The effects of four anaesthetics on haematological and blood biochemical profiles in vimba bream, *Vimba vimba*

P. Lepic, A. Stara, J. Turek, P. Kozak, J. Velisek

Research Institute of Fish Culture and Hydrobiology, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia, Ceske Budejovice, Czech Republic

ABSTRACT: The aim of this study was to compare the effect of four anaesthetics on the haematological and blood biochemical profiles of vimba bream (*Vimba vimba*). The haematological and blood biochemical profiles of vimba bream were evaluated 10 min and 24 h after anaesthesia with MS 222 (100 mg/l), clove oil (33 mg/l), 2-phenoxyethanol (0.4 ml/l), Propiscin (1.0 ml/l) and compared to non-anaesthetised controls. The 10 min exposure to any of the anaesthetics did not show any effects on haematological profiles. The exposure to 2-phenoxyethanol and Propiscin significantly ($P < 0.01$) influenced levels of glucose and ammonia, and the activity of aspartate aminotransferase compared with the control group. The level of triacylglycerols was significantly ($P < 0.01$) increased and the activity of lactate dehydrogenase was significantly ($P < 0.01$) decreased by exposure to MS 222. The use of clove oil showed no effects on the haematological and blood biochemical profiles and is recommended as a suitable anaesthetic for vimba bream. Other anaesthetics tested affected blood biochemical profiles to some extent.

Keywords: anaesthesia; tricaine methane sulphate; clove oil; 2-phenoxyethanol; Propiscin

The use of non-stressful anaesthetics is common practice in modern aquaculture. Such substance are used during handling, sorting, tagging, artificial reproduction procedures, and surgery, thus reducing stress-induced problems such as reduction in feeding and immune function (Ross and Ross 1999; Kolarova et al. 2007).

A variety of anaesthetics with differing properties have been used in aquaculture (Cho and Heath 2000; Kazun and Siwicki 2001; Velisek and Svobodova 2004a,b; Velisek et al. 2005a,b, 2006, 2007, 2009, 2011). Chemicals used in aquaculture are subject to strict control, particularly with regard to safety and efficacy (Taylor and Roberts 1999). The anaesthetics most commonly used are tricaine methane sulphonate (MS 222), benzocaine, quinaldine sulphate, methomidate, clove oil, and 2-phenoxyethanol (Velisek and Svobodova 2004a,b). Currently, only MS 222 is licensed for use in food fish in the USA and the European Union. However, compounds such as 2-phenoxyethanol, clove oil, and Propiscin have been evaluated experimentally and are being used in non-food fish and in research (Coyle et al. 2004). Their use on food fish remains illegal under EEC Regulation 2377/90, as no maximum residue levels (MRL) have been established.

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, with a recommended concentration of 100 mg/l water (Marking and Meyer 1985).

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Clove oil is derived from the stems, leaves, and buds of the *Eugenia aromatica* and *Eugenia caryophyllata* clove trees (Sato and Burhanuddin 1995; Keene et al. 1998). The active ingredient is eugenol (4-allyl-2-methoxyphenol), which constitutes 70–90% of the oil weight. It is used as a disinfectant and analgesic in dentistry (Curtis 1990) and as an additive in perfumes (Maura et al. 1989). The recommended concentration for anaesthesia of vimba bream is 33 mg/l water (Hamackova et al. 2008). 2-phenoxyethanol (ethylene glycol monophenyl ether) is used in the Czech Republic for short-term immobilisation of fish before artificial spawning at a recommended concentration of 0.40 ml/l water (Hamackova et al. 2008). Propiscin was developed at the Inland Fisheries Institute in Poland and is routinely used for immobilisation of fish in that country (Szkudlarek and Zakes 1996). The active substance of Propiscin is etomidate [etomidate (1)-ethyl 1-(α-methylbenzyl) imidazole-5-carboxylate] (Kazun and Siwicki 2001). The recommended concentration is 1.0 ml/l water (Szkudlarek and Zakes 1996).

Although anaesthesia of fish may mitigate against the biochemical and physiological stress due to handling, the anaesthetic can itself induce alterations in haematological and biochemical values. The purpose of this study was to compare the effects of clove oil, 2-phenoxyethanol, Propiscin, and MS 222 on haematological and blood plasma biochemical profiles in vimba bream, with particularly reference to stress.

MATERIAL AND METHODS

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

Experimental procedures. For assessment of the haematological profiles and the biochemical profiles of blood plasma, 54 vimba bream (339.21 ± 75.79 g body weight and 34.94 ± 3.56 cm total length) were used. During a 10-day acclimatisation period, an suitable amount of food was offered at a rate appropriate to maintain growth. Fish were not fed for 24 h before the experiments. Water temperature was maintained at 17.6–18.2 °C throughout the experimental period, and fish were maintained on a 12L : 12D regime. Nine groups of three fish each were compared:

For the control – no anaesthetic group blood was sampled prior to the treatment of anaesthetised groups. Blood was sampled immediately after 10 min of anaesthesia in the four experimental groups: MS 222 (10 min) (100 mg/l), clove oil (10 min) (33 mg/l), 2-phenoxyethanol (10 min) (0.40 ml/l), and Propiscin (10 min) (1.0 ml/l). In a further four groups blood was sampled 24 h after 10 min anaesthesia: MS 222 (24 h), clove oil (24 h), 2-phenoxyethanol (24 h), and Propiscin (24 h).

Each group was held in a separate tank containing freshwater plus the anaesthetic for experimental groups. Each treatment was duplicated. There were no mortalities during the study.

Blood was drawn from the *vena caudalis* using an 18G 1½ in syringe with heparin as anticoagulant (Heparin inj., Leciva, Czech Republic) at a concentration of 5000 IU heparin sodium salt in 1 ml. Erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and leukocyte count (Leuko) were determined according to Svobodova et al. (2012).

Blood was separated in a cooled centrifuge (4 °C, 837 × g), and the plasma was stored at –80 °C until analysis on a VETTEST 8008 analyzer (IDEXX Laboratories Inc., USA). Biochemical indices determined 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). Biochemical indices were assayed using the method of Kolarova and Velisek (2012).

Statistical analysis. Statistical analysis was carried out using Statistica software 10.0 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 vari-

ance (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 (Zar 1996).

RESULTS

Haematological profile

The 10 min exposure to the anaesthetics (MS 222, 2-phenoxythenalo, clove oil, and Propiscin) were not different from the control with respect to erythrocyte count, hematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, or leukocyte count (Table 1).

Biochemical blood profile

The biochemical profiles are given in Table 2. The level of glucose was significantly ($P < 0.01$) greater with 2-phenoxyethanol (10 min and 24 h) and Propiscin (10 min and 24 h) compared to controls.

Ammonia levels were significantly higher ($P < 0.01$) with 2-phenoxyethanol (10 min and 24 h) and Propiscin (24 h) compared with the control group.

The levels of triacylglycerols were significantly increased ($P < 0.01$) with MS 222 (24 h) compared with the control group.

The activity of aspartate aminotransferase showed a significant increase ($P > 0.01$) with 2-phenoxyethanol (10 min and 24 h); however after anaesthesia with Propiscin (10 min and 24 h) the activity of aspartate aminotransferase was significantly decreased ($P < 0.01$) compared to the control group.

Immediately after anaesthesia induced by MS 222 (10 min), fish showed significantly lower ($P < 0.01$) lactate dehydrogenase activity compared with the control group and with all other treatments. Lactate dehydrogenase activity returned to the control level within 24 h (MS 222 24 h).

The values for total protein, albumin, total globulin, alanine aminotransferase, creatine kinase, calcium, magnesium, inorganic phosphate, alkaline phosphatise, and lactate were similar among all groups.

Table 1. Effects of MS222, clove oil, 2-phenoxyethanol, and Propiscin anaesthesia on haematological indices of blood plasma in vimba bream

Indices	Control	MS222		Clove oil		2-phenoxyethanol		Propiscin	
		10 min	24 h	10 min	24 h	10 min	24 h	10 min	24 h
RBC (T/l)	1.68 ± 0.15	1.58 ± 0.12	1.53 ± 0.35	1.52 ± 0.20	1.70 ± 0.32	1.54 ± 0.22	1.66 ± 0.40	1.55 ± 0.12	1.65 ± 0.24
Hb (g/l)	83.38 ± 6.92	89.28 ± 5.79	93.52 ± 6.37	81.64 ± 11.68	94.30 ± 8.15	88.33 ± 11.95	95.49 ± 9.52	87.67 ± 9.78	91.13 ± 12.63
PCV (l/l)	0.45 ± 0.06	0.52 ± 0.05	0.47 ± 0.06	0.46 ± 0.06	0.49 ± 0.04	0.48 ± 0.03	0.48 ± 0.04	0.46 ± 0.05	0.49 ± 0.04
MCV (fl)	268.02 ± 51.67	326.46 ± 29.29	334.92 ± 135.3	306.45 ± 40.69	295.39 ± 59.03	317.47 ± 35.62	309.30 ± 77.21	299.64 ± 46.33	301.20 ± 41.95
MCH (pg)	49.93 ± 5.23	56.72 ± 4.65	65.73 ± 19.61	54.39 ± 8.29	57.77 ± 13.87	57.74 ± 5.85	60.16 ± 11.62	57.19 ± 9.56	57.25 ± 16.04
MCHC (g/l)	191.81 ± 33.24	174.63 ± 16.57	203.96 ± 34.75	177.87 ± 17.09	195.30 ± 23.32	182.73 ± 15.90	197.17 ± 12.05	190.67 ± 11.46	188.88 ± 34.97
Leuko (G/l)	14.47 ± 3.49	14.58 ± 3.11	11.82 ± 5.15	11.57 ± 3.57	13.22 ± 3.07	14.32 ± 2.27	12.53 ± 2.94	13.45 ± 3.95	12.15 ± 2.67

All values are mean ± SD, $n = 6$

Table 2. Effects of MS222, clove oil, 2-phenoxyethanol, and Propiscin anaesthesia on biochemical indices of blood plasma in vimba bream

Indices	Control	MS222		Clove oil		2-phenoxyethanol		Propiscin	
		10 min	24 h	10 min	24 h	10 min	24 h	10 min	24 h
GLU (mmol/l)	4.50 ± 0.67	6.50 ± 1.24	4.78 ± 1.04	6.95 ± 1.63	4.90 ± 2.22	7.54 ± 1.22**	8.34 ± 1.51**	8.72 ± 0.78**	11.21 ± 2.56**
TP (g/l)	42.33 ± 2.74	43.33 ± 2.87	44.00 ± 3.92	42.50 ± 2.36	43.67 ± 2.36	46.00 ± 1.41	42.67 ± 2.56	44.17 ± 2.27	44.67 ± 1.60
ALB (g/l)	8.17 ± 1.77	7.83 ± 0.37	7.67 ± 0.75	8.17 ± 1.67	7.83 ± 0.69	9.33 ± 1.25	8.00 ± 0.58	8.50 ± 0.96	8.00 ± 0.03
GLOB (g/l)	34.33 ± 2.21	34.67 ± 1.60	36.00 ± 3.51	34.33 ± 1.80	35.83 ± 2.11	36.67 ± 1.87	34.67 ± 2.69	35.67 ± 2.36	36.67 ± 1.60
NH_3 (µmol/l)	277.67 ± 99.93	351.83 ± 141.37	267.67 ± 89.86	393.83 ± 75.19	285.50 ± 52.36	410.83 ± 55.01**	506.17 ± 112.43**	368.67 ± 101.16	620.3 ± 110.7**
TAG (mmol/l)	3.49 ± 0.57	4.17 ± 0.80	5.68 ± 1.16**	3.41 ± 0.55	3.68 ± 1.16	3.38 ± 0.46	3.84 ± 1.66	3.16 ± 1.04	3.52 ± 1.06
AST (µkat/l)	1.61 ± 0.59	1.21 ± 0.50	1.46 ± 0.41	1.03 ± 0.13	1.58 ± 0.44	2.00 ± 1.52**	2.87 ± 0.62**	0.90 ± 0.25**	0.73 ± 0.31**
ALT (µkat/l)	0.85 ± 0.31	0.53 ± 0.13	0.77 ± 0.29	0.68 ± 0.14	0.81 ± 0.45	0.72 ± 0.28	0.73 ± 0.35	0.75 ± 0.30	0.76 ± 0.29
LDH (µkat/l)	22.13 ± 1.27	14.56 ± 3.60**	21.96 ± 2.60	20.23 ± 2.58	21.64 ± 2.73	22.30 ± 2.77	23.15 ± 3.18	23.61 ± 3.56	20.67 ± 1.39
CK (µkat/l)	16.79 ± 1.09	16.93 ± 1.28	16.75 ± 1.79	16.43 ± 1.26	16.90 ± 2.25	16.48 ± 1.17	17.57 ± 1.27	17.25 ± 1.60	17.39 ± 2.37
Ca^{2+} (mmol/l)	2.29 ± 0.16	2.26 ± 0.14	2.41 ± 0.34	2.36 ± 0.19	2.56 ± 0.56	2.39 ± 0.24	2.62 ± 0.29	2.46 ± 0.28	2.70 ± 0.25
Mg (mmol/l)	1.13 ± 0.12	1.15 ± 0.06	1.33 ± 0.35	1.19 ± 0.08	1.41 ± 0.32	1.27 ± 0.07	1.24 ± 0.21	1.38 ± 0.25	1.16 ± 0.40
PHOS (mmol/l)	2.26 ± 0.38	2.44 ± 0.16	2.45 ± 0.34	2.68 ± 0.39	2.67 ± 0.54	2.52 ± 0.25	2.64 ± 0.42	2.58 ± 0.30	2.74 ± 0.57
ALP (µkat/l)	0.57 ± 0.22	0.53 ± 0.34	0.57 ± 0.25	0.64 ± 0.20	0.59 ± 0.25	0.59 ± 0.22	0.56 ± 0.18	0.59 ± 0.21	0.60 ± 0.23
LACT (mmol/l)	5.18 ± 1.31	5.23 ± 0.81	5.27 ± 1.01	5.48 ± 0.98	5.17 ± 1.11	5.57 ± 0.73	5.39 ± 1.13	5.47 ± 0.80	5.36 ± 0 51

Significance levels observed are *$P < 0.05$, **$P < 0.01$ in comparison with the control group. All values are mean ± SD, $n = 6$

DISCUSSION

Because fish species may differ widely in their response to anaesthetics, the screening of dosages is often necessary. The anaesthetics tested in this study were effective as sedatives for routine weighing and measuring procedures and handling for spawning of vimba bream.

The analysis of blood parameters is one of the most valuable methods available for modern diagnostics (Anver Celik 2004), and can provide important information about the internal environment of the organism (Anver Celik 2004; Velisek et al. 2005a,b, 2006, 2007, 2009, 2011; Kristan et al. 2012). Haematological and biochemical profiles are frequently used for evaluation of the effect of anaesthetics (Iwama et al. 1989; Velisek and Svobodova 2004a,b; Velisek et al. 2005a,b, 2006, 2007, 2009, 2011; Kristan et al. 2012). To our knowledge, no other data on biochemical and haematological profiles in vimba bream anaesthetized with MS 222, Propiscin, 2-phenoxyethanol, or clove oil are available.

Anaesthesia with MS 222, Propiscin, 2-phenoxyethanol, and clove oil showed no effect on the haematological profile of vimba bream. These results correspond with the results of Velisek et al. (2005b) and Velisek et al. (2006) who found no changes with clove oil anaesthesia in common carp (*Cyprinus carpio* L.) and European catfish (*Silurus glanis* L.). However, our data differ with findings reported in other species of fish. Velisek et al. (2007) observed changes in MCHC and PCV with 2-phenoxyethanol anaesthesia on European catfish. Similar results were obtained by Kristan et al. (2012) in their study of the effects of anaesthesia on pikeperch (*Sander lucioperca*).

Biochemical indices of blood plasma were affected by the action of anaesthetics. The level of glucose was significantly higher with 2-phenoxyethanol (10 min and 24 h) and Propiscin (10 min and 24 h) compared with the control group. Increases in glucose concentrations after 2-phenoxyethanol were also detected by Ortuno et al. (2002) in gilthead sea bream, *Sparus aurata*, and Park et al. (2008) in kelp grouper (*Epinephelus bruneus*). Increased glucose levels were also reported by Kristan et al. (2012) with MS 222 and clove oil anaesthesia in pikeperch. On the other hand, Iversen et al. (2003) found no change in blood glucose concentrations in Atlantic salmon (*Salmo salar*) following clove oil anaesthesia. Velisek and Svobodova (2004a) and Velisek et al. (2007) also found no changes in the concentration of glucose in common carp and European catfish following 2-phenoxyethanol (0.30 ml/l) anaesthesia. The increases in blood glucose concentrations found in the present study reflected the response of anaesthetised fish to metabolic stress. Increases in plasma glucose are mediated by the release of catecholamines, presumably in response to the hypoxia caused by cessation of respiration in anaesthetised fish (Gingerich and Drottar 1989; Iwama et al. 1989).

2-phenoxyethanol (10 min and 24 h) and Propiscin (24 h) were associated with higher levels of ammonia compared to the control group. Alteration in levels of NH_3 in blood indicates a change in protein catabolism and/or disturbance in NH_3 elimination (Svoboda 2001). In contrast to our results, Kristan et al. (2012) reported lower ammonia levels in pikeperch following anaesthesia with 2-phenoxyethanol (0.3 ml/l), MS 222 (150 mg/l), clove oil (33 mg/l), and Propiscin (1.5 ml/l). Gomulka et al. (2008) also reported decreased ammonia levels in Siberian sturgeon (*Acipenser baerii*) after eugenol (0.075 ml/l) and MS 222 (125 mg/l) anaesthesia. No changes in the levels of ammonia in rainbow trout, carp, European catfish, or perch following clove oil (30 mg/l) and 2-phenoxyethanol (0.30 ml/l) anaesthesia were observed by Velisek and Svobodova (2004a), and Velisek et al. (2005b, 2007, 2009). Our differing results may be associated with the concentrations of the tested substances and the fish species used.

In the present study, the triacylglycerol levels were significantly increased 24 h after MS 222 anaesthesia. Changes in blood triacylglycerol levels indicate a change in protein metabolism. Similar results were reported by Gomulka et al. (2008) after MS 222 (125 mg/l) and eugenol (0.075 ml/l) anaesthesia in Siberian sturgeon. Velisek et al. (2006) reported increases in triacylglycerol levels in European catfish after clove oil (0.3 mg/l) anaesthesia. Conversely, Kristan et al. (2012) found decreased triacylglycerol levels after 2-phenoxyethanol (0.3 ml/l), MS 222 (150 mg/l), clove oil (33 mg/l), and Propiscin (1.5 ml/l) anaesthesia in pikeperch.

Enzyme activity in blood plasma can be a stress indicator. The enzymes analysed for this purpose were LDH, CK, and the transaminases ALT and AST. A significant change in the activity of the fore-mentioned enzymes indicates tissue damage, which may be stress-induced (Svoboda 2001). In

our experiment AST activity was significantly increased with 2-phenoxyethanol (10 min and 24 h) and significantly decreased with Propiscin (10 min and 24 h) compared with the control group. The activity of LDH was significantly lower with MS 222 compared with controls. The altered transaminase activity observed in the present study suggests amplified or attenuated transamination processes. Velisek and Svobodova (2004a) and Velisek et al. (2005a) reported decreased AST activity in rainbow trout after clove oil and 2-phenoxyethanol anaesthesia. A similar change in LDH activity was reported by Velisek et al. (2009) with clove oil anaesthesia in perch. Velisek et al. (2011) reported increased activity of AST with clove oil and 2-phenoxyethanol anaesthesia in rainbow trout. Velisek and Svobodova (2004a,b) and Velisek et al. (2007) found no changes in LDH activity in rainbow trout, common carp, and European catfish with 2-phenoxyethanol (0.30 ml/l) anaesthesia.

The results of this study suggest that the internal organs and tissue of vimba bream are not altered by clove oil anaesthesia, but are slightly affected by MS 222, 2-phenoxyethanol, and Propiscin anaesthesia. However, the effects of MS 222, 2-phenoxyethanol, and Propiscin anaesthesia did not significantly differ in any measured variable. Although on the basis of this experiment, it seems that clove oil would be preferred to MS 222, the final choice of anaesthetic must take into account legislation, availability, cost-effectiveness, ease of use, and safety for the user and the environment. As clove oil, 2-phenoxyethanol, and Propiscin are not approved for use on food fish, we do not advocate their use on any fish until MRL (EEC Regulation 2377/90) standards are determined and proper licensing is enacted.

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Corresponding Author:

Ing. Pavel Lepic, University of South Bohemia Ceske Budejovice, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, Research Institute of Fish Culture and Hydrobiology, Zatisi 728/II, 389 25 Vodnany, Czech Republic

Tel. +420 38777 4765, E-mail: lepic@frov.jcu.cz