Journal of Animal and Veterinary Advances 9 (14): 1925-1933, 2010
ISSN: 1680-5593

The Effects of Natural (Clove Oil) and Synthetical (2-phenoxyethanol) Anesthesia Substances on Hematology Parameters of Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta fario*)

Arzu Ucar and Muhammed Atamanalp
Department of Fisheries, Faculty of Aquaculture Enginering, Ataturk University, Erzurum, Turkey

Abstract: In the study in which the effects of clove oil and phenoxyethanol in fish farming are examined, the most cultured rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*) are used as material. As a result of the treatment, blood biochemical hematology and enxyme activity values are studied. The interaction of treatment, species and treatment species are found insignificant among hematological parameters; hemoglobin, hematocrite, erythrocyte, average hemoglobin amount per erythrocyte (MCH), average volume of erythrocyte (MCV) and average hemoglobin concentration per erythrocyte (MCHC) ($p>0.05$). Although, leukocyte values are significantly ($p<0.01$) effected by treatment and treatment species interaction the effect of species is insignificant. While, the effect of treatment and species are insignificant in erythrocyte sedimentation ratio compared with control group, the interaction between treatment and species is significant ($p<0.05$). While, the effect of treatment and species on the number of thrombocyte is significant, the effect of treatment x type interaction is detected to be insignificant.

Key words: Clove oil, 2-phenoxyethanol, blood, hematology, rainbow trout, brown trout

INTRODUCTION

Anesthetics are physical or chemical agents that act on an animal by initially inducing a calming effect and subsequently inducing loss of equilibrium, mobility, consciousness and reflex action (Summer and Smith, 1990). The welfare of fish has been the focus of less research than that of higher vertebrates such as birds and mammals (Braithwaite and Huntingford, 2004).

An ideal anesthetic should induce anesthesia rapidly with minimum hyperactivity or stress. It should be easy to administer and should maintain the animal in the chosen state. When the animal is removed from the anesthetic, recovery should be rapid. The anesthetic should be effective at low doses and the toxic dose should greatly exceed the effective dose so that there is a wide margin of safety (Coyle *et al*., 2004).

Clove oil is a dark-brown liquid, a distillate of flowers, stalks and leaves of the clove tree *Eugenia aromatica* (Sato and Burhanuddin, 1995). According to Isaacs (1983); Briozzo *et al*. (1989) and Keene *et al*. (1998), clove oil is distilled from stems, leaves and flower buds of *Eugenia caryophyllata* and its active ingredient, i.e., eugenol (4-allyl-2-methoxyphenol), makes up 70-90% by weight of clove oil. Clove oil also contains eugenol acetate (>17%) and kariofilen 5 (>12%).

Clove oil is highly effective even at low doses. Keene *et al*. (1998) report that it induced anaesthesia faster and at lower concentrations than MS-222 while Munday and Wilson (1997) found clove oil only marginally less effective than quinaldine and more effective than 3 other chemicals, except at high doses. Clove oil provides a much calmer induction to anaesthesia than the other chemicals. Recovery time after clove oil anaesthesia is substantially longer than recovery time from other anaesthetics.

Clove oil is much less expensive than other chemicals. For example, Keene *et al*. (1998) showed clove oil to be <1.15 the price of MS-222 when preparing solutions of each capable of inducing stage 5 anaesthesia in <3 min in rainbow trout.

At present, 2-phenoxyethanol is used in the Czech Republic for short-term immobilization of fish before artificial spawning and whenever fish is handled outside water. The generally recommended concentration is 0.20 mL of water bath. For big breeding fish, the recommended concentration is 0.30 mL^{-1} of water bath. At the recommended concentrations, anaesthesia is induced within 5-10 min. When transferred to clean water, fish will recover within 10 min (Svoboda and Kolarova, 1999; Hamackova *et al*. 2001). 2-Phenoxyethanol is an opaque, oily liquid. This drug is moderately soluble in water but

Corresponding Author: Arzu Ucar, Department of Fisheries, Faculty of Aquaculture Enginering, Ataturk University, Erzurum, Turkey

Table 1: Stages of anesthesia and recovery

Stages of anesthesia	Description
I	Loss of equilibrium
II	Loss of gross body movements but with continued opercular movements
III	As in Stage II with cessation of opercular movements
Stages of recovery	
I	Body immobilized but opercular movements just starting
II	Regular opercular movements and gross body movements beginning
III	Equilibrium regained and preanesthetic appearance (Iwama *et al*., 1989)

freely soluble in ethanol. The solution is bactericidal and fungicidal and is therefore, useful during surgery. It is relatively inexpensive and remains active in the diluted state for at least 3 days. About 2-phenoxyethanol has a relatively large margrin of safety and has been reported to produce a range of effects from light sedation to surgical anesthesia at concentrations of 100-600 mg L^{-1}. Concentrations of 300-400 mg L^{-1} are useful for short procedures and lower concentrations of 100-200 mg L^{-1} are considered safe for prolonged sedation such as during transport. About 2-phenoxyethanol is not approved by FDA for use on food fish in the U. S (Coyle *et al*., 2004).

Because the efficacy of most anesthetics are affected by species, body size, the density of fish in the bath as well as water quality (e.g., hardness, temperature or salinity), it is imperative that preliminary tests be performed with small numbers of the fish to determine the optimal dosage and exposure time. Due care should be taken to control the level of anesthesia desired, through the application of the appropriate concentration and to maintain constant observation of the fish as they go through the various stages of anesthesia (Table 1).

Since hematological parameters reflect the poor condition of fish more quickly than other commonly measured parameters and since they respond quickly to changes in environmental conditions (Atkinson and Judd, 1978; Atamanalp and Yanik, 2003), they have been widely used for the description of healthy fish (Blaxhall, 1972) for monitoring stress responses (Soivio and Oikari, 1976) and for predicting systematic relationships and the physiological adaptations of animals. The aim of the study was to investigate acute toxicity of clove oil and 2-phenoxyethanol for brown trout and rainbow trout using values of haematology profiles of blood to assess the effects of the fish exposure to the anaesthetics.

MATERIALS AND METHODS

The active substance of 2-phenoxyethanol is ethylene glycol monophenyl ether. Its summary formula is $C_8H_{10}O_2$, the molar weight 138.17 g•l-1, density 1.107-1.108 g•dm-3, peroxide content <0.005% and the boiling temperature is 245°C. The anaesthetic is slightly soluble in water (26.7 g•l-1) at 25°C but readily soluble in ethanol. The anaesthetic affects fish through the skin and gills. A group of 48 fish with an average weight of 180±25 g, reared in well water with a constant temperature of 8.5°C at the farm located at the Research and Extension Center in Ataturk University was transferred to the Central Laboratory in the Aquarium Fish Rearing Facility and was exposed to a anesthesia dose of 0.5 ppm of clove oil and 0.2 ppm of 2-phenoxyethanol 4 weeks in circular fiberglass tanks 780l in volume (100 cm diameter and 100 cm depth) under natural light conditions with a constant flow (1.5 l min^{-1}) of aerated dechlorinated tap water at 9-11°C and with no recirculation. The dissolved oxygen and pH levels and total water hardness were 8-9 ppm, 7.8 and 102 mg in $CaCO_3$, respectively. The tanks were aerated with an air pump. Eight fish were placed into each of 6 tanks, one for testing the clove oil, 2-phenoxyethanol and the other as the control. At the end of each day exposure, 8 fish from the control tank, 8 fish from the clove oil treatment tank and 8 fish from the 2-phenoxyethanol tank (Ahmad *et al*., 1995; Shakoori *et al*., 1996) were taken out and their blood was subjected to hematological analysis (Aziz *et al*., 1993; Shakoori *et al*., 1996; Santhakumar *et al*., 1999; Atamanalp *et al*., 2002). Approximately 2 cc venous blood was drawn from each subject for the determination of hematological parameters using heparin as an anticoagulant and for the estimation of the Red Blood Cell (RBC) count (Blaxhall and Daisley, 1973; Atamanalp *et al*., 2002), the total White Blood Cell (WBC) count (Blaxhall and Daisley, 1973; Atamanalp *et al*., 2002), the Hemoglobin (Hb) concentration (Kocabatmaz and Ekingen, 1984) and the Packed Cell Volume (PCV) (Schalm *et al*., 1975) whereas the Mean Corpuscular Volume (MCV), the Mean Corpuscular Hemoglobin (MCH) and the Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated according to Reddy and Bashamohideen (1989).

Statistical analysis: The results are presented as means ±SD. Differences between parameters were analyzed by one-way Analysis Of Variance (ANOVA) and significant means were subjected to a multiple comparison test (Duncan) at the $\alpha = 0.05$ level (26).

RESULTS AND DISCUSSION

Hematological parameters: Effects of clove oil and 2-phenoxyethanol anaesthesia on haematological indices on rainbow trout and brown trout as shown in Table 2.

Table 2: Effects of clove oil and 2-phenoxyethanol anaesthesia on hematological indices on rainbow trout and brown trout

		Trial		Species		Significant		
Parameters	Control	2-Phenoxyethanol	Clove oil	Brown trout	Rainbow trout	Trial	Species	TxS
RBC	0.68±0.0210	0.63±0.016	0.68±0.0210	0.64±0.016	0.67±0.0150	NS	NS	NS
WBC	3.950±0.200^{b}	4.089±0.15^{b}	5.083±0.200^{a}	4.228±0.15^{b}	4.521±0.150^{b}	**	NS	**
Plt	1.017±0.065^{a}	0.82±0.050^{ab}	0.767±0.065^{b}	0.79±0.051^{a}	0.952±0.048^{b}	*	*	NS
Hb	6.75±0.5200	5.85±0.320	6.033±0.420	5.903±0.36	6.52±0.3400	NS	NS	NS
Hct	21.25±1.4000	19.57±0.870	21.00±1.1400	21.50±0.970	19.71±0.9200	NS	NS	NS
MCV	319.48±28.430	314.88±17.82	310.80±23.210	339.29±19.72	290.82±18.720	NS	NS	NS
MCH	100.83±8.2700	93.92±5.180	88.51±6.7500	92.89±5.740	95.94±5.4500	NS	NS	NS
MCHC	32.048±2.980	30.46±1.870	28.82±2.4300	27.78±2.070	33.105±1.967	NS	NS	NS
ESR	2.37±0.1600^{b}	2.40±0.100^{b}	2.32±0.1300^{ab}	2.28±0.110^{a}	2.45±0.1100^{a}	NS	NS	*

*p<0.05; **p<0.01

The number of erythrocyte (RBC): Average number of erythrocyte in the blood samples of brown trout and rainbow trout in the control group are detected to be 0.649×10^{6} mm^{-3}, 0.703×10^{6} mm^{-3}. This value shows compatibility with the value (min 0.538×10^{6} mm^{-3}, max 1.185×10^{6} mm^{-3} ort 0.782×10^{6} mm^{-3}) Kocabatmaz and Ekingen (1984) and Atamanalp *et al.* (2002) have expressed for a healthy rainbow trout. Ezzat *et al.* (1974) reported this value to be 1.8×10^{6} mm^{-3} in *Tilapia zilli*, Satake *et al.* (1986) to be between 0.66-2.01 $\times10^{6}$ mm^{-3} in armoured cat fish (*Hypostomus paulinus*), Favaretto *et al.* (1978) to be 0.69×10^{6} mm^{-3} in cultured *Hypostomus regani*, Satake *et al.* (1986) to be 1.04×10^{6} mm^{-3} wild *Hypostomus regain*, Torres *et al.* (1986) to be 1.00×10^{6} mm^{-3} in *Hypostomus punctatus*, Santhakumar *et al.* (1999) to be 4.09×10^{6} mm^{-3} in *Anabas testudineus*.

Average number of erythrocyte in rainbow trout and brown trout which are 2-phenoxyethanol anesthetics are found to be 0.608±0.04 × 10^{6}, $0.641\pm0.03\times10^{6}$ and $0.716\pm 0.05\times10^{6}$ mm^{-3} and $0.657\pm 0.07\times10^{6}$ mm^{-3} in rainbow trout and brown trout which are applied with clove oil. According to the results, 2-fenoksietanol decreased the number of erythrocyte in rainbow trout and brown trout but clove oil increased.

Velisek *et al.* (2005) detected the erythrocyte values of rainbow trout which is applied with clove oil as $1.28\pm0.26\times10^{6}$ mm^{-3} right after the anesthetics and as $1.16\pm0.25\times10^{6}$ mm^{-3} 24 h later. The value detected right after the anesthetics is lower compared with control group; values taken 24 h later are higher than control group 2-fenoksietanol application decreasing the number of erythrocyte in carp shows parallelism with the current study.

No significant increases are observed in RBC number, Hb value and Hct ratio of scald fish (*Scophthalmus aquosus*) from Scophthalmidae family that are exposed to chemicals when compared with control group. It is reported that in the study carried out with bull head (*Siluris glanis*) there is an increase in the number of erythrocyte right after and 24 h later the anesthetics with clove oil (Velisek *et al*., 2006). This situation calls complication with both the results and literature results as well. This complexity can be explained with the species of fish being different in the studies.

The number of leukocyte (WBC): Average number of leukocyte in the blood samples of brown trout and rainbow trout in the control group are detected to be $4.033\pm0.30\times10^{4}$ mm^{-3}, $3.867\pm0.25\times10^{4}$ mm^{-3}. This value shows suitability with the value (min 3.0×10^{4} mm^{-3}, max 6.5×10^{4} mm^{-3} and avr. 4.6×10^{4} mm^{-3}) Kocabatmaz and Ekingen (1984) and Atamanalp *et al.* (2002) have expressed for a healthy rainbow trout (*Oncorhynchus mykiss*).

WBC change according to anesthetic materials applied is $4.350\pm0.38\times10^{4}$ mm^{-3} in 2-phenoxyethanol applied brown trout, $3.829\pm0.35\times10^{4}$ mm^{-3} in rainbow trout, $4.300\pm1.01\times10^{4}$ mm^{-3} in clove oil applied brown trout. According to these results it is detected that the number of leukocyte increased in brown trout as a result of phenoxyethanol anesthetics, decreased in rainbow trout treated with only phenoxyethanol.

From the average results gathered from study, it is detected that the difference between control group and clove oil and phenoxyethanol groups and the difference among groups do not have significance, however the effect of clove oil on rainbow trout is found to be significant.

Velisek *et al.* (2005) detected the erythrocyte values of rainbow trout as $23.70\pm4.69\times10^{4}$ mm^{-3} right after the anesthetics and as $19.60\pm9.96\times10^{4}$ mm^{-3} 24 h later. No significant change is observed in the hematological profile of carp and rainbow trout which is anesthetized with clove oil. Significant amount of leukocyte decrease is detected right after and 24 h later the treatment with clove oil in bullhead (*Siluris glanis*) (Velisek *et al*., 2006).

In their studies Noyan (1980); Roberts (1989); Yilmaz and Otlu (1989) and Isoda and Fujimaki (1990) expressed that there is a decrease in the number of leukocytes in sick fish which are exposed to any kind of infection and increase related with defend in leukocyte cells. While there is no change in rainbow trout right after the

anesthesia, an increase is detected 24 h later (Velisek *et al*., 2005). In the analyses carried out right after the anesthesia as a result of the treatment with 2 phenoxyethanol in carp and rainbow trout, significant increase is reported in the amount of leukocyte (Velisek *et al*., 2007).

The number of WBC detected in the study is lower than the value of Atamanalp and Yanik (2003) detected for the rainbow trout (*Oncorhynchus mykiss*), for Israeli bream (*Oreochromis niloticus*), Kocabatmaz and Ekingen (1984) for abant trout (*Salmo trutta abanticus*), Quentel and Obach (1992) for turbot (*Scophthalmus maximus*), higher than the value Blaxhall and Daisley (1973) found for brown trout (*Salmo trutta*), Das and Mukherjee (2003) for Indian carp (*Labeo rahita*), Hasiloglu and Atamanalp (2002) for chub (*Leuciscus cephalus*), Van Vuren and Hattingh (1978) for carp (*Cyprinus carpio*). The value is found similar to the value Kocabatmaz and Ekingen (1984) found for rainbow trout.

The number of trombocytes (Plt): Erythrocyte values of $0.967\pm0.20\times10^4$ mm^{-3} and $1.067\pm0.15\times10^4$ mm^{-3} detected for brown trout and rainbow trout in control group show compatibility with the average values (min 0.4×10^4 mm^{-3}. max 2.41×10^4 mm^{-3} and avr. 0.94×10^4 mm^{-3}) Kocabatmaz and Ekingen (1984) and Atamanalp *et al*. (2002) expressed. It is also expressed by the researchers that trombocyte values are effected significantly by the stress and the value of 2.1×10^4 mm^{-3} before stress increased to 4.3×10^4 mm^{-3} in rainbow trout after stress. Satake *et al*. (1986) expressed that this value is$1.657\pm0.341\times10^4$ mm^{-3} for armored catfish (*Hypostomus pulinus*).

For the fish in the group of 2-phenoxyethanol anesthetics applied; trombocyte values are found to be $0.700\pm0.08\times10^4$ mm^{-3} in brown trout. While, $0.957\pm0.20\times10^4$ mm^{-3} in rainbow trout. Here, $0.700\pm0.10\times10^4$ mm^{-3} in brown trout treated with clove oil and $0.833\pm0.05\times10^4$ mm^{-3} in rainbow trout.

As a result of the study it is detected that the number of trombocyte decreased in both species of fish at the end of the treatment. As a result of the statistical analysis carried out; it is detected that the difference between treatment and species are significant and treatment x type interaction is insignificant. In the study about the number of trombocyte, Lester and Budd (1983) detected the number of trombocyte as 26,103 mm^3 in coho salmons, Casillas and Smith (1977) detected it as 21.103 mm^3 before the stress and as 43.103 mm^{-3} after the stress in rainbow trout. Velisek *et al*. (2006) reported that the change of trombocyte numbers in bullhead (*Siluris glanis*) is not significant after being exposed to 30 mg L^{-1}. clove oil concentration.

MCV: Average MCV value is detected to be 377.965 ± 42.23 μm^3 in control group brown trout and 261.010 ± 3.54 μm^3 in rainbow trout. As a result of the statistical analysis treatment, species and treatment x type interaction is found to be insignificant. MCV value is found to be 313.398 ± 67.58 μm^3, 316.66 ± 45.56 μm^3 in 2-phenoxyethanol anesthetics applied brown trout and rainbow trout and 326.517 ± 90.12 μm^3, 295.100 ± 46.34 μm^3 in clove oil applied brown trout and rainbow trout.

Compared with the control group while there is a decrease in MCV values in brown trout applied with 2-phenoxyethanol and clove oil there is an increase in rainbow trout. In their study with clove oil; Velisek *et al*. (2006) detected MCV values to be 344.05 ± 57.67 μm^3 right after the anesthetics and 339.79 ± 56.96 μm^3 24 h later in bullhead (*Siluris glanis*). Compared with the control; there is an increase in the measurements right after the anesthetics and 24 h later the anesthetics. After the anesthetics with 2-phenoxyethanol, there is an increase in MCV values in carp and rainbow trout compared with the control group (Velisek *et al*., 2007). This situation can be explained with the species of fish and anesthetic materials being different in the studies.

Velisek *et al*. (2005) reported that there is a decrease in MCV values of rainbow trout after treatment with clove oil. This result shows incompatibility with current findings.

MCV value obtained in this study is higher than the values found for (*Pagrus auratus*) by Canfield *et al*. (1994), chub (*Leuciscus cephalus*) found by Hasiloglu and Atamanalp (2002); Martinez *et al*. (1994) for rainbow trout (*Oncorhynchus mykiss*), Mughal *et al*. (1993) and Shakoori *et al*. (1996) for grass carp (*Ctenopharyngodon idella*), Sandnes *et al*. (1988) for salmon (*Salmo salar*), Pages *et al*. (1995) for bream (*Sparus aurata*), Schutt *et al*. (1997) for swordtail fish (*Xiphophorus helleri*), Yamawaki *et al*. (1986) and Van Vuren and Hattingh (1978) for carp (*Cyprinus carpio*), Quentel and Obach (1992) for turbot (*Scophthalmus maximus*), Yone *et al*. (1986) for common seabream (*Chrysophrys major*); lower than the value Atamanalp *et al*. (2002) found for rainbow trout (*Oncorhynchus mykiss*).

MCH: Average MCH value is detected to be 111.730 ± 8.089 pg in control group brown trout and 89.930 ± 5.572 pg in rainbow trout. No significant differences are detected either between treatment and species or among the groups. Compared with the control group while there is a decrease in MCH values in brown trout applied with 2-phenoxyethanol and clove oil there is an increase in rainbow trout. Velisek *et al*. (2006) detected MCH values to be 49.15 ± 11.55 pg right after the

anesthetics and 51.06±9 pg 24 h later in rainbow trout after clove oil treatment. Compared with the control while there is an increase in the values right after the anesthetics, there is decrease 24 h later. In the studies carried out with carp there is an increase in the values right after the anesthetics and 24 h later the anesthetics. 45.60±4.24 and 5.25±6.28 pg values are detected in the treatments with 2-phenoxyethanol in rainbow trout and carps. It is reported that there is an increase of values compared with control group in both situations (Velisek *et al*., 2007).

As a result of the treatment with clove oil in bullhead (*Siluris glanis*), there is a decrease compared with control group and 24 h later there is an increase compared with the control group (Velisek *et al*., 2006). Average MCH value obtained in this study is higher than the values of Atamanalp *et al.* (2002) found for rainbow trout (*Oncorhynchus mykiss*), Hasiloglu and Atamanalp (2002) for chub (*Leuciscus cephalus*), Mughal *et al.* (1993) for grass carp (*Ctenopharyngodon idella*), Pages *et al.* (1995) for bream (*Sparus aurata*), Quentel and Obach (1992) for turbot (*Scophthalmus maximus*), Sandnes *et al.* (1988) for salmon (*Salmo salar*) and Yamawaki *et al.* (1986) for carp (*Cyprinus carpio*).

MCHC: Average MCH value is calculated as 29.625±1.66 g/100 mL in control group brown trout and 34.470±2.602 g/100 mL in rainbow trout. Although, the difference among species is significant in the sense of average MCHC values, treatment and treatment species interaction is found to be insignificant. This value is higher than the value (20.5-28.4 g/100 mL) Kocabatmaz and Ekingen (1984) and Atamanalp *et al.* (2002) found for healthy trout.

When MCHC values are observed it is detected that there is decrease in both the treatment groups and fish species. Velisek *et al.* (2006) calculated MCHC values to be 141.71±10.79 g/100 mL right after the anesthetics and 150.46±15.87 g/100 mL 24 h later in rainbow trout. There is a significant decrease in MCHC value after anesthetics with clove oil in fishcat. It is reported that the value right after the anesthetics is 236.99±6.96 g/100 mL in rainbow trout treated with 2-phenoxyethanol and 269.14±16.02 g/100 24 h later the treatment; and 139.56±15.09 g/100 mL and 141.66±13.74 g/100 mL, respectively in carp. These results are different from the results of the study.

Average MCHC value obtained in this study is higher than the values of found for rainbow trout (*Oncorhynchus mykiss*), Hasiloglu and Atamanalp (2002) for chub (*Leuciscus cephalus*), Mughal *et al.* (1993) for grass carp (*Ctenopharyngodon idella*), Pages *et al.* (1995) for bream (*Sparus aurata*), Quentel and Obach (1992) for turbot (*Scophthalmus maximus*), Sandnes *et al.* (1988) for salmon (*Salmo salar*) and Yamawaki *et al.* (1986) for carp (*Cyprinus carpio*), lower than the value found for turbot (*Scophthalmus maximus*) by Quentell and Obach (1992).

It reported that the change of hematological parameters among fish are influenced by the fish species as well as season and temperature, reproduction period and nutrition condition, size and weight of the fish, age, moreover salinity of the water, disease, toxic materials, heavy metal and industrial wastes and stress factors.

Hemoglobin: Average hemoglobin value is calculated as 6.950±0.35 g/100 mL in control group brown trout and 6.550±0.49 g/100 mL in rainbow trout. The difference among groups is not significant in the sense of average hemoglobin value. This value is between the value Kocabatmaz and Ekingen (1984) and Atamanalp *et al.* (2002) have reported (min. 4.3 max. 10.9 and avr. 6.5) for a healthy truot.

In this study the highest level is detected in clove oil treated brown trout (5.033±0.23 g/100 mL) and the lowest value is detected on clove oil treated rainbow trout (7.033±2.33 g/100 mL). These findings show that rainbow trout and brown trout give different reactions to clove oil. While the hemoglobin value increase for clove oil treated rainbow trout there is no change in other treatments.

In order to detect the effects of infection on blood parameters of fish which is thought to be a stress factor; carp (*Cyprinus carpio*) is anesthetized (25% phenoxyethanol) and analyzed by taking blood samples. According to the values obtained, it is seen that hemoglobin values have increased (7.47+0.35 g dL^{-1}) compared with the control group (5.16+0.20 g dL^{-1}).

Goel *et al.* (1981) have carried out comparative hematological studies on some of the India fresh-water fish and observed the changes of hematological parameters of fish related to environmental effects. According to their findings with the decrease of oxygen content of water, erythrocyte and hemoglobin amount increase in blood.

Average Hb value obtained in this study is lower than the values of Canfield *et al.* (1994) for sea bass (*Pagrus auratus*), Martinez *et al.* (1994) for rainbow trout (*Oncorhynchus mykiss*), Hasiloglu and Atamanalp (2002) for chub (*Leuciscus cephalus*), Sandnes *et al.* (1988) for salmon (*Salmo salar*) and Yamawaki *et al.* (1986) for carp (*Cyprinus carpio*), similar to the values of (Kocabatmaz and Ekingen, 1984) for Abant trout (*Salmo trutta abanticus*), Pages *et al.* (1995) for bream (*Sparus aurata*), Van Vuren and Hattingh (1978) for swordtail fish (*Xiphophorus helleri*), carp (*Cyprinus carpio*). The value is higher than the value of

Atamanalp *et al.* (2002) found for rainbow trout (*Oncorhynchus mykiss*), Azizoglu and Cengizler for Israeli bream (*Oreochromis niloticus*), Blaxhall and Daisley (1973) for brown trout (*Salmo trutta*), Dawson for scaldfish (*Scophthalmus aquasus*), Lie *et al.* (1989) for gadoid fish (*Gadus morhua*), Martinez *et al.* (1994) for grass carp (*Ctenopharyngodon idella*) and turbot (*Scophthalmus maximus*) by Quentel and Obach (1992).

Haematocrit: Average haematocrit percentages are calculated as 23.50±2.121 in control group brown trout and 19.00±2.04 in rainbow trout. These values show compatibility with the value (Min. 19.0. Max. 41.3 and avr. 28.0) Kocabatmaz and Ekingen (1984) and Atamanalp *et al.* (2002) found for healthy trout. This value is calculated as 20.00±3.916 for phenoxyethanol treated brown trout and 19.14±2.34 for rainbow trout. Haematocrit levels are found to be 21.00±3.606 for clove oil treated salmo fontinalic and 21.00±2.00 for rainbow trout. Haematocrit value is not influenced from species, treatment and treatment x type interaction.

Although, both the anesthetics treatments increase haematocrit level in rainbow trout compared with control group, this ratio is too low for brown trout. Although, it is known that HT ratio changes according to the species of fish; average Ht ratio obtained in this study is lower than the values of Kocabatmaz and Ekingen (1984) and Atamanalp *et al.* (2002) for rainbow trout (*Oncorhynchus mykiss*), Chen *et al.* (2003) for Israeli bream (*Oreochromis niloticus*) for salmon (*salmo salar*), Blaxhall and Daisley (1973) for brown trout (*Salmo trutta*), Canfield *et al.* (1994) for sea bass (*Pagrus auratus*), Kocabatmaz and Ekingen (1984) for Abant trout (*Salmo trutta abanticus*), Pages *et al.* (1995) for bream (*Sparus aurata*), Van Vuren and Hattingh (1978) for swordtail fish (*Xiphophorus helleri*), Yone *et al.* (1986) for common sea bream (*Chrysophrys major*). These values are similar to the values of Dawson for turbot (*Scophthalmus aquasus*) and Nelson *et al.* (1996) for gadoid fish (*Gadus morhua*).

In the studies carried out with brown trout (*Salmo trutta*), garyling (*Thymallus thymallus*), carp (*Condrostoma nasus*), gray mullet (*Leusiscus cephalus*), barbus barbus (*Barbus barbus*) in natural environment, it is detected that hematological parameters such as haematocrit, hemoglobin values and number erythrocyte change due to biotic and abiotic factors, individual sexual differences and reproduction cycle is effective in this (Luskova, 1997). It is asserted that sex, healthiness and acclimation have effects on blood parameters and must be taken into regard while evaluating the hematological parameters of the species (Gabriel *et al.* 2004). It is asserted that haematocrit, hemoglobin and total plasma protein levels of *C. gariepinus* show significant differences in 14 and 41°C acclimation temperatures compared with 29°C control group (Adeyemo *et al.*, 2003).

Erythrocyte sedimentation ratio: Sedimentation values are calculated as 2.475±0.3304 mm h^{-1} in control group brown trout and 2.275±0.2630 mm h^{-1} in rainbow trout. These values show compatibility with the value (0.0-8.0 and 1.8-6.0 mm h^{-1}) Kocabatmaz and Ekingen (1984) and Atamanalp *et al.* (2002) found for healthy trout. In the treatments carried out with phenoxyethanol and clove oil; the highest value is detected in clove oil treated rainbow trout with 2.750±0.3728 mm h^{-1}, the lowest value is detected in clove oil treated brown trout with 1.900± 0.2191 mm h^{-1}. As a result of statistical analysis the difference between treatment groups and species are found to be insignificant, the result of treatment x type interaction is significant.

While the sedimentation value decreases for brown trout as a result of clove oil and 2-phenoxyethanol treatment, it increases for rainbow trout. Kocabatmaz and Ekingen (1984) reported the sedimentation value as 0.8-2.3 mm h^{-1} for common carp, 1.8-2.5 mm h^{-1} for bullhead, 2.0-3.3 mm h^{-1} for chub; reported it as 6.0±2.0 mm h^{-1} for fresh water catfish (*Heteropneustes fossilis*).

Erythrocyte sedimentation ratio increase in the events such as acute infections, heavy metal toxicities and kidney deformations (Blaxhall and Daisley, 1973). Infection existence and sedimentation speed increase in fish (Kocabatmaz and Ekingen, 1984). It is thought that these values showing difference with the values given in literature is based on the fish species taken into experiment, gender and age of the fish used and the study conditions (Kocabatmaz and Ekingen, 1984). Changes in physical and chemical features of water cause change in the hematological parameters of fish as well.

CONCLUSION

It is expressed that Ph of water moving towards acidic side increase haematocrit and hemoglobin levels of fish and the number of erythrocyte and this may be based on the swelling of blood cells as a result of deformation in the regulation of plasma electrolyte with hemoconcentration (Heath, 1990).

REFERENCES

Adeyemo, O., S.A. Agbede, A.O. Olaniyan and O.A. Shoaga, 2003. The haematological response of *Clarias gariepinus* to changes in acclimation temperature. Afr. J. Biomed. Res., 6: 105-108.

Ahmad, F., S.S. Ali and and A.R. Shakoori, 1995. Sublethal effects of Danitol (Fenpropathrin), a synthetic pyrethroid, on freshwater Chinese grass carp, *Ctenopharyngodon idella*. Folia. Biol. (Krakow), 43: 151-159.

Atamanalp, M. and T. Yanik, 2003. Alterations in hematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb. Turk. J. Vet. Anim. Sci., 27: 1213-1217.

Atamanalp, M., T. Yanik, H.I. Haliloglu and M.S. Aras, 2002. Alterations in the hematological parameters of rainbow trout, *Oncorhynchus mykiss*, exposed to cypermethrin. Bamidgeh, 54: 99-103.

Atkinson, J. and F.W. Judd, 1978. Comparative haematology of *Lepomis microlophus* and *Cichlosoma cyanogutatum*. Copeia, 12: 230-237.

Aziz, F., M. Amin and A.R. Shakoori, 1993. Toxic effects of cadmium chloride on the haematology of fish, *Tilapia mossambica*. Proc. Pak. Congr. Zool., 13: 141-154.

Blaxhall, P.C. and K.W. Daisley, 1973. Routine haematological methods for use with fish blood. J. Fish Biol., 5: 771-781.

Blaxhall, P.C., 1972. The haematological assessment of the health of freshwater fish. Review of selected literature. J. Fish Biol., 4: 593-604.

Braithwaite, V.A. and F.A. Huntingford, 2004. Fish and welfare: Do fish have the capacity for pain perception and suffering. Anim. Welf., 13: 87-92.

Briozzo J.L., J. Chirife, L. Herzage, M. Daquino, 1989. Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. J. Appl. Bacteriol., 66: 69-75.

Canfield, P.J., N. Quartararo, D.L. Griffin, G.N. Tsoukalas and S.E. Cocaro, 1994. Haematological and biochemical reference values for captive Australian snapper, *Pagrus auratus*. J. Fish Biol., 44: 849-856.

Casillas, E. and L.S. Smith, 1977. Effect of stress on blood coagulationand haematology in rainbow trout (*S. Gairdneri*). J. Fish Biol., 10: 481-491.

Chen, C.Y., G.A. Wooster, R.G. Getchell, P.R. Bowser and M.B. Timmons, 2003. Blood chemistry of healthy, nephrocalcinosis-affected and ozone-treated tilapia in a recirculation system, with application of discriminant analysis. Aquaculture, 218: 89-102.

Coyle, S.D., R.M. Durborow and J.H. Tidwell, 2004. Anesthetics in Aquaculture. SRAC Publication, Texas, pp: 6.

Das, B.K. and S.C. Mukherjee, 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: Biochemical enzymatic and haematological consequence. Comp. Biochem. Physiol. Toxicol. Pharmacol., 134: 109-121.

Ezzat, A.A., M.B. Sharana and A.M. Farghaly, 1974. Studies on the blood characteristics of tilapia zilli blood cells. J. Fish. Biol., 6: 1-12.

Favaretto, A.L.V., P. Sawaya, S.O. Petenusci and R.A. Lopes, 1978. Hematologia do *Cascudo plecostomus* regani. I. Serie vermelha. Biologica, 4: 12-17.

Gabriel, U.U., G.N.O. Ezeri and O.O. Opabunmi, 2004. Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). Afr. J. Biotech., 3: 463-467.

Goel, K.A., A.K. Awasthi and J.K. Tyagi, 1981. Comparative haematological studies in some freshwater Indian fishes. 2. Tierphysiology tierernahrgu futtermittelkde. Muzafffarnagar, 46: 202-206.

Hamackova, J., J.M. Sedova, S.V. Pjanova and A. Lepicova, 2001. The effect 2-phenoxyethanol, clove oil and *Propiscin anaesthetics* on perch (*Perca fluviatilis*) in relation to water temperature. Czech. J. Anim. Sci., 46: 469-473.

Hasiloglu, M.A. and M. Atamanalp, 2002. Demirdoven baraj golu (Erzurum) tatlisu kefali (*Leuciscus cephalus*) populasyonu hematolojik parametrelerinin belirlenmesi. OMU Zir. Fak. Der., 17: 34-38.

Heath, A.G., 1990. Summary and perspective. Am. Fish. Soc. Symp., 8: 183-191.

Isaacs, G., 1983. Permanent local anaesthesia and anhidrosis after clove oil spillage. Lancet, 1: 882-883.

Isoda, M. and Y. Fujimaki, 1990. Fine-structural study of leucocytes in the goldfish (*Carassius auratus*). J. Fish. Biol., 36: 821-831.

Iwama, G.K., J.C. McGeer and M.P. Pawluk, 1989. The effects of five fish anaesthetics on acid-base balance, hematocrit, blood gases, cortisol and adrenaline in rainbow trout. Can. J. Zool., 67: 2065-2073.

Keene, J.L., D.L.G. Noakes, R.D. Moccia and C.G. Soto, 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res., 29: 89-101.

Kocabatmaz, M. and G. Ekingen, 1984. Degsik tur baliklarda kan ornegi alinmasi ve hematolojik metotlarin standardizasyonu. Doga Bilim. Derg., 8: 149-159.

Lester, R.J.G. and J. Budd, 1983. Soma changes in the blood cells of diseased coho salmon. Can. J. Zool., 57: 1458-1464.

Lie, Q., E. Lied and G. Lambertsen, 1989. Haematological values and fatty acid composition of erythrocyte phospholipids in cod (*Gadus morhua*) fed at different water temperatures. Aquaculture, 79: 137-144.

Luskova, V., 1997. Annual cycles and normal values of hematological parameters in fishes. Acta Sc. Nat. Brno, 31: 1-70.

Martinez, F.J., M.P. Garcia-Riera, M. Canteras, J. de Costa and S. Zamora, 1994. Blood parameters in rainbow trout (*Oncorhynchus mykiss*): Simultaneous influence of various factors. Comp. Biochem. Physiol., 107: 95-100.

Mughal, A.L., M.J. Iqbal and A.R. Shakoori, 1993. Toxicity of short term exposure of sublethal doses of a synthetic pyrethroid, fenvalerate, on the Chinese grass carp, *Ctenopharyngodon idella*. Proceeding of the Seminar Aquaulture Development Pak., pp: 49-74.

Munday, P.L. and S.K. Wilson, 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. J. Fish Biol., 51: 931-938.

Nelson, J.A., Y. Tank and R.G. Boutilier, 1996. The effects of salinity change on the exercise performance of two atlantic cod (*Gadus morhua*) populations inhabiting different environments. J. Exp. Biol., 199: 1295-1309.

Noyan, A., 1980. Fizyoloji Ders Kitabi. Vol. 2, Meteksan Publisher, Anadolu Unv. Yayinlari, Izmir, pp: 591.

Pages, T., E. Gomez, O. Suner, G. Viscor and L. Tort, 1995. Effects of daily management stress on haematology and blood rheology of the gilthead seabream. J. Fish Biol., 46: 775-786.

Quentel, C. and A. Obach, 1992. The cellular composition of the blood and haematopoietic organs of turbot *Scophthalmus maximus* L. J. Fish Biol., 41: 709-716.

Reddy, P.M. and M. Bashamohideen, 1989. Fenvalerate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio*. Acta. Hydrochim. Hydrobiol., 17: 101-107.

Roberts, R.J., 1989. Fish Pathology. 2nd Edn., Bailliere Tindall, London, pp: 467.

Sandnes, K., I. Lie and R. Waagbi, 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. J. Fish Biol., 32: 129-136.

Santhakumar, M., M. Balaji and K. Ramudu, 1999. Effect of sublethal concentrations of monocrotophos on erythropoietic acticity and certain haematological parameters of fish Anabas testudineus. Bull. Environ. Contam. Toxicol., 63: 379-384.

Satake, T., A. Nuti-Sobrinho, O.V. Paula-Lopes, R.A. Lopes and H.S.L. dos Santos, 1986. Haematological study of brazilian fish. III. Blood parameters in armored catfish *Hypostomus paulinus* Ihering 1905 (Pisces, Loricariidae). Ars Vet., 2: 179-183.

Sato, C.G. and S. Burhanuddin, 1995. Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). Aquaculture, 136: 149-152.

Schalm, O.W., N.C. Jain and E.J. Carrol, 1975. Veterinary Haematology. 3rd Edn., Lea and Febiger Publication, Philadelphia, pp: 807-807.

Schutt, D.A., J. Lehmann, R. Goerlich and R. Hamers, 1997. Haematology of swordtail, *Xiphophorus helleri* I: Blood parameters and light microscopy of blood cells. J. Applied Ichthyol., 13: 83-89.

Shakoori, A.R., A.L. Mughal and M.J. Iqbal, 1996. Effects of sublethal doses of fenvalarate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscles of a freshwater fish, *Ctenopharyngodon idella*. Bull. Environ. Contam. Toxicol., 57: 487-494.

Soivio, A. and A. Oikari, 1976. Haematological effects of stress on a teleost *Esox lucius* L. J. Fish Biol., 8: 397-411.

Summer, F.R.C. and L.S. Smith, 1990. Anesthesia, Surgery and Related Techniques. In: Methods for Fish Biology, Schreck, C.B. and P.B. Moyle (Eds.). American Fisheries Society, Bethesda, MD.

Svoboda, M. and J. Kolarova, 1999. A survey of anaesthetics used in the fish farming (in Czech). Proceeding of Health Protec-tion of Fish, (HPF'99), Research Institute of Fish Culture and Hydrobiology, Czech Republic, pp: 49-72.

Torres, F.I.P., E.G. Moura, C.C.A. Nascimento, Jr. Contaifer, C.F. Ramos, M.A. Pimenta and E.B. Torres, 1986. Parametros bioquimicos e hematologicos de cascudos (*Hypostomus punctatus*). Ciencia Cultura, 38: 825-830.

Van Vuren, J.H.J. and J. Hattingh, 1978. A seasonal study of the haematology of wild freshwater fish. J. Fish Biol., 13: 305-313.

Velisek, J., T. Wlasow, P. Gomulka, Z. Svobodova, L. Novotny and A. Ziomek, 2006. Effects of clove oil anaesthesia on catfi sh (*Silurus glanis* L.). Acta Vet. Brno., 75: 99-106.

Velisek, J., Z. Svobodova and V. Piackova, 2005. Effects of clove oil anesthesia on rainbow trout (*Oncorhynchus mykiss*). Acta Vet. Brno., 74: 139-146.

Velisek, J., Z. Svobodova and V. Piackova, 2007. Effects of 2-phenoxyethanol anaesthesia on haematological profile on common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). Acta Vet. Brno., 76: 487-492.

Yamawaki, K., W. Hashimoto, K. Fujii, J. Koyama, Y. Ikeda and H. Ozaki, 1986. Hemochemical changes in carp exposed to low cadmium Concentrations. Bull. Jpn. Soc. Sci. Fish., 52: 459-465.

Yilmaz, K. and A. Otlu, 1989. Veteriner Hematoloji El Kitabi. irat Unv.Veteriner Fak. Hatiboglu Yayinevi, Ankara, pp: 120.

Yone, Y., M.A. Hossain, M. Furuichi and F. Kato, 1986. Effect of fermented scrap meal on proximate compositions of muscle and liver hematological characteristics and chemical components in blood plasma of rea sea bream. Bull. Jpn. Soc. Sci. Fish., 52: 1461-1464.