Hormonal and Glucose Levels *in Clarias gariepinus* Exposed to Synthetic Anaesthetic Drugs

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ABSTRACT: Nine hundred and sixty African catfish (*Clarias gariepinus),* which consists of 480 each of juveniles (mean length 26.64cm ± 1.02 SE; mean weight 356.21g ±1.86 SE) and adults (mean length 52.13cm ±1.01SE; mean weight 1100.38g ± 3.04SE) were exposed to four anaesthetics drugs in the laboratory. The anaesthetics used were: Tricaine methane sulfonate (MS-222); Metomidate hydrochloride; Eugenol and Sodium bicarbonate. The exposure of this fish to different anaesthetics caused a significant alterations (P <0.05) in hormonal (cortisol, triiodothyronine – T_3, and thyroxine – T_4) and glucose levels in the blood of this species. The highest hormonal response (155.20 ± 6.16) was in cortisol in the adult fish exposed to Sodium bicarbonate and the lowest in T_4 in the fish exposed to eugenol. However, glucose levels increased significantly (P < 0.05) with increasing concentration of the anaesthetics.

Key Words: Fish, Anaesthetics, Biochemical, Hormones, Haematology

I. INTRODUCTION

In Aquaculture operations some of the procedures imposed stress on the fish. Therefore, it has become necessary to introduce a number of practical approaches, in order to ameliorate the detrimental consequences of stressful conditions in fish [1-2]. One of the method commonly use to reduce the risk of routine stress in day to day aquaculture practice, is treatment of fish with anaesthetics[3]. However, higher dosage of anaesthetics can trigger stress in fish [4]. The resulting stress response of fish to various degrees of anaesthesia can be classified as endocrine, metabolic and whole animal responses [5]. Hence, it is of prime importance that reliable indicators are used to assess anaesthetics application in fish in relation to their stress responses. Hormonal assay and glucose have been identified as major stress indicators in fish [6].

Stress-related changes reflect in blood chemistry within a short time when fish are disturbed in the culture medium. These include changes in plasma hormones, energy metabolism, enzymatic reactions and electrolytes balance. Ichthyologists make use of blood chemistry indices for the evaluation of fish responses to stress, nutritional conditions, tissue damage due to handling procedures and health status[7]. Researchers usually evaluate fish responses to drug application in aquacultural practice by studying blood biochemical properties[8]. Several studies have confirmed that anaesthetics such as MS-222, clove oil, metomidate and others affect blood biochemical parameters including glucose, cortisol and hormones such as thyroids when applied at higher dosages [9-11].

In higher vertebrates, thyroid hormones (TH) are known to influence an extensive array of physiological processes, such as development, metabolism, homeostasis, reproduction and adaptation[12]. While in lower vertebrates, its role as a metamorphosis inducing hormone in amphibians and fishes is probably the best known characteristic of TH [13]. The production of TH is controlled by thyroid stimulating hormone (TSH) from the pituitary. It is a small liposoluble molecule with non species specifically and has two bioactive forms, tetraiodo-L-thyronine (T_4) and triiodo-L-thyronine (T_3), thus containing four or three iodine atoms in a molecule[14]. Fishes are known to recognize stressor of inherent and external origins. They respond to these stressors with an intricate network of neuroendocrine and physiological responses. Cortisol is the major corticosteroid in teleost fishes and its plasma concentrations rises considerably during traumatic circumstances [15]. Although many studies have assessed the relative effects of anaesthetics on fish, few to date have evaluated the associated impacts of

these sedative agents on thyroid hormones in fish. This paper therefore evaluates hormonal and glucose levels in fish exposed to some commonly used anaesthetics in the laboratory.

II. EXPERIMENTAL WORK

SOURCES OF EXPERIMENTAL FISH

A total of nine hundred and sixty (960) apparently healthy *C. gariepinus*, consisting of 480, 12 weeks old juvenile fish (mean length 26.64cm ± 1.02SEM; mean weight 356.21g ±1.86SEM) and 480, 24 weeks old adult sizes (mean length 52.13cm ±1.01SEM; mean weight 1100.38g ± 3.04SEM) were sampled from African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt rearing concrete tanks adjacent to the experimental site. These tanks are being stocked intermittently for at least two production cycles annually. The fish were harvested from the tanks, after drainage. They were immediately transferred into holding tanks in the hatchery.

FISH ACCLIMATION

In the hatchery, the fish were acclimated to laboratory conditions for a period of seven days, following the method of Gabriel *et al.* [12], who recommended that fish for experimental purposes must be handled carefully and stocked in a well aerated holding tank, so as to reduce the incidence of stress to the barest minimum. During this period the fish were fed daily with ARAC feed (40% CP) at 5% body weight and the water in the holding tanks were renewed daily.

ANAESTHETICS

Four synthetic anaesthetics namely: Tricaine methane sulfonate (MS-222), metomidate hydrochloride sold as 'Tranquil', eugenol and sodium bicarbonate were used for the experiment these were purchased off shelf from Agric Consultants Shop in Port Harcourt, Rivers State, Nigeria.

PREPARATION OF TEST SOLUTION

A stock solution of the anaesthetics was prepared by adding 1ml of the anaesthetic concentrate to 1 liter of water. Exposure concentration of anaesthetics were 0.00 (control); 50, 100, 150, and 200ml/L. Thirty, 50L plastic containers were labeled and each filled with water from the borehole to the 30L mark, and another 30 plastic containers were filled with fresh water without anaesthetics were placed side by side. The different concentrations were prepared by serial dilution by measuring 50, 100, 150 and 200 of the stock solutions (x30) that was made into 30L with the borehole water that gave the desired concentrations. The same thing was done for each of the eight anaesthetics.

EXPERIMENTAL PROCEDURE

The anaesthetic solution was then stirred with a glass rod (50cm in length) for homogeneous mixture. Within 10 minutes the tanks were randomly stocked with four juveniles per tank, while four adult fish were equally stocked per tank, using a scoop net. Three tanks each were used for each concentration and the control in each of the fish sizes. The tanks were not aerated during the experimental period. Duration of fish exposure to various anaesthetics at different concentrations depends on the induction and recovery time. The same procedure was repeated for all the eight anaesthetics. A total of 480 fish were sampled for blood, 240 each from Juvenile and adult sizes. In each of the eight anaesthetics under consideration, 60 fish were sampled with 30 each for juvenile and adult sizes making it two fish in each experimental tank (in triplicates). Blood samples were taken at the deepest anaesthesia, when the fish was completely immobilized.

CORTISOL

Cortisol assay was done in the laboratory using time resolved floroimmuno assay (TR - PM) technique. Using kits obtained from Sorrin Biomedical Division, New Delhi, India following the method described by Small and Davis [16].

THYROID HORMONES (T_3 and T_4)

The plasma levels: T_3 and T_4, were measured with the aid of radioimmunology assay kits, RIAK5/5A, for T_4 (sensitivity 0.625mg/ml, based on 93.67% B/Bo intercept) and RIAK4/4A for T_3 (sensitivity 0.0375ng/ml, based on 90.77% B/Bo intercept). Radioimmunoassay (RIA) for T_4 and T_3 were conducted following the manufacturer protocols, using the methods of Chopra (1979). The RIA was validated using hormone-free fish plasma. The

radioactivity in the bound fraction was counted with the help of a well-type gamma counter (Electronic Corporation of India, Hyderabaz, India). The concentrations of total T_4 and T_3 were expressed as ng/ml of plasma.

STATISTICAL ANALYSIS

The data obtained from the study were collated and analyzed using statistics software 8.0 for windows. Data were first tested for normality (Kolmogorov - Smirnov test) and homosesdasticity of variance (Bartetts test). When these conditions were satisfied, a two way analysis of variance (ANOVA) was employed to reveal significant differences in measured variables among control and experimental groups. When a difference was detected ($P < 0.05$), Tuckey's multiple comparison test was applied to identify which treatment were significantly different [17].

III. RESULTS AND DISCUSSION

The hormonal and glucose levels in *C. gariepinus* exposed to various anaesthetic agents indicated that the cortisol level in the fish exposed to eugenol increased significantly ($P<0.05$) from control 79.23±l.15 ($nmolL^{-1}$) and 102.13±2.00 ($nmolL^{-1}$) and peaked at 94.00±4.70 ($nmolL^{-1}$) and 112.33±2.10 $nmolL^{-1}$ for juvenile and adult fish respectively (Table 1). The thyroid-stimulating hormones, triiodothyronine (T_3) and thyroxine (T_4) levels in both sizes of fish, revealed significant differences obtained at higher concentrations of 150.0 and 200.0mlL^{-1}. Moreover, glucose mean values were found to be elevated from 2.13±0.57$mgdL^{-1}$ (control) to 5.03±0.11$mgdL^{-1}$ (200mlL^{-1}) in juvenile fish, and 3.67±0.57$mgdL^{-1}$ (control) to 5.80±0.20$mgdL^{-1}$ (200mlL^{-1}) in adult size (Table 1). In the fish exposed to metomidate (Table 2), the mean values of cortisol were within the same range with no significant difference ($P>0.05$) at 50, 100, 150 and 200mlL^{-1} in both life stages of the fish. The thyroid stimulating hormones, T_3, T_4 and the glucose levels increased significantly when compared to the control values. This level of significant ($P <0.05$) were recorded from 100mlL^{-1} concentration of metomidate.

The mean cortisol values in *C. gariepinus* treated with anaesthetic MS-222 (Table 3) revealed that the cortisol levels in juvenile fish, increased significantly ($P<0.05$) from 79.80±0.60 in control to 102.00±4.05$nmolL^{-1}$ (200mlL^{-1}), while in the adult fish, the cortisol level was significantly ($P<0.05$) elevated from 10l.80±l.57 (control) to 122.61±1.15nmol (200mlL^{-1}). The thyroid stimulating hormones, T_3 and T_4 along with the glucose values were elevated significantly as the concentration of MS-222 increased. An indication that these elevations were dose dependent (Table 3). The fish exposed to sodium bicarbonate experienced high levels of cortisol, when compared to the control (Table 4). The cortisol levels increased significantly ($P<0.05$) as the concentrations of sodium bicarbonate increased. A similar trend of dose dependent increase was noted in the values of T_3, T_4 and glucose levels, which were more pronounced at higher concentrations from l00mlL and above of the chemical.

The functions of thyroid hormones (THS) in stress response in fishes are important, mainly because of the contribution of THS in virtually all aspects of physiological activities. This has prompted the pioneers of thyroid research to set off numerous trials to define the THS action during stress situations, particularly upon treatment with different drug concentrations [18]. A well defined and multi step regulation of THS homeostasis exists in fish. This is because of the ability of the thyroid axis to respond to the stimuli, even at the slighted provocation [18]. In the present study, the levels of T_3 and T_4 were elevated as the concentrations of the anaesthetics increased. These dose dependent increments were consistent with that of Holloway *et al.* [18] in rainbow trout (*Oncorhynchus mykiss*) exposed to clove oil and MS-222. Also, Power *et al.* [19] reported a significant increase in the levels of serum T_3 and T_4 in climbing perch, (*Anabas testudineus)* exposed to nimbecidin a neem based bio-pesticide. This response modified the metabolic pattern of the fish, suggesting a role for THS in stress acclimation. On the contrary, there are reports on inhibition of circulating thyroid hormones in common carp (*Cyprinus carpio)* exposed to eugenol, tricaine methane sulphonate and thiopental sodium [20]. On the hand, activation and inactivation of thyroid functions during stressor exposure has been reported [20].

Several authors have reported different views on the status of plasma glucose during anaesthetic application in fish. For instance, Ortuno *et al.* [21] tested four anaesthetics (MS-222, benzocaine, 2-phenoxyl-ethanol and quinaldine) in gilthead seabream (*Sparus aurata*) and reported that basal glucose level was 3.6$mmolL^{-1}$ and increased to 5.6, 11.1, 11.9 and 16.4$mmolL^{-1}$, when exposed to MSS-222, bencocaine, 2-phenoxyethanol and quinaldine respectively. Despite this, Iversen *et al.* [21], did not observed any changes in glucose levels in Atlantic salmon (*Salmo salar*) exposed to anaesthetics such as, metomidate, clove oil, Aquis and Benzoak.However, Velisek *et al.* [22] reported a significant increase in plasma glucose of rainbow trout (*Oncorhynchus mykiss*) when

anaesthetized with clove oil. Interestingly, Wagner *et al.* [23] observed that clove oil and MS-222 blocked cortisol secretions, but increased glucose levels in rainbow trout. These different results which suggests elevation or reduction of glucose levels is anaesthetics and species dependent.

The plasma level of cortisol is often considered as a measure of the magnitude of stress response and under acute stress it can easily rise up many folds, to enhance the mobilization of balance energy reserves, metabolic rate and electrolytes[24]. The basal plasma cortisol levels in *C. gariepinus* held under small – scale rearing conditions in the present study was between 78 – 82 nmoll^{-1} for juveniles and 100-106 nmoll^{-1} for adults within the range generally considered representative of unstressed fish [24]. In the present study, the sample fish in both juveniles and adult, regardless of the type of anaesthetic used, had significantly elevated plasma cortisol levels after exposure, especially at higher concentration (200.0 mlL^{-1}) when compared with fish sampled for basal levels (control) except in eugenol and metomidate. Among the synthetic anaesthetics under consideration, elevation in cortisol levels was more pronounced in fish exposed to sodium bicarbonate. These results demonstrated that sodium bicarbonate exacerbates the cortisol stress response in *C. gariepinus* as in channel catfish (*Ictalurus punctatus)* exposed to synthetic anaesthetics. In the present work, cortisol levels remains unchanged in fish exposed to metomidate, as was evident in the findings of Iversen *et al.* [25] in Atlantic salmon (*Salmo salar*), exposed to metomidate and Davis and Griffin [26], in sunshine bass . (*Morone saxatilis*) exposed to seven synthetic anaesthetics. They noted that exposure to a sedating concentrations in all the anaesthetic used except metomidate, resulted in a significant increase of plasma cortisol. Also in assessing the effects of various anaesthetics on cortisol secretion in channel catfish, Small [27] found metomidate to be effective at suppressing stress induced by cortisol increase.

Table 1: Hormonal and Glucose Levels in *C. gariepinus* Treated with Eugenol (Mean ± SD)

		Concentrations (mlL^{-1})				
Life Stage	Variables	0.00	50.00	100.00	150.00	200.00
Juvenile	Cortisol(nmol/L^{-1})	79.23±1.15^{ab}	79.07±1.60^{a}	82.4±2.25^{a}	91.17±3.00^{a}	94.00±4.70^{a}
	T_3 (nmol/L^{-1})	5.00±0.36^{a}	5.20±0.36^{a}	5.30±0.10^{a}	7.63±0.45^{ab}	8.90±0.30^{c}
	T_4 (nmol/L^{-1})	3.10±0.26^{a}	2.36±0.37^{a}	3.96±0.11^{a}	5.26±0.50^{ab}	5.80±0.40^{ab}
	Glucose (mgdl)	2.13±0.57^{a}	3.12±0.72^{b}	4.20±0.10^{ab}	4.80±0.00^{ab}	5.03±0.11^{c}
Adult	Cortisol (nmol/L^{-1})	102.13±2.00^{a}	104.93±1.61^{a}	106.96±1.68^{a}	110.66±1.41^{a}	112.33±2.10^{ab}
	T_3 (nmol/L^{-1})	10.23±0.41a	11.60±1.05^{a}	12.20±1.00^{a}	16.63±0.37^{ab}	17.53±0.30^{ab}
	T_4 (nmol/L^{-1})	6.67±0.83^{a}	7.53±0.61^{a}	8.13±0.57^{ab}	9.36±0.21^{ab}	12.43±0.35^{c}
	Glucose (mgdL^{-1})	3.67±0.57^{a}	4.00±0.10^{ab}	4.33±0.57^{ab}	5.16±0.21^{c}	5.80±0.20^{c}

Mean within the row with different superscripts are significant (P<0.05)

Table 2: Hormonal and Glucose Levels in *C. gariepinus* Treated with Metomidate (Mean ± SD)

		Concentrations (mlL^{-1})				
Life Stage	Variables	0.00	50.00	100.00	150.00	200.00
Juvenile	Cortisol(nmol/L^{-1})	79.3±1.05^{a}	79.60±1.21^{a}	78.37±3.28^{a}	78.40±2.30^{a}	73.33±2.19^{a}
	T_3 (nmol/L^{-1})	5.10±0.43^{a}	5.73±0.70^{a}	6.53±0.30^{ab}	8.13±0.30^{c}	9.86±0.57^{d}
	T_4 (nmol/L^{-1})	3.16±0.35^{a}	3.93±0.30^{a}	4.43±0.32^{ab}	6.13±0.20^{c}	6.33±0.30^{c}
	Glucose (mgdl)	2.13±0.57^{a}	2.86±0.66^{b}	4.00±0.00^{ab}	4.63±0.57^{ab}	4.93±0.24^{ab}
Adult	Cortisol (nmol/L^{-1})	101.80±1.70	100.53±2.11^{a}	100.47±1.22^{a}	78.33±2.73^{a}	96.60±1.91^{b}
	T_3 (nmol/L^{-1})	10.06±1.27a	12.03±0.90^{ab}	14.43±1.33^{ab}	17.50±0.43^{c}	18.23±0.35^{c}
	T_4 (nmol/L^{-1})	7.06±0.31^{a}	8.17±0.55^{a}	9.16±0.25^{ab}	10.70±0.26^{c}	13.60±0.20^{d}
	Glucose (mgdL^{-1})	3.60±0.01^{a}	3.90±0.10^{a}	4.13±0.57^{ab}	5.06±0.11^{c}	5.80±0.52^{c}

Mean within the row with different superscripts are significant (P<0.05)

Table 3: Hormonal and Glucose Levels in *C. gariepinus* Treated with MS-222 (Mean±SD)

		Concentrations (mlL^{-1})				
Life Stage	Variables	0.00	50.00	100.00	150.00	200.00
Juvenile	Cortisol($nmol/L^{-1}$)	79.80 ± 0.60^{a}	85.50 ± 1.25^{ab}	85.67 ± 4.08^{ab}	94.13 ± 2.8^{c}	102.00 ± 4.05^{d}
	T_3 ($nmol/L^{-1}$)	5.13 ± 0.25^{a}	6.33 ± 0.45^{ab}	7.56 ± 0.47^{c}	9.13 ± 0.30^{d}	10.20 ± 0.2^{e}
	T_4 ($nmol/L^{-1}$)	3.20 ± 0.26^{a}	4.33 ± 0.50^{a}	6.36 ± 0.45^{ab}	7.06 ± 0.94^{c}	8.76 ± 0.15^{d}
	Glucose (mgdl)	2.16 ± 0.11^{a}	3.43 ± 0.12^{b}	4.50 ± 0.10^{ab}	5.56 ± 0.12^{ab}	6.30 ± 0.11^{c}
Adult	Cortisol($nmol/L^{-1}$)	101.80 ± 1.57^{a}	112.43 ± 2.07^{a}	118.07 ± 1.20	119.73 ± 3.97^{ab}	122.6 ± 1.15^{c}
	T_3 ($nmol/L^{-1}$)	$10.06\pm0.94a$	13.56 ± 0.15^{ab}	15.00 ± 1.62^{ab}	19.73 ± 0.11^{c}	21.57 ± 1.68^{c}
	T_4 ($nmol/L^{-1}$)	7.06 ± 0.41^{a}	8.50 ± 0.26^{ab}	12.40 ± 0.11^{c}	12.53 ± 0.23^{c}	14.43 ± 0.61^{d}
	Glucose ($mgdL^{-1}$)	3.56 ± 0.57^{a}	5.03 ± 0.37^{a}	6.40 ± 0.30^{c}	8.33 ± 0.15^{d}	9.36 ± 0.25^{c}

Mean within the row with different superscripts are significant (P<0.05)

Table 4: Hormonal and Glucose Levels in *C. gariepinus* Treated with Sodium Bicarbonate (Mean±SD)

		Concentrations (mlL^{-1})				
Life Stage	Variables	0.00	50.00	100.00	150.00	200.00
Juvenile	Cortisol($nmol/L^{-1}$)	81.23 ± 0.15^{a}	87.00 ± 0.51^{a}	94.83 ± 3.74^{ab}	99.50 ± 0.75^{ab}	114.10 ± 1.50^{c}
	T_3 ($nmol/L^{-1}$)	5.20 ± 0.34^{a}	7.20 ± 0.79^{ab}	8.70 ± 0.52^{ab}	10.47 ± 0.55^{c}	11.36 ± 1.81^{d}
	T_4 ($nmol/L^{-1}$)	3.23 ± 0.31^{a}	4.50 ± 0.40^{ab}	5.36 ± 0.25^{ab}	6.90 ± 0.20^{c}	8.13 ± 0.64^{d}
	Glucose (mgdl)	2.16 ± 0.57^{a}	4.20 ± 0.10^{ab}	5.90 ± 0.12^{ab}	6.93 ± 0.11^{c}	8.00 ± 2.88^{d}
Adult	Cortisol($nmol/L^{-1}$)	102.43 ± 2.15^{a}	116.90 ± 4.32^{b}	123.50 ± 1.27^{ab}	128.36 ± 3.61^{ab}	156.20 ± 6.16^{c}
	T_3 ($nmol/L^{-1}$)	$10.43\pm0.41a$	14.06 ± 0.15^{b}	15.16 ± 0.83^{ab}	16.43 ± 0.25^{ab}	22.20 ± 0.56^{c}
	T_4 ($nmol/L^{-1}$)	7.30 ± 0.26^{a}	9.23 ± 0.35^{b}	10.60 ± 0.30^{ab}	11.40 ± 0.20^{ab}	15.76 ± 1.05^{dc}
	Glucose ($mgdL^{-1}$)	3.56 ± 0.15^{a}	5.80 ± 0.10^{b}	7.03 ± 0.21^{b}	9.36 ± 0.83^{ab}	11.56 ± 1.10^{c}

Mean within the row with different superscripts are significant (P<0.05)

CONCLUSION

The anaesthetics under consideration altered significantly the physiological variables s in *C.gariepinus*, which were more pronounced in MS -222, sodium bicarbonate, at higher concentration of 200.0 mlL^{-1}. The physiological parameters assessed were mild in eugenol, and metomidate. The responses of *C. gariepinus* to application of these anaesthetics were size related, with the effects more pronounced in adult fish than the juveniles.

REFERENCES

1. Bauqurer, S.H., Greenwood, J., and Whittem, T. Evaluation of the sedative and anaesthetic effects of five different concentrations of alfaxalone in gold fish (*Carassius auratus).* Aquaculture, 2013, 399:119-123.
2. Gabriel, U.U ., and Akinrotimi O.A. Management of stress in fish for sustainable aquaculture development. Researcher, (2011), 3 (4): 28-36.
3. Akinrotimi, O.A., Edun, O.M., and Eddie Dan, M. Effects of Clove seed as anaesthetics agents in two species of grey mullets (*Liza falcipinnis* and *Liza grancisquamis). Journal of Aquatic Sciences*, 2013,1(1): 7-10.
4. Barton, B.A. Stress in fish; A diversity of responses wish particular references to changes in circulating corticosteroids. Integrative Comparative Biology, 2002, 42: 517-528.
5. Sneddon, L.U. Trigeminal somato sensory innovations of the head of a teleost fish with particular reference to nociception. Brain Research, 2003, 972: 44-52.
6. Miller, S.M., Mitchell, M.A., Hartley, J.J., Wolf, T., Lapuz, F., and Smith J.A.C. Clinical and cardio respiratory effects of protocol in the spotted bamboo shark (*Chiloscyllium plagiosum).* Journal of Zoology and Wild Animal Medicine, 2005, 36: 673-676.
7. Wagner, E., Arndt, R., and Hilton, B. Physiological stress haematological and biochemical response in rainbow trout brood stock anaesthesia with clove oil, tricaine sulfonate. Aquaculture, 2008, 211: 353-366.
8. Feng, G., Zhoang, P., Zhang, L., Kynand, B., Liu J., and Huang, X. Effects of anaesthetics MS-222 and clove oil on blood biochemical parameters of juvenile Siberian sturgeon, (*Acipenser baerii).* Journal of Applied Ichthyology, 2011, 27: 595-599.

9. Akinrotimi, O.A., Gabriel, U.U., and Orokotan, O.O. Changes in enzymes activities of *Clarias gariepinus* brood fish exposed to anaesthetics metomidate. *Applied Ecology and Environmental Science*, 2013, 1(3): 37-40.
10. Haga, Y., Suzuki, T., and Takevehi, T. Retinoic acid isomers produce malformations in post embryonic developments of Japanese flounder (*Paralicthys olivaceus).* Zoological Science, 2002, 19: 1105-1112.
11. Akinrotimi, O.A., Aranyo, A.A. and Ibemere I. F. Effects of capture, handling and confinement on the glucose levels of black jaw tilapia (*Sarotherodon melanotheron)*. Advances In students Research, 2011, *6* (2): 27-30.
12. Gabriel, U.U., Deekae, S.N., Akinrotimi, O.A., & Orokotan, O.A. Haematological response of *Clarias gariepinus* exposed to anaesthetic metomidate. Continental Journal of Pharmacology and Toxicology Research, 2012, 4 (1): 18-29.
13. Greenblatt, M. Changes in thyroid hormone levels in eggs and larvae and iodide uptake by eggs of coho and chinook salmon. Fish Physiology and Biochemistry, 1989, 6:261-278.
14. Dapeng, L., Ping, X., and Xuezhen, Z. Changes in plasma thyroid hormones and cortisol levels in crucian carp, exposed to the extracted microcystins. Chemosphere,2008, 74: 13-18.
15. Sattari, A., Mirzargar, S.S., Abrishamifa, A., Lourakzadega, R., and Miasari, A. Comparison of Electroanaesthesia with chemical anaesthesia (MS-222 and clove oil) in rainbow trout (*Oncorhychus mykiss) using* plasma cortisol and glucose responses as physiological stress indicators. Asian Journal of Animal and Veterinary Advances, 2009, 4 (6): 306-313.
16. Small, B.C., and Davis, K.B. Validation of a time – resolved fluuroimmunanoassay for measuring Plasma cortisol in channel, catfish (*Ictalurus punctatus).* Journal of World Aquaculture Society, 2002, 33: 184-187.
17. Zar, J.H *Biostatistical Analysis (*4th edition*)*, New Jersey, U.S.A. Prentice Hall, publishers. 1996.
18. Holloway, A. C., Keene, J., Noakes, D. G., and Moccia, R. D. Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout (*Oncorhynchus mykiss).* Aquaculture Research, 2004, 35: 1025-1030.
19. Power, D.M; Liwellyn, L., Fausho, M., Nowell, M.A., Bjornsson, B.T and Sweney G.E Enzymes and hormones in growth and development of fish. Comparative Biochemistry and Physiology, 2010,140: 879-903.
20. Hikasa, Y., Takase, T., Ogasawara, T., and Ogasawara, S. Anaesthetic recovery with tricaine methane sulfonate, and energy and thiopental sodium in the carp (*Cyprinus Carpio)*. Japanese Journal of Veterinary Science, 1986, 48: 341-351.
21. Darras, V.M., Geris, K.I., Buyse, L.R., and Kuhn, E.R. Thyroid hormone secretion and peripheral deiodination in fish, hormonal and nutritional influences. Journal of Endocrinology, 2007, 102: 317-328.
22. Velisek, J., Svobodova, Z., and Piackova, V. Effects of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss).* Acta Vetrinary Bruno, 2005, 74: 139-146.
23. Wagner, G. N. Singer, T. D., and Mckinley, R.S. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus* mykiss). Aquaculture Research, 2003, 34: 1139-1146.
24. Williams,T.D., Rollins, M., and Block, B.A. Intramuscular anaesthesia of bonito and pacific mackerel with ketamine and metomidine and reversal of anaesthesia with antipamazole. Journal of Vetrinary Medicine Association, (2004), *225*, 417-421.
25. Iversen, M., Eliassen, R.A.,and Fibstad B. Potential benefit of clove oil sedation on animal welfare during salmon smolt, (*Salmo salar)* transport and transfer to sea. Aquaculture Research, 2009, 40: 233-241.
26. Davis, K.B., and Griffin B.R. Physiological responses of hybrid stiped bass under sedation by several anaesthetics. *Aquaculture,* 2004, 233, 531-548.
27. Small, B. C. Effect of isoeugenol sedation on plasma cortisol, glucose, and lactate dynamics in channel catfish (*Ictalurus punctatus)* exposed to three stressors. Aquaculture, 2008, 238, 469-481.