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PROOF

Haematological and Biochemical Blood Profile in Russian Sturgeon Following Propofol and Eugenol Anaesthesia

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Abstract

Sturgeon production involves unavoidable handling procedures inducing stress response. Clove oil is the anaesthetic commonly used in sturgeon culture to minimise stress and pain. Propofol is one of the most frequently used anaesthetic in humans. Propofol is a promising candidate anaesthetic for fish. The aim of the study was to compare the effect of propofol and eugenol on haematological and biochemical blood profile of Russian sturgeon. Determined indices covered: PCV, RBC, Hb, MCV, MCHC, MCH, leucogram, and concentration of calcium cations, inorganic phosphates, total proteins, albumins, globulins, ammonia, triacylglycerols, glucose, and the activity of creatine kinase, alkaline phosphatase and aspartate aminotransferase. Exposure to both anaesthetics caused a stress response in Russian sturgeon expressed as changes in WBC system and increased values of PCV, Hb, glucose, triacylglycerols, inorganic phosphates, calcium and ammonia. The impact of eugenol was more sever and longer being. No damage of internal organs was stated. According to our results, propofol is safe anaesthetic for Russian sturgeon.

Keywords: 2'6 diisopropylophenol, 2-metoksy-4-(2-propenylo) phenol, handling stress, biochemical blood profile.

Introduction

Caviar is the main reason of Russian sturgeon rearing. Caviar production needs many handling procedures like sorting, sampling of gonad clippings for sex determination, small surgery procedures, etc. Anesthetics are used routinely for these procedures. From the other hand, the use of anesthetics involves a risk of undesirable drug residues in fish tissue. According to Pawar *et al*. (2011) there is a need for quickly metabolized anesthetics for aquaculture.

Clove oil is one of the most frequently used anesthetic for sturgeons and many other cultured fish species Eugenol (4-allyl-2-methoxy-phenol) is a major volatile component of clove oil (Kamatou *et al*. 2012). It is reported to have anti-bacterial activity (Karapmar and Aktug 1987, Briozzo *et al*. 1989), anti-fungal activity (Bullerman *et al*. 1977) and anti-oxidant capabilities (Gülçin *et al*. 2012). Clove oil has a broad spectrum of applications in human medicine. This drug is effective and cheap, and it is allowed food additive. However, clove oil has one important disadvantage; therapeutic index for this drug is as low as 2.5-2.7 (Velišek *et al*. 2005a, b). According to Guénette *et al*. (2007) the plasma half-life time of eugenol in rainbow trout was 12.1 h in 4°C of water temperature and authors suggest its potential tendency to accumulate in trout tissues.

Clove oil can cause some hypersensitivity symptoms in hatchery staff (skin irritation, nusea) (own observations). Buckley *et al*. (2002) found that 16.3% of health care workers and 39.39% metalworkers were allergic to eugenol due to frequent contact with eugenol containing cosmetics.

Propofol (2'6'diisopropylphenol) is a potent anesthetic used in both human and veterinary medicine. Propofol quickly induce general anaesthesia and is quickly metabolized in mammals. Propofol is considered as very safe and thus it is the most frequently used anesthetic for intravenous infusion in humans (Murthy 2008).

Propofol is a potentially good anesthetic for fish

(Fleming *et al*. 2003, Miller *et al*. 2005, Gomułka 2008). However, there is almost lack of reports on propofol influence on fish physiology. Gomułka et. al (2014) reported that propofol anaesthesia can reduce the negative impact of handling stress in European whitefish. The induction of anaesthesia is quick and accompanied by good myorelaxation. Progressive bradypnoe, down to the arrest of respiratory movement, can be observed during prolonged exposure or over dosage.

The aim of this study was the comparison of the effect of both propofol and clove oil on Russian sturgeon (*Acipenser gueldensaedtii*) by means of hematological and biochemical blood profile.

Materials and Methods

Fish and Drugs

Russian sturgeon juveniles were used for the experiment (n = 90). The mean length of fish was 31.5 ± 1.5 cm and mean weight was 116.3 ± 21.2 g. Fish were obtained from the Experimental Fish Hatchery "Dgał" (Polish Inland Fisheries Institute in Olsztyn, Poland). Till the experiment, fish were kept in 1.5 m^3 tank. Water temperature was 18.0 ± 0.1 °C. Fish were fed with commercial pellet feed (E-1p Stella, Skretting). Fish were starved 24 hours before the experiment.

Both propofol (>97%) and eugenol (>98%) were supplied by Sigma-Aldrich (USA). Stock solutions of propofol and eugenol in ethyl alcohol were prepared before the experiment, 25 mg ml^{-1} and 106 mg ml^{-1} respectively.

Experiment Protocol

Two procedures were applied. In the procedure I, fish were exposed to the anesthetic for 10 min and the blood was sampled immediately after the exposure. In this procedure, control fish were sampled without anaesthesia in less than 2 min after removal from the tank. In the procedure II, blood was sampled 24 h after 10 min of exposure. The control fish, in this procedure, were exposed to anaesthetic free water.

Each procedure involved 3 groups of fish (n = 15); control group (C), eugenol exposed group (E) and propofol exposed group (P). Depending on the procedure, each group was marked as "0" or "24".

The exposure to the anesthetic was done in 30 l tank. Anesthetic bath temperature was 18 ± 0.5 °C and oxygen saturation was over 80%. Anesthetic concentrations 10 mg l^{-1} and 42.4 mg l^{-1} were used for propofol and eugenol respectively. Above concentrations were chosen in preliminary test.

Blood was sampled with a syringe covered with heparin lithium salt from caudal vessels. Hematological indices were determined according to Svobodova *et al*. (1991) and covered hematocrit (PCV), red blood cell count (RBC), hemoglobin concentration (Hb), mean cell valume (MCV), mean cell hemoglobin concentration (MCHC) and mean cell hemoglobin content (MCH), differential white blood cell count (Leukogram).

Blood samples were centrifuged with StatSpin centrifuge for 30 s in 15800 rpm (12 000 x g). Biochemical indices were determined with VetTest Chemistry Analyzer (Idexx Lab., USA) and covered blood concentration of albumins (ALB), globulins (GLOB), total proteins (TP), ammonia (NH3), glucose (GLU), triacylglycerols (TRIG), calcium (CA), phosphates (PHOS) and activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP) and creatinine kinase (CK). All above parameters were determined in one run to avoid repeated thawing/freezing of the sample.

Statistic Analysis

Data were tested for the normal distribution using Shapiro-Wilk test and for the homogeneity of variance by Leven's test. Data which met assumptions of variance analysis were tested by ANOVA procedure and *post hoc* Tuckey test. Kruskal-Wallis ANOVA and Mann-Whitney U test were used for the others. Differences were considered as significant when $P<0.05$.

Arcsin transformation (Sokal and Rohlf 1969) was applied for leucogram data before the statistical analysis, according below formula:

$$t\,(p) = \arcsin\sqrt{p}$$

where: p means proportion of blood cell of given type.

Results

Both propofol and eugenol effectively induced anesthesia in Russian sturgeon. Fish were anesthetized within approximately 3 min. No mortalities was observed 24 h following the anaesthesia.

No significant differences in MCV, MCHC and MCH were found between experimental groups in both procedure. Significantly higher values of PCV and Hb were determined in sturgeons exposed to procedure I when compared to respective control (C0) (Table 1). After 24 h, PCV and Hb were recovered to initial values. RBC values were significantly lower 24 h after exposure in all groups subjected to procedure II when compared to C0 (Table 1).

No significant difference in leucogram was observed between anaesthetic exposed fish and respective control subjected to the procedure II . However, significant decrease of the percentage of lymphocytes was observed in E24 group when

compared to initial control (C0). Significant increase of the contribution of neutrophiles and significant decrease in case of eosinophiles was observed in all groups subjected to procedure II when compared to C0. The contribution of eosinophiles was also decreased in P0 group (Table 2).

PHOS concentration was increased ($P<0.05$) in sturgeon immediately following anaesthesia, however it was recovered within 24 h. CA concentration was elevated in P0 only and it was significantly lower in P24 fish when compared to both controls (Table 3).

Significant decrease of TP was found in P24 and E24 group when compared to C0, it resulted from significant decrease of GLOB in these groups.

Glucose level was significantly elevated in fish exposed to both anesthetics in procedure I. It was recovered in P24 group. TRIG was increased in all groups sampled 24 h after anesthesia, however, the increase was significant only in E24 fish when compared to C0. Significant increase in NH_3 was determined in the blood of E0 fish (Table 3).

In all tested fish ALT activity level was below the range of linearity of determination (>10 UI/L). No significant change in the activity of AST and CK was found in blood plasma. ALKP activity was significantly decreased in P24 fish when compared to C24.

Discussion

Exposures to both propofol and eugenol moderately affected the hematological and biochemical profile of blood of Russian sturgeon.

Sudden increase in the number of circulating erythrocytes is a result of spleen evacuation (Caldwell and Hinshaw 1994). Newly released cells can consist even 90% of RBC and may be released within several minutes (Pearson and Stevens, 1991) in response to stress. There was no significant increase of RBC immediately after exposure to both anesthetics, however, increase of Hb could result from RBC destruction which equalized RBC realize. Significantly lower RBC number in fish exposed to procedure II seems to support this hypothesis. The used Hb determination method measures all blood volume haemoglobin not specifically internal erythrocyte haemoglobin. The exposure of Siberian sturgeon to both MS-222 and eugenol resulted in erythrocyte swelling and destruction (Gomułka *et al*. 2008). The apparent growth of MCH value was observed in this study. Although no significant increase of MCV and MCH in anaesthetic exposed fish was observed in the present study, it seems that the same phenomena was observed, however, less sever.

Table 1 Hematological indices of Russian sturgeon exposed to both propofol and eugenol anaesthesia

Indicator	Group					
	procedure I			procedure II		
	Control 0	Eugenol 0	Propofol 0	Control 24	Eugenol 24	Propofol 24
PCV [l/l]	0.25±0.02 (0.21-0.28)	0.29±0.03* (0.25-0.33)	0.29±0.03* (0.24-0.35)	0.24±0.02 (0.2-0.28)	0.23±0.02 (0.2-0.28)	0.23±0.02 (0.2-0.29)
Hb [g/l]	49.4±6.2 (36.6-57.3)	58.6±4.2* (52.4-66.8)	58.2±5.9* (48.7-69.6)	46.5±5.5 (38.4-58.2)	50.1±8.6 (37.2-63.7)	45±5.2* (38.4-56.1)
RBC [T/l]	0.45±0.08 (0.32-0.6)	0.46±0.03 (0.41-0.49)	0.47±0.03 (0.41-0.54)	0.40±0.03* (0.34-0.46)	0.40±0.05* (0.32-0.48)	0.37±0.03*# (0.31-0.42)
MCV	565±103 (420-781)	647±68 (544-774)	631±85 (500-793)	600±70 (533-750)	588±80 (456-734)	627±67 (538-784)
MCHC	0.20±0.01 (0.17-0.22)	0.20±0.02 (0.17-0.24)	0.20±0.02 (0.17-0.23)	0.20±0.02 (0.15-0.25)	0.21±0.02 (0.18-0.25)	0.20±0.02 (0.17-0.22)
MCH	112±22 (82-165)	129±12 (109-152)	125±13 (103-145)	118±17 (96-150)	126±24 (87-179)	123±14 (101-151)

Results are presented as mean ± SD (range). Asterisk * indicates the result significantly different ($P<0.05$) from Control 0 group; and hash # indicates results significantly different from Control 24 group.

Table 2 Leucogram of Russian sturgeon exposed to both propofol and eugenol anaesthesia

White blood cell type	Group					
	Procedure I			Procedure II		
	Control 0	Eugenol 0	Propofol 0	Control 24	Eugenol 24	Propofol 24
Lymphocytes	0.67±0.02 (0.23-0.84)	0.69±0.02 (0.41-0.86)	0.69±0.01 (0.49-0.9)	0.59±0.01 (0.43-0.81)	0.51±0.02* (0.31-0.77)	0.64±0.01 (0.53-0.74)
Monocytes	0±0 (0-0.01)	0±0 (0-0)	0±0 (0-0)	0±0.00 (0-0.01)	0±0 (0-0.01)	0±0 (0-0.01)
Myelocytes	0.01±0.00 (0-0.02)	0±0.00 (0-0.01)	0±0.00* (0-0.01)	0.01±0.00 (0-0.03)	0.01±0.00 (0-0.03)	0±0.00 (0-0.01)
Neutrophiles	0.14±0.02 (0-0.32)	0.18±0.01 (0.04-0.41)	0.23±0.021 (0.06-0.48)	0.33±0.02* (0.13-0.51)	0.43±0.024* (0.15-0.66)	0.31±0.01* (0.15-0.45)
Eosinophiles	0.16±0.041 (0.05-0.77)	0.12±0.01 (0.06-0.31)	0.06±0.01* (0.02-0.18)	0.06±0.01* (0.01-0.19)	0.04±0.01* (0-0.08)	0.04±0.00* (0.01-0.13)

Values are presented as mean±SD (range). Asterisk indicates the result significantly different ($P<0.05$) from the Control 0 group.

Table 3 Biochemical blood profile of Russian sturgeon exposed to both propofol and eugenol anaesthesia.

Indicator	Group					
	Procedure I			Procedure II		
	Control 0	Eugenol 0	Propofol 0	Control 24	Eugenol 24	Propofol 24
PHOS [mmol/l]	2.45±0.26 (2.00-2.97)	2.81±0.53* (1.84-3.52)	2.94±0.56* (2.00-3.84)	2.26±0.34 (1.61-2.77)	2.43±0.41 (1.81-3.03)	2.36±0.41 (1.71-3.16)
CA [mmol/l]	1.83±0.13 (1.63-2.05)	1.81±0.15 (1.53-2.03)	2.11±0.32* (1.75-2.65)	1.91±0.39 (1.18-2.60)	1.88±0.28 (1.45-2.40)	1.68±0.28*# (1.20-2.45)
TP [g/l]	21.6±6.4 (13-35)	23.4±6.0 (13-34)	20.6±5.5 (10-31)	18.1±5.8 (9-30)	16.9±5.0* (9-25)	15.8±4.7* (9-26)
ALB [g/l]	1.67±1.84 (0-5)	2.20±1.47 (0-4)	2.33±1.54 (0-5)	2.40±2.5 (0-8)	1.73±1.79 (0-5)	1.33±1.35 (0-4)
GLOB [g/l]	19.8±4.8 (13-30)	21.2±4.8 (13-30)	18.2±4.1 (10-26)	15.9±4 (9-24)	15.2±3.6* (9-21)	14.4±3.5* (9-22)
NH3 [µmol/l]	121±28 (77-170)	162 ±30* (126-224)	148±32 (93-214)	115 ±34 (71-193)	129 ±36 (71-191)	110±35 (72-195)
TRIG [mmol/l]	1.77±0.83 (0.60-3.77)	1.83 ±0.65 (1.00-3.61)	2.64±0.78 (1.46-4.22)	2.69±1.21 (0.60-4.22)	2.93±0.89* (1.31-4.22)	2.34±0.79 (1.05-4.03)
GLU [mmol/l]	2.57 ±0.50 (1.72-3.50)	3.59±0.50* (2.89-4.72)	3.84±0.45* (3.22-4.94)	3.22±0.60* (2.44-4.56)	3.14 ±0.49* (2.33-4.00)	2.93±0.54 (2.11-4.11)
CK [U/L]	408 ±397 (4-1427)	275±235 (26-772)	321±244 (31-827)	302 ±309 (11-1190)	207 ±166 (49-597)	294 ±218 (78-777)
AST [U/L]	198±77 (88-389)	204±89 (90-361)	200±81 (80-347)	257±96 (87-475)	270 ±187 (117-899)	253±93 (120-406)
ALKP [U/L]	173 ±167 (85-738)	187±112 (72-418)	183±143 (78-638)	269 ±238 (89-893)	171±98 (55-404)	122±69 # (53-332)

Values are presented as mean±SD (range). Asterisk * indicates the result significantly different (P<0.05) from Control 0 group; and hash # indicates results significantly different from Control 24 group.

According to Ainsworth *et al*. (1991), acute stress in fish is usually followed by a decrease of the percentage of lymphocytes and eosinophiles and an increase in neutrophiles contribution in circulating blood. Cortisol, secreted during stress reaction, shortens the life span of lymphocytes and promotes their apoptosis (Wyets *et al*. 1998, Verburg van Kemenade 1999). Thus a decreased lymphocyte count is often observed effect of stress. Eugenol anesthesia in Russian sturgeon was followed by significant decrease of percentage of lymphocytes when compared to C0. Similar decrease in lymphocyte contribution was observed in European catfish *Silurus glanis* following clove oil anaesthesia (Velišek *et al*. 2006). However, no effect of clove oil anaesthesia on lymphocyte count was found in Siberian sturgeon, rainbow trout and common carp (Gomułka *et al*. 2008, Velišek *et al*. 2005 a,b).

Glucose is considered as the main source of energy for fish cells and rapid increase of blood glucose follows acute stress in fish (Barton 2002). Both eugenol and propofol exposures were followed by immediate significant increase of blood glucose. However, in procedure II, control and eugenol anesthetized sturgeon also revealed increased glucose level. Moreover, it was accompanied by increased level of TRIG (Table 3) in E24 fish. TRIG are synthesized from carbohydrates in liver and stored in fat tissue as an energy source (Tocher 2003). Bayea *et al*. (2006) and Gomułka *et al*. (2008) suggest that hiperlipidemia is an alternative pathway of energy stores mobilization in sturgeons under stress conditions. Increased TRIG and unchanged blood glucose were found in Siberian sturgeon (Gomułka *et al*. 2008) and European catfish (Velišek *et al*. 2006) exposed to clove oil. Unchanged TRIG level was found in rainbow trout and common carp anesthetized with both eugenol and 2-phenoxyetanol (Velišek *et al*. 2004a, 2005a,b, Velišek and Svobodova 2004b).

Increased level of blood ammonia can be attributed to protein catabolism and gluconeogenesis which is activated to meet the demand for glucose in response to stress. However, no significant decrease of protein was observed at the same time. From the other hand, the increase of ammonia level can also resulted from respiratory acidosis and subsequent difficulties in ammonia excretion.

Increased level of PHOS and CA following anesthesia can be attributed to acute respiratory acidosis, and opposite, the decrease in both indices is observed in case of respiratory alkalosis (Ghosh and Joshi 2008). Exposure to both anesthetics was followed by hyperphosphatemia in Russian sturgeon which was recovered within 24 h. Similar pattern was found for CA in sturgeons exposed to propofol, however, CA level was significantly lower 24 h after anesthesia when compared to both controls. In this group of fish ALKP activity was also decreased.

Unchanged activity of AST, ALT and CK reflects no tissue damage following both propofol and eugenol anaesthesia.

Conclusions

Eugenol anesthesia caused more severe physiological disturbances in Russian sturgeon when compared to propofol anesthesia. We can recommend propofol as a save drug for inducing general anesthesia in Russian sturgeon.

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