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RELATIONSHIPS OF CHEMICAL COMPOSITION, QUANTITY OF MILT TO FERTILITY AND HATCHABILITY OF *CLARIAS GARIEPINUS (BURCHELL, 1822)*

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ABSTRACT

The biochemical composition of milt and the effect of its varying quantity on fertility and hatchability of *Clarias gariepinus* (African catfish) eggs were investigated. The study aimed at determining the right quantity of milt that can be used to fertilize certain quantity of eggs. The study further examined the effect of varying volume of milt on the water quality during the incubation of *C. gariepinus* eggs in order to provide baseline information on chemical composition, which can be used as a basis for sperm-mediated gene transfer. Result obtained from this study showed that 0,035ml of milt gave the best hatchability 2041±90.9, while 0.175ml of milt gave the least hatchability 1341± 384.2 .There was a positive significant correlation between the quantity of milt used and hatchability ($P< 0.05$) and there was a significant difference among the treatments and within the hour of the experiment ($P< 0.05$). The investigation revealed that the quantity of milt used for artificial reproduction in fish significantly affect water quality used for incubation ($P <0.05$). The higher the quantity of milt, the lower the Dissolved Oxygen (DO) level, there was equally a buildup of pollutants as the quantity of milt increased. Biochemical analysis of milt showed that glucose at a concentration of 8585±6.05mg/l is the major organic substance that supplies the spermatozoa with energy. Sodium and chloride are the major electrolytes with 113:7±0.88 mg/l and 100± 1.73mg/l respectively and the pH of the milt was 6.2±0.17mg/l. The albumin content was low, with a mean value of 1.3±0.9mg/l. The glucose level was high having a mean value of 85.0±6.08mg/l and the cholesterol level was equally high with a mean value of 188.0± 13.67mg/l. The triglyceride content of the milt was high having a value of 202±19.88mg/l. The high-density lipoprotein cholesterol was low, with a mean value of 13± 6.01 when compared with the low density lipoprotein cholesterol which had a mean value of 134 ± 11.37. There was low motility of sperm in fish milt which may be due to a number of factors ranging from exposure to light, to low pH and aerobic breakdown of glucose to supply energy for the spermatozoan (which is very low).

Key words: Biochemical, *Clarias gariepinus*, milt, fertility, hatchability

INTRODUCTION

It has been estimated that about ten million people die every year in the world either through starvation or malnutrition [1]. As the world population increases, the demand for fish in the world also grows [2]. Inspite of high preference for fish and fishery products in Africa, the *per capita* consumption of fish in this part of the world is still very low [2]. A decline in fish availability will have a detrimental effect on the nutritional status of man as fish contributes significantly to the protein intake of the people in most countries of Africa. In Nigeria, fish constitutes 40% of animal protein intake [3]. This means that any short fall in fish supply will affect the animal protein intake of Nigerians. From a global perspective, over 50% of the world's fish stocks are either fully or over exploited [4, 5]. Analysis of trends by FAO [6] show that many marine fish stocks are in decline, inland fish stocks are under threat from environmental change and impacts, while aquaculture continues to develop and expand in many parts of the world. *C. gariepinus* had already been firmly entrenched as one of the world's most important fish by the start of the twentieth century. With increased emphasis on fish culture in Nigeria and the advent of modern techniques, *C. gariepinus* has become even more valuable to man [7].

Fingerling production and availability of quality fish feeds have been bottlenecks for development of fish farming in Nigeria for the past 40 years [8]. Over the past several years, private sector fingerling production has increased from some 3 million per year in 2001 to more than 30 million per annum at present with several large producers delivering more than 300, 000 fingerlings monthly [8]. There are 24 species of Clarias [9] in Nigeria and only the *Clarias gariepinus* has been singled out as the best fish for culture instead of the *Clarias Lazera* from central Africa which was first cultured intensively by Dutch Scientists working in Bangui, Central African Republic in the late 1970's. The cultivation of many economically important species has been helped greatly by the growing use of artificial fertilization and incubation. The artificial spawning and fertilization of many species to which this technique could not be applied formerly is now possible due to hypophysation. The eggs of the fish obtained in this way are generally incubated artificially to guarantee success.

Nevertheless, the study of artificial fertilization is particularly important in species whose gamete are hand-stripped since this may decrease the variability of reproduction yield as noted in many commercial hatcheries in Nigeria.

There is, therefore, need to investigate the volume of milt that can be used to fertilize effectively certain quantity of eggs (sperm-egg ratio) in order to improve rate of fertilization and hatchability.

MATERIALS AND METHOD

The experiment was carried out at the research laboratory of the Department of Wildlife and Fisheries Management, University of Ibadan, Oyo State, Nigeria. Oyo state is located in the southwestern part of Nigeria, within 2°31′ - 5°30′ E and 6° 45′ – 9°15′ N. Thirty Brooders with mean weight of 500± 0.5g were obtained from a

reputable fish farm in Oyo State, Nigeria. The fish were sexed using the Holden and Reed [10] and Viueen *at al.* [11] methods.

Experimental procedure

The female spawners were injected using Ovaprim^{TM}; 0.5ml/kg. Ovaprim^{TM} is exclusively distributed by Syndel Laboratories Ltd and manufactured by The Aquatic Sciences companies. Ovaprim^{TM} is a potent ovulating/spermiating agent to promote and facilitate reproduction of many species of fish. The hormone (Ovaprim^{TM}) was drawn into syringe and injected intra-muscularly into the female brooder at the angle of 30^0 -45^0 in the dorsal muscle. The fishes were then separated individually into separate bowls and covered with nets to prevent them from escaping.

After about 10 hours of injection, the females were stripped of their eggs into dried plastic containers; the males were sacrificed to remove their testes. 10g of eggs were weighed out into each of five separate plastic containers. Small incisions were made into the lobes of testes, the milt was squeezed out into a Petri dish and volume of the milt (ml) was measured using a syringe and a drop of milt from the syringe is equivalent to 0.035ml. The testes was lacerated and 0.035 ml of milt released into the first plastic with eggs and labeled T_1, 0.070ml of milt into the second plastic with eggs and labeled T_2, 0.105ml into the third and labeled T_3, 0.140ml into the fourth and labeled T_4, and 0.175ml into the fifth and labeled T_5. Each batch of eggs with milt was stirred individually and then transferred into hatching net in separate 46cm x 26cm x 30cm glass tanks. Flow through system was used for water circulation using 2mm diameter drip-hose. The experiment was carried out in triplicate.

Water quality analysis

The following water quality parameters were analysed using the method described by Boyd [12] and AOAC [13]; Dissolved oxygen, (DO), Temperature; Biological Oxygen Demand (BOD); Nitrites, Hydrogen sulphide and Hydrogen ion concentration (pH). The pH was measured by the use of Hanna instrument pH 211-micro processor pH-meters. The interval between collection of water samples for the analysis of the above parameters were as follows: the first collection was done at the zero hour i.e. immediately the fertilized eggs were incubated in the tanks; the second collection was done at the 12th hour and the third collection was done at 24th hour.

Chemical composition of milt

The males were sacrificed to remove their testes. The testes were stored using saline solution and immediately taken for assessment at the surgery and reproduction laboratory of the Faculty of Veterinary medicine, University of Ibadan. Sperm mass activity: progressive motility, live-dead ratio and morphology were determined by conventional methods [14]. The biochemical analysis of milt was done, at the chemical pathology laboratory of the University College Hospital, Ibadan using standard analytical technique described by AOAC [13]. The following analyses were also conducted: sodium, potassium, chloride, calcium, inorganic phosphate, total protein, albumin, glucose, lipid profile: cholesterol, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol of the milt.

Morphological observation was determined from a total count of 100 spermatozoa in smears obtained with nigrosin and eosin for live-dead count. Dead spermatozoa picked up the stain while live spermatozoa did not pick up the stain. The hatchability ratio was determined as:

$$\text{Hatchability} = \frac{\text{Number of eggs hatched (Fry)}}{\text{Number of eggs incubated (fertilized)}}$$

$$\%\text{Egg Hatching} = \frac{\text{Number of whitish broken eggs} \times 100}{\text{Number of eggs fertilized}}$$

STATISTICAL ANALYSIS

Data generated were subjected to a non- parametric t- test (Mann-Whitney U-Wilcoxon Rank sum W-test) for comparison of means with in treatments while the Tukey HSD one- way ANOVA was used for between treatments comparison at the 5% level of significance. All these were done by using STATISTICA for windows XP 2000 ON PC (Linear version).

RESULTS

The results of the water quality analysis at zero hour, 12 hours and 24th hours are presented in Tables 1, 2 and 3.

The results showed that the highest pH of 7.9± 0.06 was observed at the zero hour in treatment 1, while other treatments had a mean value of 7.8± 0.020. However, there was a drop in the pH values in all the treatments at the 12th and 24th hours. There was an increase in the dissolved oxygen level from T_1 (2.14 ±0.009) to T_2 (2.27±0.02) but a significant decrease ($p<0.05$) from T_3 (2.06±0.006) to T_5 (1.20±0.06) for the zero hour. There was an increase in the dissolved oxygen level as the experiment progressed. The highest dissolved oxygen level was recorded in the treatments at the 24th hour. The various dissolved oxygen levels observed in the treatments were significantly different ($P<0.05$) for the treatment at the different hours. Ammonia level at the zero hour, in T_1, T_2 and T_3 had a mean value of 0.01±0.003, while T_4 and T_5 had 0.02mg/l and 0.03mg/l respectively. At the 12th hour, no ammonia was observed in T_1,T_2 and T_3 (0.03mg/l), while T_4 had 0.1±0 .003mg/l and T_5 had 0.02±0.003mg/l respectively. At the 12th hour, no ammonia was observed in T_1, T_2 and T_3 (0.0 mg/l) while T_4 had 0. 1±0.003mg/l and T_5 had 0.02 ±0.003mg/l. The ammonia level at the 24th hour in all the treatments, ranged from 0.01± 0.003mg/l for T_1 to 0.004± 0.003mg/l for T_5. The nitrite level increased from T_1 (0.021±0.001mg/l) all through to T_5 (0.091±0.001mg/l) at the zero hour. The same trend was also observed at the 12th hour with T_1 having 0.042±0.001mg/l and T_5 0.095±0.001mg/l. The highest level of nitrate was observed at the 24th hour, with T_1 having 0.078±0.00mg/l and the level increasing up to T_5 with 0.126±0.001mg/l. The level of H_2S was high at the zero hour for T_1 (0.003±0.003mg/l) all through to T_5 (0.23±0.003mg/l). The level of H_2S reduced from zero hour to 24th hours. At the 12th

hour T_1 had 0.03±0.003mg/l with higher values for other treatments up to T_5 that had 0.14±0.003mg/l. The lowest levels of H_2S was observed at the 24th hour with T_1 having the least value 0.03±0.003mg/l and increasing all through to T_5 with value of 0.12±0.006mg/l. There was a significant difference ($P< 0.05$) within the treatments. There was an increase in the Biological Oxygen Demand requirement in all the treatments, from 2.11±0.006mg/l for T_1 to 5.62±0.09mg/l for T_5 at the zero hour. There was a steady increase in the observed BOD values for the 12th and 24th hours. T_1 at 12th hour had 4.23±0.009mg/l and T_5 had 9.12±0.006mg/l. The highest BOD values were observed from the 24th hour. T_1 had 6.02±0.012mg/l and there was an increase for each of the treatments up to T_5, which was 10.07±0.012mg/l. There was a significant difference among the treatments and within the hours of the experiment ($P<0.05$). The temperature recorded for the treatment was not affected by treatments, as there was a uniform temperature for all the treatments at a particular hour T_1 to T_5 had 25^0c at the zero hour, at the 12th hour the temperature had risen to 27^0c while at the 24th hour it was 26^0c.

Relationship between sperm quantity, fertility and hatchability

Table 4 shows the relationship between sperm quantity, fertility and hatchability.
T_1 with one drop of milt, which is equivalent to 0.035ml, had 5.166 $x10^8$ sperm cells. There was a steady increase in the sperm concentration as the quantity of milt increased from T_1 to T_5. T_5 had 0.175ml of sperm used for fertilization with a sperm concentration of 2.2583 x 10^9. The fertility observed for the treatment, however, showed that T_1 had the highest percentage fertility of 30.0±1.15%, closely followed by T_2 which had 29.6±5.17% fertility. The lowest fertility was observed for T_5 with percentage fertility of 16.0± 5.68%). These equally affected the hatchability, as T_1 had the highest hatchability of 2041±90.9, T_2 ranked next with value of 2018±373.6, the downward trend occurred with T_5 having the lowest hatchability of 1341±38.2.2. The sperm to egg ratio was lowest in T_1 with 7380: 1 while T_5 had the highest value 3.69 $x10^4$:1.

Biochemical composition

The results of the biochemical composition of *Clarias gariepinus* milt are presented in Table 5. Sodium, one of the four electrolytes analysed had the highest values of 113.7±0.88 followed by chloride which had a mean value of 100± 1.73.

The potassium level was low having a mean value of 29± 0.69 while the least electrolytes was calcium which had a value of 11.1 ±1.69. The inorganic phosphate content was 28.8± 1.08, the total protein being 4.0±0.12. The albumin content was low, with a mean value of 1.3±0.9. The glucose level was high having a mean value of 85.0±6.08 and the cholesterol level was equally high with a mean value of 188.0± 13.67. The triglyceride content of the milt was high having a value of 202±19.88. The high-density lipoprotein cholesterol was low, with a mean value of 13± 6.01 when compared with the low density lipoprotein cholesterol which had a mean value of 134 ± 11.37. The PH was 6.2±0.17.

Sperm motility and live-dead ratio

The sperm motility taken showed a very low spermatozoa movement (15%). For the live-dead ratio, the count showed that for every 100 spermatozoa 93 sperm cells were alive with 7 dead.

DISCUSSION

The motility of the sperm cells was generally low (15%) especially when compared with the motility rate of 88-94% observed in West African Dwarf Goats [15]. This might be because of testicular semen used in the artificial reproduction in fish whereas in higher animals it is the epididymal semen. The low motility may also be as a result of the low pH (6.2) of the fish milt. In higher animals, optimum survival of sperm is seen at a pH of 7 with progressive decline in motility and metabolism below the optimum [16]. Furthermore, the major substance utilized for energy in fish is glucose while in other animals fructose is utilized [17].

This glycolytic process is important to sperm because it allows for their survival and for the production of utilizable energy, the major portion of which is utilized in motility [16]. However, conversion of hexose (Glucose) to lactate is slower under aerobic conditions than under anaerobic conditions [16]. This may also be responsible for the difference in motility of fish when compared with that of other animals. Exposure of milt to light may also affect its motility and fertility [13]. The quantity of milt used significantly affected the water quality parameters, fertility and hatchability of *Clarias gariepinu*s eggs in this study. The reduction in pH level with time may be as a result of increase of metabolite produced during egg development. This, however, fall within the range recommended by Laszalo *et al*. [18] as optimal level for hatchery production of fish. According to Boyd and Lichtkcopper [19], transfer of Oxygen from air to water will occur when water is under-saturated with DO. Only treatments 1 and 2 had DO values falling within the 5-12mg/l range by [18]. This may account for the better hatchability recorded for the treatments. Treatment 5 with the lowest dissolved oxygen equally had the lowest hatchability.

The values of BOD increased with increasing quantity of milt in each treatment. This may probably be due to pollution of the water through decomposition of the excess spermatozoon used for the treatments. The level of H_2S recorded in this study was higher than the 0.0mg/l recommended by [18], which might have affected hatchability and egg survival.

Aquaculture and Inland Fisheries project (AIFP) [7] further observed that un-ionized H_2S is extremely toxic to fish at concentrations that may occur in natural water. The presence of ammonia at the zero hour may be because of the presence of some sperm cells that were not used for fertilization along with the saline solution's effect at the time of incubation. The reoccurrence of ammonia at the 24^{th} hour may be as a result of decomposition of unhatched eggs. Un-ionized ammonia is toxic to fish [19]. In this study, the nitrate level showed that nitrate increased from the zero hour to the 24^{th} hour and equally increased with increasing milt quantity in the treatments. This agrees with the findings of [18]. In that study T_1 with the lowest sperm to egg ratio, gave the

best hatchability [7] in *Clarias gariepinus* being 40.001:1. The fertility or the hatchability record was not given. Chemical analysis of fish sperm shows that glucose is the chief substance utilized for energy in the fish semen (Table5) whereas in most other animals it is fructose [16]. Sodium and chloride are the principal electrolytes in fish milt, which is similar to that reported in dog seminal plasma [7]. Though a certain amount of electrolyte is necessary for normal sperm irritability, the precise action of electrolytes is not well understood [16]. The level of potassium is higher in fish milt when compared with the level in dog seminal plasma but falls within reference standard. High levels of potassium improve motility of washed spermatozoa in 0.15M of sodium chloride solution [16]. The calcium content of fish milt is higher than that of dog seminal plasma. The function of protein, lipoproteins and starch in higher animals is to give protective action against dilution and presumably act by preventing loss of intracellular constituents [16].

CONCLUSION

It can be concluded from the study that the treatment with the least sperm drop gave the highest hatchability. It, therefore, suggests that in commercial breeding, the more the number of female brooders (which may consequently give large quantity of eggs) to very little quantity of milt, the better the result. For better fertility and hatchability, milt should not be exposed to light as this may affect their fertilizing capacity. Also a flow through system could be used during incubation as this enables the pollutants in the incubation tanks to be flushed out thus increasing the level of dissolved oxygen in the system.

The present study provides information on sperm and egg that will lead to more efficient gamete management, and hopefully, an increase in the yield of catfish fry in the hatchery.

Table 1: Mean water quality for the treatments at zero hour (values ± S.E.M)

Trt	pH	DOmg/l	NH_3mg/l	$N0_2$mg/l	H_2Smg/l	BODmg/l	T^OC
1	7.9±0.06^c	2.14±0.0009^c	0.0±0.003^a	0.21±0.001^d	0.003±0.003^a	2.11±0.006^a	25±0.0
2	7.8±0.06^b	2.27±0.020^b	0.01±0.000^{ab}	0.019±0.001^a	0.01±0.003^b	3.03±0.009^b	25±0.0
3	7.8±0.06^b	2.06±0.006^b	0.01±0.003^{ab}	0.034±0.001^b	0.05±0.003^b	4.05±0.029^c	25 ±0.0
4	7.8±0.03^b	1.28±0.006^a	0.02±0.0^b	0.071±0.001^b	0.16±0.003^c	5.33±0.003^d	25±0.0
5.	7.3±0.03^a	1.20±0.006^a	0.02±0.003^b	0.091±0.001^c	0.23±0.003^d	5.62±0.009^d	25±0.0

Legend:

Trt =treatment

pH =Hydrogen ion concentration

NH_3=Ammonia

No_2=Nitrite

H_2S=Hydrogen sulphate

BOD= Biochemical Oxygen Demand

T^OC=Temperature

Means with same alphabet within a column are not significantly different ($p>0.05$).

Table 2: Mean Water quality analysis for the treatment at 12th hours: (value± S.E.M)

Trt	pH	DOmg/l	NH_3mg/l	$N0_2$mg/l	H_2Smg/l	BODmg/l	T^OC
1.	7.1±0.03[a]	5.05±0.009[c]	0.00±0.00[a]	0.042±0.001[a]	0.03±0.03[a]	4.23±0.009[b]	27±0.0
2	7.2±0.03[a]	6.3±0.006[d]	0.00±0.000[a]	0.051±0.001[a]	0.06±0.003[b]	2.14±0.003[a]	27±0.0
3	7.1±0.009[a]	3.21±0.012[a]	0.00±0.000[a]	0.076±0.000[b]	0.09±0.003[c]	5.23±0.0009[c]	27±0.0
4	7.1±0.03[a]	4.3±0.009[b]	0.01±0.003[b]	0.084±0.001[bc]	0.12±0.006[d]	6.06±0.009[d]	27±0.0
5.	7.1±.0.03[a]	3.65±0.014[c]	0.02±0.003[c]	0.093±0.001[c]	0.14±0.003[d]	9.12±0.006[e]	27±0.0

Legend:

Trt = treatment

pH =Hydrogen ion concentration

NH_3=Ammonia

No_2=Nitrite

H_2S=Hydrogen sulphate

BOD= Biochemical Oxygen Demand

T^OC=Temperature

Means with same alphabet on the same vertical column are not significantly different ($p>0.05$).

Table 3: Mean water quality analysis for the treatment at 24th hours (values ± S.E.M)

Trt	pH	DOmg/l	NH_3mg/l	$N0_2$mg/l	H_2Smg/l	BODmg/l	T^OC
1.	7.1±0.03[a]	7.10±0.006[d]	0.01±0.003[b]	0.01±0.001[b]	0.03±0.03[a]	6.02.±0.012[a]	26±0.0
2	7.1±0.00[a]	6.71±0.012[c]	0.003±0.003[a]	0.088±0.001[a]	0.04±.0.006[a]	7.31±0.012[b]	26±0.0
3	7.1±0.06[a]	4.55±0.014[b]	0.02±0.000[b]	0.096±0.000[a]	0.043±0.003[a]	7.54±0.012[b]	26±0.0
4	7.1±0.03[a]	4.42±0.006[b]	0.03±0.003[c]	0.120±0.001[b]	0.05±0.006[b]	9.16±0.006[c]	26±0.0
5.	7.1±.0.03[a]	3.595±0.006[a]	0.04±0.003[c]	0.126±0.001[b]	0.12±0.006[c]	10.07±0.012[d]	27±0.0

Legend:

Trt = treatment

pH =Hydrogen ion concentration

NH_3=Ammonia

No_2=Nitrite

H_2S=Hydrogen sulphate

BOD= Biochemical Oxygen Demand

T^OC=Temperature

Means with same alphabet with in column are not significantly difference ($p>0.05$).

Table 4: Relationship between sperm quantity, fertility and hatchability

Parameters	T_1	T_2	T_3	T_4	T_5
Quantity of milt drop	1	2	3	4	5
The volume of milt in milliter (ml)	0.035	0.070	0.015	0.140	0.175
Number of sperm cell	5.16 $6x10^8$	$1.03x10^9$	$1.549x10^9$	$2.066x10^9$	$2.583x10^9$
Sperm to egg ratio	7380:1	14700:1	$2.2x10^4$:1	$2.29x10^4$:1	$3.62x10^4$:1
Hatchability ratio± S.E	2041±90.9	2018±373.6	1679±325.7	11612±262.9	1341±384.2

Table 5: Biochemical Composition of *C. gariepinus* milt

Parameters	Fish milt ±S.E. mg/l
Sodium	113,7±. 088
Potassium	29. ±0.69
Calcium	11.1±1.69
Chloride	100±1.73
Phosphate	28.8±1.08
Total protein	4.0 ±0.12
Albumin	1.3±0.9
Glucose	85.0. ±6.08
Cholesterol	188.0± 13.67
Triglyceride	202 ±19.88
HDL-chol	13±6.01
LDL0chol	134±11.37
PH	6.2±0.17

- HDL – Chol. High density lipo protein cholesterol

  LDL- Chol. Low-density lipoprotein cholesterol

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