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The effect of clove oil as sedative to hematophysiology of hybrid grouper (*Epinephelus* sp.) in close transportation in tropical country

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Abstract
A problem often faced by grouper fish exporters is the high mortality rate (97%) of the fish due to stress during transport. The present study was conducted to analyzethe effect of clove oil on blood glucose levels and the number of erythrocyte during close transport system. This experimental design used complete randomized design (CRD). The data obtained in the study were analyzed using statistical test ANOVA (Analysis of Variance) to determine whether there were differences among the treatments, followed by Duncan's multiple range test. Results of the study showed blood glucose value of $P< 0.05$, indicating significant difference in the treatment of clove oil. Duncan's multiple range test showed that there were significant differences among the treatments P2 (MS-222 70 ppm) with P1 (negative control), P3 (clove oil 5 ppm), P4 (clove oil 10 pp) and P5 (clove oil 15 ppm). Total erythrocyte estimation shows the value of $p>0.05$, indicating no significant difference in clove oil treatment. In this study,Duncan's multiple range test showed no significant differences among treatments.

Keywords: grouper, clove oil, MS-222, blood glucose

1. Introduction
Grouper is a type of fish that has important economic value to be cultivated in Indonesia. [15] According to Indonesian Fisheries Statistic (2015), grouper production by 2013 was 113 368 MT, consisting of farmed grouper of 13.464 MT and catches grouper of 99 904 M. Grouper exports value was US $ 19.8 million with a volume of 2,552 MT.

Transport plays an important role in distributing the fish to consumers. One of the best method used is enclosed transportation. [13] The biggest challenge is to minimize the stress level of the fish. According to Harmon, [9] fish tend to be stress due to exposure to various stressors during trans portation. Stressors are associated with irregular stocking density, poor physical handling, deterioration of water quality and with adaptation to new environment.

Behavioral parameters of fish, such as breathing, opercular movement, surfacing, general activity, restlessness, loss of equilibrium, lethargy, unconsciousness, burst of erratic swimming, vertical position, bottom dwelling, ventral lay down, feeding, death mimicry and death, were observed carefully through direct contact. Blood parameters such as hematocrit, hemoglobin, red blood cell count, blood glucose, and chortisol, were also observed. [11]

The level of stress fish during transport can be reduced with sedation/anesthesia. [26] One of the natural ingredients that are often used in fish anesthesia is clove oil. [20] Clove oil contains eugenol, a component in large quantities that have a nature benefit as local anesthetics, stimulant, carminative, antimetic, antiseptic and antipasmedic. [17] Clove oil is highly effective even in low doses, in the process of induction, and longer recovery time. [14] One anesthetic material often used is tricaine methanesulfonate (MS-222). [2] MS-222 is an anesthetic substance used in the transport of fish that is tentatively anesthetized, causesthe fish to become insensitive to vibration, easy to use, fast induction time, and does not cause negative impacts to fish and humans at certain levels. [5] This study was conducted to determine the effect of clove oil and MS-222 to the physiological response of grouper seed hybrid grouper *Epinephelus* sp. transported by a closed system.

2. Materials and Methods

2.1 Fish

Before being used in this experiment, thegroupers were selected for their healthiness by stressing them in any condition of environmental changing.Fish were selected according to their length(7-9 cm) and abnormality, and they were subjected to acclimatization for 7 days. Fish was observed everyday during the course of the experiment.

2.2 Transportation

Packing was done at night at 10:00 pm to avoid direct sunlight. Anesthesia was poured according to preliminary study dose for 10 fish in plastic bag containing 2 lt sea water, added with oxygen at a ratio of 1: 1, and ten plastic bags were tied up and put in a cool box with crushed ice. Transportation took place for 10 hours from Situbondo to Surabaya (±500 km) vice versa. Water quality was measured before and after 10 hours of transportation.

2.3 Blood Sampling

Blood sampling was performed before and after transportation. The blood was drawn from caudal vein located in the base of the fish using a 1 ml syringe size. The syringe and microtube had been rinsed earlier with 10% EDTA as an anticoagulant. Blood drawn was stored in microtube of 1.5 ml.

2.4 Blood Glucose

Blood glucose measurements was performed using Blood Glucose Monitoring System (Easy Touch GCU). Samples of blood were dripped on glucose test strips, then the screen was read.

2.5 Total Erythrocytes

Calculation of erythrocytes used a toma pipette with a scale of 0.5 and added with Hayem's solution up scale 101. Pipette was driven with a figure of eight to homogenize the solution for 3-5 minutes. The first droplets were discharged and dropped into the next drop haemocytometer and covered with a glass lid. The number of red blood cells counted was converted by the formula: The number of red blood cells = Σ red blood cells counted x 10^7sel/mm^3

2.6 Total Leukocytes

Leukocyte was counted using a method of Blaxhall and Daisley (1973). The first blood was sucked by a pipette containing heparin to a scale of 0.5. Then, Turk's solution was added to scale 11, and the the pipette was swung to form a figure 8 for 3-5 minutes in order to mix the blood properly. One drop solution was put on haemocytometer, then it was covered with a glass lid. Fluid filled the count in capillaries. The number of leukocytes was calculated with microscope with a magnification of 400 X. The number of leukocytes was calculated using the following formula: The number of white blood cells = Σ white blood cells counted x 50 cells/$mm^{3.}$

2.7 Water Quality

Water quality measurement was performed twice, before and after transport. Parameters measured were dissolved oxygen (DO), pH, temperature, salinity and CO_2. The measurement was performed as follows: temperature was measured using thermometer, pH by pHmeter and other by water kit (Merck, 2017).

2.8 Survival Rate

Calculation of survival rate was done after the experiment was completed. Groupers were set free to recovery. According to Effendi, [6] the fish survival rate can be calculated using the following formula:

$$SR = \frac{\text{Number of fish seed at the beginning of the study}}{\text{Number of fish seed at the end of the study}} \text{ x } 100\%$$

3. Results and Discussion

Results of the analysis of variance (ANOVA) showed that the survival rate had $P < 0.05$, showing significant differences among the treatments. Statistical test results can be seen in Table 1.

Table 1: Survival rates of grouper after tranp**o**rt

Treatment	Survival Rate (%) ± Standard Deviation
P 1 Control Negative	60.00 ± 0.58^{c}
P 2 Control Positive	90.00 ± 0.00^{a}
P 3 Clove oil 5 ppm	76.67 ± 0.00^{bc}
P 4 Clove oil 10 ppm	86.67 ± 0.58^{ab}
P 5 Clove oil 15 ppm	80.00 ± 0.00^{bc}

Table 1 shows that the survival rate had $P < 0.05$, indicating significant differences among the treatments. Ten ppm doses is suitable for long distance anesthesia during transportation.

Table 2: Blood glucose (mg/dL) of grouper

Treatment	Doses	Average of blood glucose (mg/dL) (Mean± Standard Deviation)
P1	Control	$55.66^{b} \pm 10.69268$
P2	MS-222 70 ppm	$124^{a} \pm 32.44996$
P3	Clove oil 5 ppm	$33.33^{b} \pm 4.72582$
P4	Clove oil 10 ppm	$37^{b} \pm 6.55744$
P5	Clove oil 15 ppm	$38{,}33^{b} \pm 11.93035$

Results of measurement of blood glucose showed that after transport it elevated to 124 mg/dL, which was significantly different from the control P1 (55.66 mg/dL), P3 (33.33 mg/dL), P4 (37 mg/dL) and P5 (38.33 mg/dL). Whereas, P1 was not significantly different from P3, P4 and P5. The highest blood glucose was in P2 (MS-222 70 ppm) with 124 mg/dL and lower blood glucose was in P3 (Clove oil is 5 ppm) with 33.33 mg/dL (Table 2).

Table 3: Mean leucocyte count of grouper

Treatment	Total Leucocyte (10^4 sel/mm^3) ± Standard Deviation
P1 Control	2.56 ± 0.0125^{a}
P2 MS-222 70 ppm	2.28 ± 0.009^{b}
P3 Clove oil 5 ppm	2.38 ± 0.0230^{ab}
P4 Clove oil 10 ppm	2.35 ± 0^{b}
P5 Clove oil 15 ppm	2.36 ± 0^{b}

Table 4: Total erythrocyte count of grouper (× 10^7 sel/mm^3)

Treatment	Doses	Average ± Standard Deviation Transformation log y
P1	Control	$23.26^a \pm 0.00000$
P2	MS-222 70 ppm	$35.33^a \pm 0.00000$
P3	Clove oil 5 ppm	$12.33^a \pm 0.57735$
P4	Clove oil 10 ppm	$13.66^a \pm 0.57735$
P5	Clove oil 15 ppm	$17.66^a \pm 0.00000$

Results of measurement of mean leukocyte count showed that there was no significant difference between P2, P4, and P5. However, P2, P4, and P5 were different from P1 and P3 (Table 3). Results of analysis of variance (ANOVA) on the erythrocyte count showed $p > 0.05$, indicating no significant difference between treatments. Red blood cell count of the grouper after being transported for 10 hours are presented in Table4. It shows that red blood cells production is supressed or in anemic condition. Table 5 shows water quality during the course of the experiment. It is a normal condition for fish life.

Table 5: Water quality during the experiment

Treat ment	Water Quality									
	Temperature		Disolved Oxygen		Salinity (ppt)		Carbon Dioxide		pH	
	1	2	1	2	1	2	1	2	1	2
P1	25	28	5	3,8	32	32	0,22	6,67	8,1	8
P2	25	28	5	4,1	31	30	0,21	6,33	7,8	7,1
P3	25	28	5,2	3,8	32	31	0,22	7,1	8	7,9
P4	24	28	5	3,9	31	30	0,22	6,77	7,8	7,2
P5	24	28	5	3,9	32	31	0,23	6,83	8,03	7,6

1: before transport, 2: after transport

Clove oil influences blood glucose level during transport. Blood glucose of MS-222 showed the highest levels at 124 mg/dL. High blood glucose level showed that the grouper was in the state of stress. The drug causes eliminated consciousness of the fish as a results of the decrease in nerve function by blocking the action and conduction of nerve impulses [3]. This condition is the basis for the use of anaesthesia. Anesthetized fish will cause numbness or fainting.

During fainting, physiological processes occur in the fish. At this time the fish will normally secretes cortisol and epinephrine, and a further increase in glucose and impaired osmoregulation as the indicator of stress [8] Increased cortisol levels will reduce the action of insulin in the blood. Reduced insulin causes stress, and the blood glucose levels continue to rise because of the limitations of insulin that mobilized blood glucose into the cells more slowly [7].

The results of total erythrocytes in P1 is (23.3 × 107 cells/mm^3) were not significantly different among treatment P2 (35.33 × 107 cells/mm^3), P3 (12.33 × 107 cells/mm^3), P4 (13.66 × 107 cells/mm^3) and P5 (17.66 × 107 cells/mm^3). The total erythrocyte P2 is highest value (35.33 × 107 cells/mm^3), followed by P1 (23.3 × 107 cells/mm^3), P5 by value (17.66 × 107 cells/mm^3), P4 (13.66 × 107 cells/mm^3) and lowest erythrocyte P3 (12.33 × 107 cells/mm^3).Clove oil also affects the total erythrocyte grouper seed during transport. The highest erythrocyte isP2 with 35.33 × 107 cells/mm^3 (Table 4). The high number of erythrocytes indicates the fish in a state of stress (Wedemeyer and Yasutake 1977). Increased red blood cells in fish is homeostasis in the effort to increase hemoglobin to bind oxygen [21].

The lowest total erythrocyte was in P3 (Clove Oil 5 ppm) of 12.33 × 107 cells/mm^3. The low value of the total erythrocytes is thought to be caused by the analgesic activity of clove oil. Eugenol compounds have pharmacological activity as an analgesic, anti-inflammatory, antimicrobial, antiviral, antifungal, antiseptic, antispamosdik, antiemetics, stimulants, and local anesthetic so that the compound is widely used in the pharmaceutical industry [19].

Water quality measurements during the study were performed before and after transportation. The parameters were temperature, pH, dissolved oxygen (DO), salinity and CO_2. Temperature is one environmental factor that may cause stress to fish. Temperature changes cause sudden stress to the fish. Stress is defined as the inability of an organism maintain homeostasis due to disruption or stimulation from the outside [16].

During the transport, the temperature rise ranges from 3-4° C. Fluctuations in water temperature harm the fish. According to Stickney [22]. temperature fluctuations that endanger fish life is 5 °C within 1 hour.

Dissolved oxygen decreased after and before the transport. The decrease of dissolved oxygen was the result of its use by fish during transport. The oxygen values were lowest in P1 and P3 treatment at 3.8 mg/L. This value was a suitable for the life of grouper. This was consistent with a study by Langkosono and Wenno [12], that the DO suitable for grouper is 3.95 - 4.28 mg/L. The average value of dissolved oxygen prior to the transport were P1, P2, P4 and P5 of 5 mg/L, and the highest was in P3 5.2 mg/L.Salinity range between 31-32 ‰, it is still within the optimum range for the survival and growth of grouper. The optimum salinity range of tiger grouper was between 22-32 ‰ [23].

The concentration of CO_2 in the water increases the during transportation. In P5, CO2 concentrations ranged from 0.23 mg/L before, and 6.83 mg/L after transport. Such CO_2 range still support fish life. Boyd [4] reported a lot of fish that live in water with CO_2 levels higher than 60 mg/L.

The decrease in pH is associated with increased fish excretion and addition of anaesthesia MS-222 into the water. This is one of the problems in the transport of the fish with the use of anaesthesia or drugs [2]. The water pH value ranged from 7 to 8.1, but the range was still supporting fish life. According to Pescod [18]. the ideal pH range is 6.5 to 8.5. Groupers in P1 (without anesthesia), P2 (addition of MS-222 70 ppm) and P5 (the addition of clove oil 15 ppm) were stressful than those in P3 (addition of clove oil 5 ppm) and P4 (the addition of clove oil 10 ppm). It was characterized by high blood glucose levels

of 124 mg/dL at P2 and 55.66 mg/dL in P1. Another parameter that indicates stress among the grouper was observed in erythrocytes. In P1, it was 32.33 × 107 cells/mm^3 and in P5 (clove oil addition of 15 ppm) it was of 23.30 × 107 cells/mm^3.

4. Conclusion

The provision of clove oil during the process of transport influences blood glucose level and increases the survival rate of grouper. Further research is needed to determine the hematologic response of the grouper *Ephinephelu*s sp. transported with clove oil in optimal doses and measurement of cortisol levels to determine stress response in fish.

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6. Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

7. References

1. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. J. Fish Biol. 1973; 5:771-781. DOI: 10.1111/j.1095-8649.1973.tb04510.x.
2. Bourne PK. The Use of MS-222 (Tricaine Methanesulphonate) as an Anaesthetic for Routine Blood Sampling in Three Species of Marine Teleostei. Aquaculture. 1984; 36:313-321.
3. Bose AN, Ghosh SN, Yang CT, Mitra A. Coastal Aquaculture Engineering. Edward Arnold. A Division of Hodder & Stoughton, London. 1991, 375.
4. Boyd CE. Water quality management for pond fish culture. 1992; 9:388.
5. Daud R, Suwardi Jacob MJ, Utojo. Penggunaan MS-222 (*Tricaine*) Untuk Pembiusan Bandeng Umpan. Jurnal Penelitian Perikanan Indonesia. 1997; 3:47-51.
6. Effendie MI. Bilogi Perikanan. Yayasan Pustaka Utama. Bogor, 2002.
7. Fujaya Y. Fisiologi Ikan Dasar Pengembangan Teknik Perikanan. 1st Ed. Jakarta: Rineka Putra, 2004.
8. Davis BK, Griffin BR. Physiological Respon of Hybrid Striped Bass Under Sedatation by Several Anasthetics. Aquacultue. 2004; 233:531-548.
9. Harmon TS. Method for reducing stressor and maintaining water quality associated with live fish transport in tanks : a review of the basics. Reviews in Aquaculture. 2009; 1:58-66.
10. Indonesian Fisheries Statistic. Laporan Kinerja Satu Tahun Kementerian Kelautan dan Perikanan, 2015.
11. Lal Shah S. Hematological changes in *Tinca tinca* after exposure to lethal and sublethal doses of Mercury, Cadmium and Lead.Iranian Journal of Fisheries Sciences. 2010; 9(3):434-443.
12. Langkosono, Wenno LF. Distribusi ikan kerapu (Serranidae) dan kondisi lingkungan perairan Kecamnatan Tanimbar Utara, Maluku Tenggara. Prosiding Lokakarya Nasional dan Pameran pengembangan agribisnis Kerapu II Jakarta, 8–9 Oktober 2002. "Menggalang Sinergi untuk pengembangan Agribisnis kerapu". Pusat Pengkajian dan Penerapan Teknologi Budidaya Pertanian, BPPT, Jakarta. 2003, 203-212.
13. Lim LC, Dhert P, Sorgeloos P. Recent developments and improvements in ornamental fish packaging system for air transport. Aquaculture Research. 2003; 34:923-935.
14. Keene JL, Noakes DLG, Moccia RD, Soto CG. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac Res. 1998; 29:89-101.
15. Kohno H, Diani S, Supriatna A. Morphological Development of Larval and Juvenile Grouper, *Epinephelus fuscoguttatus.* Japan, J. Ichthyology. 1993; 40(3):307-316.
16. Kubilay A, Ulukoy G. The Effect of Acute Stress on Rainbow Trout (*Oncorhyncus mykiss*). Turk J Zool. 2002; 26:249.
17. Nurdjannah N. Diversifikasi Penggunaan Cengkeh. Persektif. 2004; 3(2):61-70.
18. Pescod MD. Investigation of rational effluent and stream standard for tropical countries. AIT, Bangkok, 1973.
19. Pramod K, Ansari SH, Ali J. Eugenol: a natural compound with versatile pharmacological actions. Natural Product Communications 2010; 5(12):1999-2006. www.ncbi.nlm.nih.gov.com. (03 Oktober 2016).
20. Rahim SW, Nessa MN, Trijuno DD, dan Djawal I. Efektivitas Minyak Cengkeh sebagai Bahan Anaestesi Terhadap Ikan Injel Biru-Kuning (*Centropyge bicolor*). Makassar: Universits Hasanuddin, 2013.
21. Setiawati M, Winarno TI, Suprayudi MA, Mokoginta I, Manula W. Mineral Besi Sebagai Peningkat Vitalitas Ikan Kerapu Bebek (*Cromileptes altivelis*) Saat Kondisi Stres Hipoksia. Jurnal Ilmu Pertanian Indoneia. 2007; 12(1):8-14.
22. Stickney RR. Principles of warmwater aqua-culture. New York : John Wiley &Sons, New York, 1979.
23. Sutarmat T, Andriyanto W, Ismi S, Hanafi A, dan Wardoyo. Studi Kepadatan pada Pembesaran Ikan Kerapu Bebek, *Cromileptes altivelis* dengan Ukuran yang Berbeda. [Laporan Penelitian]. Balai Besar Riset Perikanan Budidaya Laut Gondol, 2003.
24. Syawal H, Siregar YI. Respon Fisiologis Ikan Jambal Siam (*Pangasius hypopthalamus*) Pada Suhu Pemeliharaan yang Berbeda. Berkala Perikanan Terubuk, Februari. 2011; 39(1):51-57. ISSN 0126-6265.
25. Wedemeyer GA, Yasutake WT. Clinical Methods for Assesment of The Effect of Environmental Stress on Fish Health. Thechnical papers of the U.S Fish and Wildlife Service, Fish and Wildlife Service. 1977; 89:1-17.
26. Zonneveld N, Huisman EA, Boon JH. Prinsip-Prinsip Budidaya Ikan. Jakarta: Gramedia Pustaka Utama, 1991, 164-298.