.41±0.48	2.09±0.97	2.42±1.10	2.30±0.81	1.68±0.84
ALT (µkat.L^{-1})	0.15±0.04	0.14±0.08	0.17±0.07	0.11±0.05	0.15±0.09	0.10±0.08	0.25±0.15	0.18±0.11	0.22±0.11
LDH (µkat.L^{-1})	20.14±2.54	19.23±2.80	21.12±6.23	19.80±3.03	17.75±2.78	20.04±2.22	17.94±3.43	18.90±1.56	18.98±2.60
CK (µkat.L^{-1})	14.90±2.30	14.08±1.36	14.12±2.94	14.13±1.23	15.49±3.37	14.10±1.26	14.67±2.36	14.17±1.49	15.83±1.74
Ca^{2+} (mmol.L^{-1})	2.83±0.15	3.05±0.10	2.73±0.11	2.82±0.12	2.81±0.19	2.84±0.49	2.67±0.04	2.56±0.35	2.78±0.10
Mg (mmol.L^{-1})	1.15±0.10	1.20±0.10	0.96±0.06	1.04±0.08	1.01±0.19	0.93±0.09	1.05±0.30	1.02±0.16	1.19±0.42
PHOS (mmol.L^{-1})	3.82±0.62	4.15±0.35	2.80±0.13**	3.32±0.33	3.13±0.35	3.32±0.77	3.18±0.36	3.31±0.52	2.94±0.36**
ALP (µkat.L^{-1})	1.17±0.11	0.96±0.15	1.03±0.12	0.93±0.23	0.95±0.29	0.96±0.16	0.88±0.17	0.88±0.12	1.19±0.21
LACT (mmol.L^{-1})	3.10±0.64	4.20±0.57	2.52±0.43	3.95±0.31	2.34±0.33	3.79±0.93	2.80±0.66	3.36±0.96	2.37±1.06

Significance levels observed are $^{*}p<0.05$, $^{**}p<0.01$ in comparison to the control group. All values are mean ± SD, n=8. See text for description of experimental procedure.

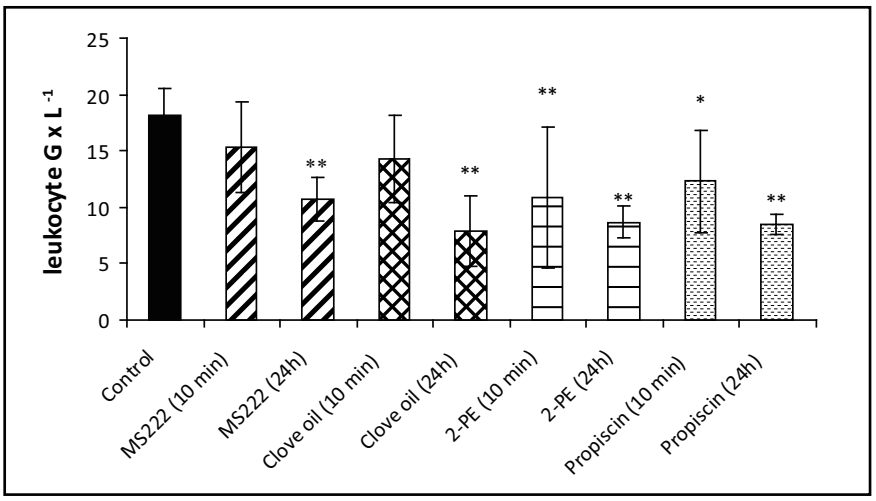

Fig. 1. Effects of MS222, clove oil, 2-phenoxyethanol and Propiscin anaesthesia on leukocytes of blood profile in pikeperch. Significance levels observed are $^{*}p< 0.05$, $^{**}p<0.01$ in comparison to the control group. All values are mean ± SD, n=8. See text for description of experimental procedure.

(2008) achieved also decreased of ammonia levels in Sibernia sturgeon (*Acipenser baerii*) with Eugenol and MS 222. Change levels of NH_3 in blood indicate a change in protein catabolism and/or some disturbances in NH_3 removal (Svoboda 2001).

Also the levels of triacylglycerols was significantly decreased ($p<0.01$) with Propiscin (10 min and 24 h), MS 222 (24 h), clove oil (24 h) and with 2-phenoxyethanol (24 h) compared with the control group (Table 2).These findings do not agree with those of Velisek *et al.* (2006) who reported increased concentration of triacylglycerols with clove oil in European catfish.

After 24 hours MS 222 (24 h) and Propiscin (24 h) anaesthesia, fish showed significantly lower ($p<0.01$) concentration of inorganic phosphate compared with the control group. These observations are not in agreement with Velisek *et al.* (2004, 2005, 2007, 2009a). The lower concentration of PHOS could be linked to redistribution of electrolytes between intra- and extra-cellular compartments and/or impairment of renal function (Svoboda 2001).

The values for TP, ALB, GLOB, AST, ALT, LDH, CK, ALP, Ca^{2+}, Mg and LACT were similar among all groups.

The results of haematological profiling are given in Table 1. Haemoglobin and haematocrit are often elevated during stress situations to increase oxygen carrying capacity and oxygen apply to the major organs in

response to higher metabolic demands (Rutten *et al.* 1992). The erythrocyte count ($p<0.01$) and haematocrit ($p<0.01$) was significantly decreased in 2-phenoxyethanol (24 h) compared with controls. The mean corpuscular haemoglobin concentration was a significant increase ($p<0.05$) in 2-phenoxyethanol (10 min), Propiscin (10 min and 24 h) compared to the control group. Similar results observed Velisek *et al.* (2007) who achieved also the changes of MCHC and PCV in 2-phenoxyethanol. Acute stress can cause significant changes in the white blood cell count. The response to environmental challenges often leads to leucopenia with lymphopenia and sometimes neutrophilia, which is similar to the classic leukocytic response to stress in mammals. The 2-phenoxyethanol (10 min and 24 h), MS 222 (24 h), clove oil (24 h), and Propiscin (24 h) showed significantly lower ($p<0.01$) leukocyte count compared with the control group (Figure 1), whereas, Velisek *et al.* (2005) observed no changes with clove oil in common carp, sheat-fish (2006) and with 2-phenoxyethanol in leukocyte count. The values for Hb, MCV and MCH were similar among all groups.

In summary, all tested anaesthetics were associated with changes in haematological and blood biochemical parameters in pikeperch. However, the effects of the anaesthetics were different from one another in the measured variables. On the basis of this experiment, it appears that clove oil was associated with the lowest effects in pikeperch and therefore would be recommended as an alternative to MS 222.

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Potential Conflicts of Interest: *None disclosed.*

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