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Comparative Study of Haematological and Biochemical Blood Profile of Grass Carp (*Ctenopharyngodon idellus*) at Using of Clove Oil and Quanaldine as Anesthetic During Ovulation Period

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ABSTRACT

Clove oil, containing the active ingredient eugenol, has been reported to be inexpensive and effective fish anaesthetic. So this study aimed to establish the lowest effective concentration of clove oil as anesthetic for grass carp (*Ctenopharyngodon idellus*) broodstock, and to determine the adverse effect of clove oil through estimation of some haematological indices and biochemical parameters and comparing with other anaesthetic as quanaldine. The current results revealed that using of clove oil at a dilution rate (7.5 ml /100 l) is the lowest concentration causing general anaesthetia with an average recovery time 3 min, 40 sec. No significant differences between values of haematological and biochemical parameters of clove oil and control one, while quanaldine resulted significant increase in glucose and cortisol levels as compared with the control group.

Keywords: Grass carp,blood profile, clove oil and quanaldine.

INTRODUCTION

Anaesthetics are used with increasing frequency in aquaculture mainly to reduce the stress and to prevent mechanical damage to fish during handling. At present, clove oil is used for short-term immobilization of fish before artificial spawning. Clove oil a dark brown liquid, a distillate of flowers, stalks and leaves of clove tree *Jugema aromatica* (Soto and Burhanndin, 1995) and its active ingredient i.e. eugenol (4-allyl-2-methoxyphenol) makes up 40 to 90 % by weight (Isaacs, 1983). The most widely used anaesthetics include Ms-222 benzocaine and quanaldine. Recently clove oil has been pointed out as a potential fish anaesthetic (Iwama and Ackerman, 1994). Its main advantage lies in its low costs and its relative safety for both fish and human .The objective of the current study was

to establish the lowest concentration of clove oil causing general anaesthetia for grass carp C. idellus and investigate the possible adverse action of clove oil through estimation of some haematological and biochemical blood profile in comparison with other anaesthetic, quanaldine.

MATERIALS AND METHODS

Fish management

The present study was conducted at Abbassa Fish hatchery, Central Laboratory for Aquaculture Research Abbassa, Abu- Hammad, Sharkia during May 2011. Eighteen grass carp weighted 2.600-2.800 kg and length of 60-70 cm were harvested from earthen ponds (1/4 feddan) at Abbassa experimental ponds. Fishes were fed on crude protein ration during summer and autumn months and transported to circular fiberglass tanks with capacity of 11.5 m^3 and supplied with irrigation water and well oxygenated. The water quality was maintained at temperature of 22±0.2 °C, pH of 7.01±0.01, total ammonia 0.5 ± 0.1 and dissolved oxygen of 4.1 ± 0.5 mg/l. Fishes were stored in fiberglass tanks for 12 hrs after harvest to prevent stress effects before the beginning of the experiment. Two types of anaesthesia clove oil and quanaldine (with different doses) were used. The fishes were divided into five groups each one contains three fishes. The experimented design was shown in Table (1).

The clove oil was marketed by the Klulich Company (Jan Klulich, hr adec Kralou/Ricay, CR). The quanaldine was obtained from Al-Gomhoria Company, Egypt.

Preparation of anaesthetic solution

One ml of clove oil dissolved in 4 ml ethyl alcohol (95%) (stock solution), then the desired concentration prepared by adding the desired dose to 10 liter of aquarium water. The quanaldine anaesthetic was prepared by adding one ml to 40 liter irrigation water(Gibson, 1967) .

Each fish group was transferred to the anaesthetic jar; to calculate and record the onset of sedation and time elapsed until recovery. During the time of anaesthesia, blood samples were taken from the control and different treatments as follow:

Table 1. ***Design of the experiment***

Group NO.	Type of anaesthesia	Dose	Route of adminstration
G. I	Clove oil	10. 00 ml / 100 l *	Immersion
G. 2	Clove oil	7.50 ml / 100 l *	
G. 3	Clove oil	5.00 ml / 100 l *	
G. 4	Quanaldine	2.50 ml / 100 l	
G. 5	control	-	

Haematological profile

Blood samples were taken from the caudal blood vessels of both control fish (no anaesthetic administration) and immediately after exposure of fish to clove oil and quanaldine at different concenteration. One ml of blood samples was stabilize in aqueous solution of heparin sodium salt 5000 u/ml at a rate of 0.01 ml/1 ml blood for haematological indices (Svobodova and Vykusova, 1991). The indices used to evaluate the haematological profile include the erythrocyte count (ER), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The rest of blood was left to coagulate and centrifuged for 5 min at 3000 rpm/min to obtain the blood serum for the biochemical investigation (total protein, albumin, urea, uric acid, glucose, cortisol, ALT (Alanine amino transferase) & AST (Aspartate amino transferase) according to the method described by Bidinotto et al (1997). Blood samples were preserved in sodium fluoride for estimating blood glucose.

Statistical analysis

Data were statistically analysed according to Bailey (1981). The level of significance between means for the statistical analysis is 0.05.

RESULTS

As shown in Table (2), the higher dose of clove oil (G1) gives shorter time anaesthesia and longer time of recovery.

Results in Table (3), the results showed that Hb (8.1±0.07) and PCV % (26.6±0.5) were higher in G1 than G2 (7.1±0.1, 23.8±0.4) and G3, G4 and G5 (6.3±0.2, 22.0±0.6, 7.4±0.2, 24.8±0.2 and 7.2±0.1, 24.2±0.5), respectively. The MCV results showed that no significant difference between G1, G3 and G5 (121.7±1.5, 123.6±2.6 and 121.1±1.9) respectively. On the other hand, high significant difference appear in G4 (126.9±1.6) compared with G2 (119.5±2.2). Similar results were obtained at MCHC. The results of MCH showed a high significant difference between G3 (123.6±2.6) and

Table 2. Means time of anaesthesia and recovery of different treatments.

Groups	Time of anaesthesia (min)	Time of recovery (min)
G 1	1.2	8.6
G 2	4.3	3.4
G 3	5.3	2.00
G 4	4.3	3.5

Table 3. Effect of clove oil anaesthetia on blood hematological parameters of grass carp at comparison with quanaldine anaesthesia.

Experimental group	Hb g/dl	RBC 10^{12}/l	PCV %	MCV FL	MCH pg	MCHC %
G. I	8.1 a ± 0.07	2.2 a ± 0.01	26.6 a ± 0.5	121.7 ab ± 1.5	32.8 b ± 0.3	35.9 ab ± 1.2
G. 2	6.3 c ± 0.2	1.8 c ± 0.04	22.0 c ± 0.6	119.5 b ± 2.2	119.7 a ± 1.9	34.9 b ± 0.3
G. 3	7.1 b ± 0.1	1.9 c ± 0.02	23.8 b ± 0.4	123.6 ab ± 2.6	123.6 a ± 2.6	36.6 ab ± 0.6
G 4	7.4 b ± 0.2	1.9 c ± 0.04	24.8 b ± 0.2	126.9 a ± 1.6	33.5 b ± 0.5	37.9 a ± 0.7
G 5	7.2 b ± 0.1	2.0 0 b ±0.00	24.2 b ± 0.5	121.1 ab ± 1.9	33.6 b ± 0.4	35.9 ab ± 0.1

Values in the same row having the same latter are not significant different (P<0.05).

G2 (119.7 ± 1.9) than in G1 (32.8 ± 0.3), G4 (33.5 ± 0.5) and G5 (33.6 ± 0.4). No significant difference were recorded among G3 (1.9±0.02), G2(1.8±0.04) and G4 (1.9±0.04) in R B C s, while highly differences were observed between G1 (2.2±0.01) and G5 (2.0±0.0), G2, G3 and G4.

The results of serum biochemical parameters (Table 4) showed that highly significant difference recorded G5 (2.5± 0.01) among other groups G1 (1.6± 0.03), G3 (1.7 ± 0.1), G2 (1.8 ± 0.1) and G4 (1.8 ± 0.1) in uric acid concentration, mean while, no significant difference was observed between G1, G2, G3 and G4.

The results of TP showed higher values in G2 (4.0 ± 0.3) than those recorded in G1 (3.2 ± 0.6), G3 (3.1 ± 0.05), G4 (3.1 ± 0.08) and G5 (3.1 ± 0.05). The glucose concentration showed highly significant difference between G4 (169.6 ± 3.0) compared with G1 (102.5±1.8), G3 (130.3±5.4), G2 (100.8±2.5) and G5 (117.5±1.1).

Higher values of Alb were observed in G1 (1.7±0.02) and G2 (1.68±0.03) than G2, G4and G5 (1.56±0.04, 1.4±0.02 and 1.4±0.02), AST showed increased levels in G4 and G5 comparative with the other groups. The results of ALT showed that G2had the higher values than groups G1, G3, G4 and G5. Cortisol

Table -4. ***Effect of clove oil anaesthesia on biochemical parameters of grass carpat comparison with quanaldine anaesthetia.***

Experiment / Indices	Glucose mg/dl	TP g/dl	Alb g/dl	AST u/l	ALT u/l	Creatinine mg/dl	Urea mg/dl	Uric acid mg/dl	Cortisol µg/dl
G 1	102.5 d ± 1.8	3.2 b ±0.0	1.7 a ±0.02	9.0 b ± 1.1	8.3 b ± 0.3	0.44 b ± 0.0	7.4 a ± 0.2	1.6 b ±0.0	14.7c ± 0.6
G 2	100.8 d ± 2.5	4.0 a ±0.0	1.7a ±0.03	12.3ab ± 1.2	12.6a ± 2.3	0.48 ab ± 0.0	7.8 a ± 0.6	1.8 b ± 0.1	18.7 b ± 0.8
G 3	130.3 b ± 5.4	3.1b ±0.0	1.6b ±0.04	11.6ab ± 1.4	10.0ab ± 1.0	0.49 ab ± 0.0	6.3 b ±0.1	1.7 b ± 0.1	18.8 b ± 0.4
G 4	169.6 a ± 3.0	3.1 b ±0.0	1.4 b ±0.02	13.0ab ± 0.5	8.3 b ± 0.8	0.51 a ± 0.0	7.2ab ± 0.1	1.8 b ± 0.1	26.8 a ± 0.7
G 5	117.5 c ± 1.1	3.1 b ±0.0	1.4 b ±0.02	13.6 a ± 0.3	4.0 ab ± 0.5	0.51 a ± 0.0	7.2ab ± 0.1	2.5 a ±0.0	17. 0 b ± 0.6

Values in the same row having the same latter are not significant different ($P<0.05$).

concentrations showed a significant difference between G4 and those observed in G1, G3, G2 and G5. G3 showed higher values in urea with a high difference among other groups; G1, G3, G4 and G5. Creatinine results revealed that G5 and G4 contained higher values than G1 with a high significant difference, mean while, no significant difference was observed between G2and G3 andG1, G4 and G5.

DISCUSSION

Haematological and biochemical blood profile of grass carp can provide important information about the internal environment of the organism (Masopust, 2000). Values determined in this study suggest that internal organs of the grass carp are not altered by clove oil anaesthesia. While significant increase in blood plasma glucose level after anaesthesia with quanaldine as well as cortisol values may be returned to the difference of mode of action between of both types of compared anaesthesia.

Hikasa et al. (1986) recommended 25-100 ppm clove oil as effective anaesthesia for common carp

(*Cyprinus carpio*). It has been demonstrated that onset time of clove oil anaesthesia for individual fish as well as recovery time were concentration dependent. The same effect of anaesthesia concentration levels on anaesthesia onset times has been described by Hirata et al (1970) for the Crucian carp (*Carassias carassina*). The present study report using 75-100 mg/l clove oil as effective anaesthetic for grass carp. These results agree with Waterstrat (1999) who stated that 100 mg/l clove oil is considered a safe concentration for anaesthesia of channel catfish. The haematological and biochemical parameters in the present research shows a high significant alterations.

Iverzen et al. (2003) found no changes in glucose concentrations of Atlantic salmon following clove oil anaesthesia, while Velisek et al. (2005) stated that significant increase in blood plasma glucose immediately after ten min. clove oil anaesthesia was observed. Yet, the obtained results showed increase of blood glucose levels in control and quanaldine anaesthesia fishes than the clove oil anaesthesia fishes, which may be due to the procedure (handling) caused some stress on these fishes.

Results in the present study showed a significant difference with higher values of plasma cortisol ($P<0.05$) in quanaldine and control than clove oil anesthetic fish. This observation may be due to the short duration of handling associated with moving grass carp into water aquaria contains anaesthesia evoked a cortisol stress response. These results agree with those reported by Thomas and Robertson (1995) who stated that quanaldine exhibited dose related increase in plasma cortisol and glucose level. Also, Brian (2003) found that reflex movement severe in quanaldine anaesthesia. Taylor and Roberts (1999) noted that clove oil appear to be viable anaesthesia for aquaculture use.

CONCLUSION

Clove oil at a concentration of 7.5 ml /100 l appear to be viable, cheap and safe for grass carp broodstock fish.

REFERENCES

Bailey, N. T.(1981) : Statistical Methods in Biology. 2nd ed. (Biological Science Texts).Course on Fresh-Water Fish Diseases and Intoxications. Research Institute of Fish Culture and Hydrobiology, Vodnany, 270 pp.

Bidinotto, P.M.; Souza, R.H.S.; Moraes, G. (1997): Hepatic glycogen in eight tropical freshwater teleost fish: A

procedure for field determinations of microsamples. *Boletim Técnico do Cepta*, 10: 53-60.

Brian, C.S. (2003) : Anesthetic efficacyof metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish (*Ictalurus punctatus*). Aquaculture, 218: 177-185.

Gibson, R.N. (1967): The use of anaesthetic quinaldine in fish ecology. Journal of Animal Ecology,Vol,36 No:2 pp 295-301.

Hikasa Y., Takase K., OgasawaraT., Ogasawara S. (1986): Anaesthesia and recovery with tricane methanesulphonate, eugenol and thiopental sodium in the carp (*Cyprinus carpio*). Japanese Journal of Veterinary Science, 48:341–351.

Hirata M., Isoda S., Kanao M., Shimizu H., Abd Inouse S. (1970): Studies on anesthetics for fish. Bulletin of Japanese Society of Science Fisheries, 36: 1127–1135.

Isaacs G. (1983): Permanent local anaesthesia and anhydrosis after clove oil spillage. Lancet, 1:882–883.

Iverzen M., Finstad B., McKinley R.S., Eliassen R.A. (2003): The efficasy of metomidate, cloveoil, Agui-Sand Benzoak as anaesthetics in Atlantic salmon stress – reducing capacity. Aquaculture, 221: 549–566.

Masopust, J. (2000) : Clinical Biochemistry (in Czech) Karolinium, Prague. 832 pp.

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

Svobodova Z. and Vykusova B. (1991): Diagnostics, prevention and therapy of fish diseases and intoxications. Manual for International Training. Research Institute of fish culture and Hydrobiology, Vodnany, Czechoslovakia.

Taylor P.W and Roberts S.D. (1999): Clove oil: An alternative anaesthetic for aquaculture. North American Journal of Aquaculture, 61:150–155.

Thomas, P and Robertson, L.(1995): Plasmacortisol and glucose stress

responses of red drum (*Sciaenops occllatus*) to handling and shallow water stressors and anesthesia withMs-222, quinaldine sulfate and metomidata. Aquaculture, 96: 60- 86.

Velisek J., Svobodova Z and Piackova V. (2005): Effect of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*). Acta Veterinaria Brno, 74: 139–146.

Waterstrat, P. R. (1999) : Induction and recovery from anaesthesia in channel catfish (*Ictalurius punctatus*) fingerlings exposed to clove oil. J. World Aquaculture Society, 30: 250-255.

دراسه مقارنه لصورة الدم الهيماتولوجيه والوظائف البيوكيمائيه لمبروك الحشائش عند استخدام زيت القرنفل والكوانالدين كمخدرين اثناء فترة التبويض

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المعمل المركزي لبحوث الثروه السمكيه بالعباسه

استهدف البحث دراسة تأثير استخدام زيت القرنفل والكوانالدين كمخدرين لآمهات مبروك الحشائش اثناء فترة التبويض . استخدم لذلك البحث عدد ١٨ سمكه اوزانها تتراوح بين ٢,٦٠٠-٢,٨٠٠ كجم وطولها من ٦٠-٧٠ سم قسمت هذه الاسماك الي خمسة مجاميع متساويه كل مجموعه بها ثلاث سمكات . المجموعه الاولي تم تخديرها بزيت قرنفل ١٠ مللي/١٠٠لتر والمجموعه الثانيه تم تخديرها بزيت قرنفل ٧,٥ مللي/ ١٠٠ لتر والمجموعه الثالثه تم تخديرها بزيت قرنفل ٥مللي / ١٠٠لتر والمجموعه الرابعه تم تخديرها بالكوانالدين ٢,٥ مللي / ١٠٠لتر حيث هو المستعل دائما في المفرخات والمجموعه الخامسه استخدمت كمجموعه ضابطه (كنترول) وكانت النتائج كما يلي:

استخدام زيت القرنفل بمعدل ٧,٥ مللي لكل ١٠٠ لتر ادي الي تخدير الآسماك مع افضل زمن استرجاع ٣ دقيقه و٤٠ ثانيه و لم توجد هناك اختلافات معنويه بين مقاييس الدم المختلفه والمجموعه الضابطه. بينما استخدام الكوانالدين ادي الي اختلافات معنويه كبيره بين مقاييس الدم المختلفه وخاصة الجلوكوز والكورتيزول و المجموعه الضابطه.

مما سبق نوصي باستخدام زيت القرنفل بالمعدل السابق لعدم تأثيره علي المقاييس المختلفه للدم (كوضع امن) ولتوفره بأسعار رخيصه.