

Research Article

Effects of Four Anesthetics, Clove Extract, Thyme Extract, Lidocaine, and Sodium Bicarbonate on the Blood Parameters and Cortisol Amount in Grass Carp (*Ctenopharyngodon Idella*)

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Abstract

The anesthetic effects of two chemical compounds, lidocaine and sodium bicarbonate, and two natural compounds, clove and thyme extracts, were examined on the Red Blood Cells (RBC), White Blood Cells (WBC), hemoglobin, hematocrit, and blood cortisol levels of Grass carp at two times of 10 minutes and 24 hours after anesthesia. RBC counts had no significant increase in the samples of 10 min and 24 h after induction of anesthesia with clove extract only compared to the control group ($p > 0.05$). The number of WBC increased significantly in the samples from 10 min and 24 h after anesthesia with sodium bicarbonate only and it was not different from control samples in other treatments 24 h after anesthesia. The amount of hemoglobin indicated an increase with all treatments compared to the control in 10 min after anesthesia. The increases were not significant in thyme and clove extract ($P > 0.05$). Samples treated at 24h after anesthesia exhibited non statistical hemoglobin elevations with thyme extract only. Hematocrit increased significantly in the samples from 10 min after anesthesia with lidocaine and sodium bicarbonate but, at 24h after anesthesia, hematocrit amount with thyme and clove extracts and lidocaine treatments were not different from the control significantly ($P > 0.05$). Also, Cortisol level was significantly higher in both sampling times for lidocaine and sodium bicarbonate treatments than the control group. Accordingly, the use of natural anesthetics (thyme and clove extracts) will result in better outcomes than the synthetic compounds (sodium bicarbonate and lidocaine) in the Grass carp.

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Introduction

In today's aquaculture industry of many countries, anesthesia techniques receive special attention to reduce stress and achieve higher meat quality. The use of anesthetics and stolidity drugs in aquaculture can positively impact the quality of protein produced^[1]. The main purposes of anesthetizing fish include reducing mobility and stress in operations such as tagging, biometrics, sorting, examination, and sampling^[2]. A compound for use as an anesthetic in aquaculture should have three features of safety, cheapness, and availability^[3]. The impact of an anesthetic on fish depends on many factors such as, its concentration, water temperature, fish size, and the species^[4]. Common anesthetics in aquaculture include tricaine methane sulfonate (MS222), benzocaine, coinaldine, metomidate, clove extract, and 2-phenoxy eth-

anol^[5]. Some of these anesthetics have carcinogenic effects and, in addition, the periods of avoidance for human consumption are sometimes very long in the health legislations of countries^[6]. Furthermore, some of the compounds (e.g. MS222) currently used as anesthetics in the aquaculture industry are very expensive.

So far, a number of studies have been carried out in order to assess and describe the physiological effects of various anesthetics on aquaculture species^[7-9]. Hormonal and humoral analyses are methods of assessing the effects of anesthetic drugs. During and/or following anesthesia, the blood of fish may undergo some transient or prolonged changes. These changes can include anemia, compensatory increase in RBC, increased WBC count, and changes in blood cortisol levels caused by oxygen deficiency, and stress among other factors^[5]. The aim of this study,

therefore, was to evaluate the effects of anesthetic combination of herbs (thyme and clove extracts) and two chemical anesthetics (lidocaine and sodium bicarbonate) on the Grass carp.

Materials and Methods

This research was carried out in a warm-water fish farm located in Sari, northern Iran. A total of 48 Grass carp with an average weight of 200g and length of 18 cm were randomly divided into five groups. All the water conditions of the tanks (such as temperature, oxygen content, pH, etc.) were controlled and kept at optimal levels during the experimental period Table 1.

Table 1. Conditions of water during the study period

Parameter	Value	Optimal range
Water temperature (°C)	21-23	25-17
pH	7.7	6.5-8
Ammonia (mg/l)	0.6	0-0.02
Nitrite (mg/l)	0.0	0-0.1
Total hardness (mg/l)	285	10-400
Dissolved oxygen (mg/l)	8	5-10

Lidocaine (2 %) was obtained from the Pasteur Institute of Iran, sodium bicarbonate from Soda Kaveh Chemical Industry Co., and clove and thyme extracts from Giah Essence Pharmaceutical Co. To prepare the anesthetic solutions, an amount of each anesthetic was added to the water container [Table 2], and then the resultant solution was completely stirred and homogenized. The fish were not fed 24h prior to the start of the anesthetic trials. After preparing the anesthetic solutions with the desired concentrations [Table 2], fish were anesthetized via bathing in small tanks and then transferred to larger tanks to recover from anesthesia.

Table 2. The doses of anesthetics used in the current study 3-16-23

Anesthetic	Recommended dose	Anesthesia time (min)	Recovery time (min)
Lidocaine	150mg/l	2	5
Sodium bicarbonate	300 mg/l	5	10
Clove extract	50 mg/l	5	5
Thyme extract	100 mg/l	6	6

Blood samples were taken from the caudal vein in two steps (10 min and 24 h after recovery of fish). Five blood samples were taken from five fish of each treatment as replications. A portion of the blood was transferred to non-heparinized tubes to measure cortisol hormone, and another fraction was poured into heparinized tubes for counting blood cells^[10]. Blood samples were also taken from the control fish that had received no anesthetic. WBC and RBC number were counted manually using Neubauer hemocytometer^[11]. Hematocrit amount was measured according to microhematocrit method^[12]. Hemoglobin amount was determined using a kit and spectrophotometry at 540 nm^[13], and cortisol level in blood was measured by radioimmunoassay (Immunotech, France)^[10].

The statistical model used in this study was a completely randomized design. Within such a design, four anesthetics (clove and thyme extracts, lidocaine, and sodium bicarbonate)

and a control group were studied as treatments each with five replications. Data were analyzed using SPSS version 21 at a confidence level of 95 %. The difference between means of the data were evaluated by the Analysis of Variance (ANOVA) and compared with Duncan's test.

Results

The results of this study showed that the numbers of RBC were not significantly different in the samples anesthetized by clove extract compared to control samples after 10 min and 24h after anesthesia ($p > 0.05$). The increases of RBC numbers in the other treatments were significant at both sampling times ($p < 0.05$) Table 3.

Table 3. Comparison of differences in the studied blood parameters at different treatments

		Red blood cell (N×10 ⁶ /mm ³)	White blood cell (N/mm ³)	Hemoglobin (g/dl)	Hematocrit (%)
Control		2.08 ± 0.28 ^a	3350 ± 525.99 ^a	6.32 ± 0.62 ^b	24.75 ± 1.26 ^a
Thyme extract	10 min.	2.67 ± 0.52 ^{bc}	5100 ± 476.48 ^{ab}	6.82 ± 0.82 ^{bc}	28.00 ± 2.45 ^{abc}
	24 h	2.68 ± 0.37 ^{bc}	4075 ± 675.15 ^a	7.65 ± 0.80 ^c	27.50 ± 2.38 ^{abc}
Clove extract	10 min.	2.43 ± 0.14 ^{abc}	5400 ± 370.83 ^{ab}	6.92 ± 0.46 ^{bc}	28.75 ± 1.89 ^{abc}
	24 h	2.26 ± 0.25 ^{ab}	5200 ± 219.61 ^{ab}	6.65 ± 0.89 ^{bc}	27.25 ± 2.22 ^{ab}
Lidocaine	10 min.	2.71 ± 0.53 ^c	6075 ± 955.12 ^{ab}	7.57 ± 0.87 ^c	31.75 ± 3.40 ^c
	24 h	2.80 ± 0.19 ^c	5375 ± 269.18 ^{ab}	5.17 ± 0.76 ^a	27.50 ± 1.73 ^{abc}
Sodium bicarbonate	10 min.	2.88 ± 0.18 ^c	7300 ± 675.82 ^c	7.60 ± 0.29 ^c	30.50 ± 4.72 ^{bc}
	24 h	2.62 ± 0.19 ^{bc}	6100 ± 509.96 ^b	8.77 ± 0.80 ^d	31.25 ± 1.70 ^{bc}

*Values with the same letters in each column show no significant differences ($P > 0.05$).

The number of WBC elevated 10 min after anesthesia with thyme extract, clove extract, and lidocaine compared to the control (3350 ± 525.99 N/mm³), these elevations were not significant ($P > 0.05$). On the other hand, a statistical increase was observed 10 min and 24 h after anesthesia with sodium bicarbonate ($P < 0.05$). Sampling in 24h after anesthesia with the other treatments displayed no significant differences compared to the control group ($P > 0.05$) Table 3.

The amount of hemoglobin in the control group was 6.32 ± 0.62g/dl. This indicated an increase with all treatment groups compared to the control in 10 min after anesthesia. The increases were not significant in thyme and clove extract ($P > 0.05$). Samples treated at 24h after anesthesia exhibited non-statistical hemoglobin elevations with thyme extract only. The results showed that hematocrit increased significantly in the samples from 10 min after anesthesia with lidocaine and sodium bicarbonate in relation to control. The amounts of hematocrit in samples belonged to 24h after anesthesia with thyme and clove

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extracts and lidocaine treatments were not different from the control significantly ($P > 0.05$).

In this study, the lowest amount of cortisol was measured in the blood of fish in the control group (17.75 ± 3.70 ng/mm³). The amount of this hormone showed significant rises in the treatments of 10 min after anesthesia with lidocaine (38.05 ± 10.96) and sodium bicarbonate (31.55 ± 10.23) compared to the control group ($p < 0.05$). Cortisol values displayed non-significant higher concentrations in the samples from 10 min after anesthesia with thyme (19.75 ± 2.36) and clove (22.37 ± 2.88) extracts. The fish anesthetized with lidocaine and sodium bicarbonate showed significant greater amounts of this hormone than the control samples 24h after anesthesia [Figure 1].

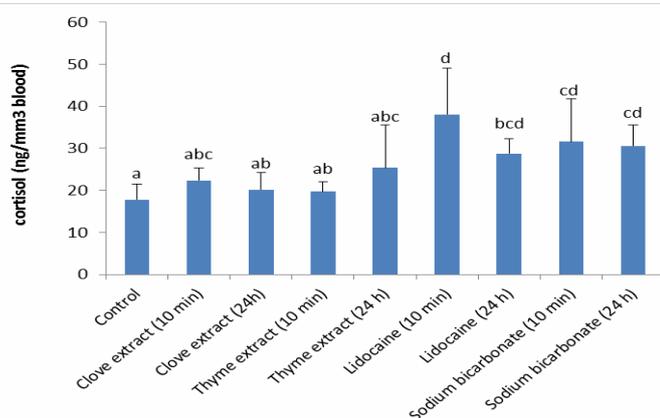


Figure1: Comparison of the cortisol hormone levels in the blood of Grass carps exposed to different anesthesia treatments.

Discussion

Since different species of fish show very different responses to anesthetic substances, determination of the type and dosage of such materials for any species often seems necessary. Analysis of blood parameters is one of the most important and reliable methods that can provide important information about an organisms healthy conditions^[9].

RBC counts, hemoglobin, and hematocrit changes are in relation to stressful environmental factors^[14]. For example, increased number of RBCs indicates high stress levels in fish. The number of RBC, and levels of hemoglobin and hematocrit will change according to the species, gender, age of the fish, and stress factors^[15]. In this study, the numbers of RBC were not significantly different in the samples anesthetized by clove extract only, after 10 min and 24h after anesthesia. Research showed that the use of clove oil in the cat fish (*Silurus glanis*) could not cause any significant difference in the number of RBC^[16]. The similar result was seen in the roach (*Rutilus rutilus*)^[17].

Stress can cause the number of white blood cells to rise. This occurs because the immune system is designed to kick into action to manage or prevent disease. When feeling stressed, the brain receives signals that there is a problem that needs to be addressed. The brain and the body of fish are in constant communication with one another. During times when stress is acute or short term, the brain can first respond to help the body deal with the situation. When the stressful event has ended the brain then sends signals to reverse the initial stress reaction, allowing the white blood cell count to return to normal^[18]. The results of WBC

count showed that only sodium bicarbonate treatment exhibited a marked increase 10 min and 24 h after anesthesia. Researchers examined anesthesia with clove oil in cat fish (*Silurus glanis* L.) and observed no significant different WBC count after 10 min and 24 h of anesthesia^[16] which is consistent with the results of the present study. The results of studies on the roach (*Rutilus rutilus*)^[17] and also on the Persian sturgeon (*Acipenser persicus*)^[19] revealed significant WBC elevations after 10 min anesthesia with clove extract, even so, no considerable differences from the control were detected following 24 h post-anesthesia contrary to the results of this study.

The incidence of stressful conditions results in an increase in the number of RBCs per unit of blood volume and, consequently, an increase in hemoglobin^[14]. Lack of oxygen is a factor in a rapid increase of hemoglobin level and other main blood components^[18]. In this study, the amount of hemoglobin in fish anesthetized with lidocaine and sodium bicarbonate showed significant increase after 10 min compared to the control group. Clove extract resulted insignificant rise in hemoglobin measures of 24 h of anesthesia compared to the control group. A study showed no significant differences in hemoglobin amount in cat-fish anesthetized with clove oil after 10 min and 24 h of anesthesia^[16] which is similar to the results of this study. The results of other studies on the roach^[17] and Persian sturgeon^[19] showed significant decreases in hemoglobin after 10 min of anesthesia with clove extract contrasting the findings of the present study.

The results from hematocrit analyses showed the significant increase in the samples from 10 min after anesthesia with lidocaine and sodium bicarbonate and in samples belonged to 24h after anesthesia with sodium bicarbonate only. At study which used clove extract as an anesthetic for the broodstock of silver pond fish (*Carassius gibelio*) caused no significant changes in hematocrit values match with the results of this study in the clove extract treatment^[20]. Similar to the results of the present study in fish treated with lidocaine, boosted RBC, WBC, hemoglobin, and hematocrit amounts were seen after exposure of gold fish (*Carassius auratus auratus*) to the lidocaine^[21].

Cortisol is known as the most important indicator of stress in animals. In this study, the amount of cortisol in the blood of fish showed a significant increase after anesthesia with lidocaine and sodium bicarbonate compared to the control group. Research showed decreased cortisol levels in the blood of rainbow trout (*Oncorhynchus mykiss*) treated with clove oil indicating diminished stressful conditions^[22]. Also, the study of effects of clove extract on the channel catfish (*Ictalurus punctatus*), found it very effective in suppressing the secretion of cortisol hormone in the fish^[23], which corroborate the results of this study about the herbal anesthetics (thyme and clove extracts). The discrepancies between the results of different studies may be due to differences in species, gender, age, size, conditions of the culture media, the type and dose of the anesthetics, the anesthesia technique applied, and so on^[24].

Conclusion

It can be concluded that the use of natural compounds (extracts of thyme and clove) will yield better results in the grass carp in comparison with synthetic substances (sodium bicarbonate and lidocaine).

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