

Effects of Clove Extract as an Anesthetic on Sperm Motility Traits and Some Hematological Parameters in Prussian Carp *Carassius Gibelio*

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ABSTRACT

To evaluate the effects of anesthesia with different concentrations of clove extract (0, 50, 200, 400 and 600 mg) on sperm function and some hematological parameters in Prussian carp, *Carassius gibelio* (Bloch, 1782), 30 mature male were examined. In this purpose, sperm motility traits and some hematological parameters (Red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (PCV), white blood cell count (WBC) and the differential leukocyte count (leukogram)) were tested. The percentage of motile sperm was unaffected by anesthetic treatments, averages ranging from 71.65% to 85.67%. However, duration of motility decreased as anesthetic concentration increased, averages ranging from 34.66 to 54.33 s. There were no significant differences between values of Hb, PCV and leukogram in each treatment ($p > 0.05$); however, WBC was significantly lower at 400 and 600 ppm clove extract treatment group ($p < 0.05$). Moreover, RBC in these group was increased significantly ($p < 0.05$), and at 600 ppm clove extract treatment group was higher than those of other groups ($p < 0.05$). The results of this study show that use of clove extract in lower dosages than 400 ppm don't affect on sperm motility traits and hematological parameters in Prussian carp.

Key words: clove extract, sperm motility, hematological parameters, Prussian carp

Introduction

The Prussian carp (*C. gibelio*) is a fresh water fish that belongs to Cyprinidae. Most Prussian carp breed in captivity, particularly in pond settings. Breeding usually happens after a significant temperature change, often in spring. Males chase females, prompting them to release their eggs by bumping and nudging them. The reproductive biology of *Carassius* species is complicated by the occurrence of gynogenesis[1].

Anesthetics are widely used in the aquaculture industry to aid in handling fish and to minimize stress. Stress can have a negative impact on captive fish, with effects including reduced

immunocompetence, increased susceptibility to disease, reduced egg quality and spermatocrit and reduced growth[2].

Since fish breathe through gills rather than lungs, anesthetic agents are greatly inhaled with gills. As a result, anesthesia must be added to the tank water and delivered through an aquatic medium[3]. Therefore the relationships between the epithelium surface of the gill and the body volume as well as thickness of epithelium affect the efficacy of anesthetics[4].

The type and the concentrations of anesthetics may affect the sperm quality during handling[5]. In rainbow trout, the percentage of motile sperm was unaffected by anesthetic treatment of male

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broodstock, but the duration of motility decreased as anesthetic concentration increased[2]. The efficacy of anesthetic agents varies both within and between species. Fish of different age, size and sex may respond differently to a particular dosage, and the response is also influenced by environmental factors such as salinity, pH, oxygen level and water temperature[6].

Clove extract is used as an anesthetic before handling or treating fish in breeding, artificial propagation, blood sampling or for some other veterinary interventions. The use of an anesthetic facilitates the handling of too big or too agile fish species[2,7,8]. At present, clove extract is used in Iran for short-term immobilization of fish before artificial spawning and whenever fish is handled outside water.

The objective of the present study was to examine effects of anesthesia with different concentrations of clove extract (0, 50, 200, 400 and 600 mg) on sperm motility characteristic (sperm movement duration and percentage of motile spermatozoa) and some hematological parameters of Prussian carp (*C. gibelio*).

Materials and methods

Broodstocks and Samples Preparation:

Male Prussian carp were obtained from a reared hatchery at Babol, Iran. To stimulate fish for spawning injected intraperitoneally: 0.5 ml kg⁻¹ Ovaprim (sGnRH_a+Dompridon). Milt samples were collected during the 2010 spawning season from 30 sexually mature two-year-old male Prussian carp (mean total weight: 64.25 ± 3.48 g). Finally the fishes were divided to five groups including control, 50 ppm (T1), 200 ppm (T2), 400 ppm (T3) and 600 ppm clove extract (T4)[9]. Fish were dried to avoid activation of sperm by water, urine and blood, and then milt was collected by applying gentle bilateral abdominal pressure. Blood was drawn from the caudal vein using a needle and syringe. EDTA was used as anticoagulant.

Sperm Motility Analysis:

The effect of clove extract on sperm motility trait was examined. One µl of milt was thoroughly mixed (for approximately 5 s) with 1000 µl of above water, and then pipetted onto a glass slide. Samples reviewed at 200× on a negative phase-contrast microscope (Leica USA) and the motility was presented as the percentage and duration of motility. The duration of sperm motility was measured immediately after initiation of sperm activation until 100 % spermatozoa were immotile and expressed as sperm movement duration. Only forward moving

sperm were judged motile, those simply vibrating or turning on their axes was considered immotile[10].

Hematological Analysis:

The indices used to evaluate the hematological profile were included; white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), hematocrite (PCV) and the differential leukocyte count (leukogram). The procedures were based on methods described for fish hematology[11].

Statistical Analysis:

Statistical analysis of data was done by One-Way ANOVA with Duncan test at the level of 95% using SPSS 16. Statistically significance was set at the level of p<0.05 with ± standard deviation (SD).

Results and discussion

Spermatological Parameters:

According to Figure 1 the maximum and minimum sperm movement duration (54.33 ± 7.10 and 34.66 ± 5.13) were recorded in control and clove extract concentration of 600 ppm (p<0.05). Indeed, sperm movement duration showed a decline trend with increasing in clove extract concentration.

The dynamics of the percentage of motile spermatozoa as a function of time post activation is shown in Figure 2. The percentage of motile sperm had not significant changes (p> 0.05) in experimental groups.

Hematological Parameters:

Effects of clove extract on the hematological indices of Prussian carp are showed in Figure 3 and Figure 4 (Table 1). Values of hematological indices in five experimental groups were compared with the corresponding values in the control group (0 ppm clove extract). The RBC was significantly increased within 400 and 600 ppm clove extract treatment groups in compare with control group (p<0.05). A significant decrease of WBC in the 400 and 600 ppm treatment groups was also observed in compare with control group (p<0.05). The other indices (Hb, PCV and leukogram) in treatment groups were not significantly differed with the control group (p>0.05).

Discussion:

The use of anesthetics for broodfish greatly facilitated the handling of both sexes during the spawning process. No delayed mortality was observed in the weeks following the tests for any of

the fish handled and bled. This study was the first to evaluate sperm viability and some hematological parameters from Prussian carp broodstock exposed to clove extract in different concentrations.

Previous investigations have identified a multiple correlation between decreasing sperm motility and increasing anesthetic concentration. Allison^[12] found a reduction of brook trout (*Salvelinus fontinalis*) sperm motility to less than 10 s at tricaine concentrations ranging from 19 to 75 mg/l, but Billard^[13] found no reductions in rainbow trout (*Oncorhynchus mykiss*) sperm motility when working with tricaine concentrations of 4–150 mg/l. According to our findings, percentage of motile sperm didn't show significant change in breeders that were exposed on anesthetic in different concentrations comparing the control. These results agree with those obtained by Wagner *et al.*^[2] and Holcomb *et al.*^[14]. Their findings on rainbow trout sperm showed incubation of milt with anesthetics did not alter sperm motility significantly. As well as they observed duration of motility decreased as anesthetic concentration increased, similarly in our study there was significant difference in duration of motility when the anesthetic concentration increased. Dietrich *et al.*^[15] reported anesthesia in rainbow trout didn't influence on sperm mobility parameters that it is not agree with our results.

Hematological indices are closely related to response of the animal to the environment^[16]. Previous studies showed that clove oil anesthesia at 30 mg l⁻¹ concentration and 10 min exposing had no effect on the hematological profile of *Cyprinus carpio*^[7] and *Oncorhynchus mykiss*^[8]. However, in Roach *Rutilus rutilus*, the 7-min exposure to clove powder caused a significant increase in hematocrit, hemoglobin and total erythrocyte count after anesthesia and then returned to normal level^[17]. Moreover, the leukocyte counts of *Silurus glanis* exposed to 30 mg ml⁻¹ of clove oil were significantly decreased 24 hours after anesthesia^[18]. Our results did not show any change in hematological indices of Prussian carp while clove extract was used at different concentrations, however clove extract at concentrations of 400 and 600 ppm could increased RBC value. Imanpoor *et al.* ^[9] (2010) to evaluate clove essence effect on *Acipenser persicus* anesthesia found that amount of White Blood Cell (WBC) showed a decline trend associated with arresting in anesthetic that their findings agree with our results. In general, physiological responses in fish to the anesthetics are different. The results of this study indicated that clove extract in lower dosages than 400 ppm don't affect on sperm motility traits and hematological characterizes in Prussian carp significantly and the findings can be useful in the performance of handling and propagation of this species.

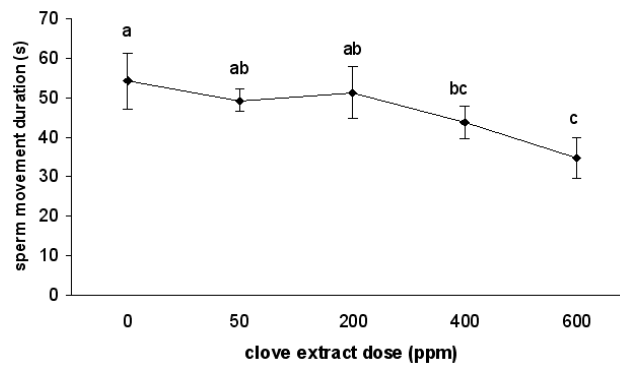


Fig. 1: Effect of different clove extract concentrations on sperm movement Duration (mean ± SD) of Prussian carp. Different letters indicate a significant difference for each variable (p<0.05).

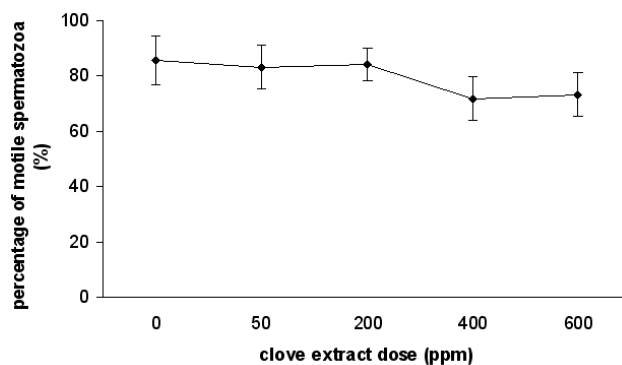


Fig. 2: Effect of different clove extract concentrations on percentages of motile spermatozoa (mean ± SD) of Prussian carp.

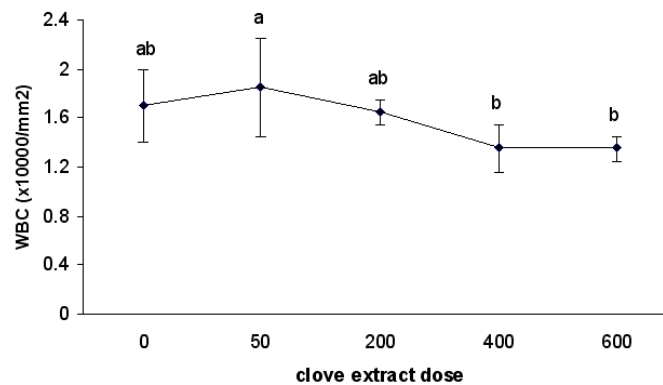


Fig. 3: Effect of different clove extract concentrations on WBC values (mean ± SD) of Prussian carp. Different letters indicate a significant difference for each variable (p<0.05).

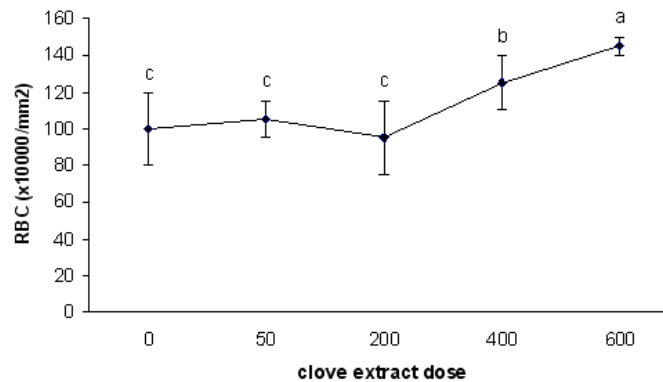


Fig. 4: Effect of different clove extract concentrations on RBC values (mean ± SD) of Prussian carp. Different letters indicate a significant difference for each variable (p<0.05).

Table 1: Effect of different clove extract concentrations as an anesthetic agent on hematological indices of Prussian carp.

Dose(ppm)	Parameters	0	50	200	400	600
Hb (g/dL)		7.95±0.94	8.71±0.90	8.82±0.75	9.20±0.50	10.25±0.74
PCV (%)		37.84±2.48	37.45±2.10	35.54±1.75	37.62±3.25	38.12±1.45
Monocyte (%)		2.00±0.25	2.40±0.25	1.90±0.45	2.40±0.24	1.86±0.55
Granulocyte (%)		12.25±1.30	14.20±1.75	13.60±2.25	14.35±0.85	13.80±0.96
Lymphocyte (%)		84±1.40	85±2.10	82±1.63	81±2.42	84±0.96

Means in the respective column are not significantly different, p>0.05

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