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The Potentials of Raphia Palm Wine (Raphia vinifera) and Oil Palm Wine (Elaeis guineensis) as Milt (Semen) Extenders in African Catfish (Clarias Gariepinus) Following Chilled Storage and their Impacts on Milt Quality, Fertilization, Hatchability and Fry Survival Rates

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ABSTRACT

This work aimed to investigate the use of palm wines (Raphia vinifera and Elaeis guineensis) as milt (semen) extenders. Mature male catfish weighing 1.6kg was used for milt collection and dilution. Another male weighing 1.5kg was used for fresh milt collection for spawning (Phase 2). Mature female catfish weighing 2.2kg was used for spawning and egg stripping to collect the eggs. The experiment consisted of 5 groups in phase 1, comprising of groups A (coconut water/control), B (Oil palm wine), C (Raphia palm wine), D (Raphia palm wine + antibiotics) and E (Oil palm wine + antibiotics). Each of the groups was extended with fresh catfish milt at dilution ratio of 1:9 and each group was shared into 10 different sterile containers of 1ml each. Each group was repeated into three and they were stored at 4°C and were observed daily for mass activity, motility and pH till 7 days. Three superior diluents were used for spawning in phase 2 after 24hrs of chilled storage consisting of group A (Coconut water), group B (Oil palm + AB), and group C (Raphia palm + AB) and group D (Fresh milt / control). Results showed that daily sperm motility had significant differences between the means across all groups. There were significant differences ($p<0.0001$) in fertilization, hatchability and fry survival rates. It was concluded that the addition of antibiotics increased the potential of both palm wines to be used as milt extenders. It was recommended to further modify the palm wines to explore their potentials as milt extenders

INTRODUCTION

In Nigeria, the local supply of fish is largely insufficient to meet the demand. Nigeria having an annual fish consumption of over 1.5 million tons, is both the largest fish consumer in Africa and one of the largest fish consumers worldwide. Nevertheless, Nigeria imports more than 900,000 metric tons of fish annually compared to its estimated 450,000 metric tons domestic catch. (Ozigbo et al., 2014; Federal Department of Fisheries, 2015). As a result of overfishing, habitat destruction, and pollution, ocean fisheries' supply began to decline, necessitating the development of aquaculture. (Adedeji et al., 2011). Aquaculture is the farming of aquatic organisms and plants in fresh, brackish or salt water. A wide variety of aquatic organisms are produced through aquaculture. These include: fishes, crustaceans, molluscs, algae, and aquatic plants (Adedeji et al., 2011). The lack of sufficient supply of high-quality fish seeds, among other things, has hampered the development of aquaculture in Nigeria and other developing nations. Stocking high-quality fingerlings in ideal conditions that promote rapid growth and harvesting as soon as possible is a key component of a successful aquaculture business. (Adewolu et al., 2008). Therefore, efforts are being made to improve fish seed production through the use of extenders in an effort to increase the quantity and quality of fish production, with the long-term objective of lowering the production cost. Extenders have been identified as liquid diluents that, when added to semen, preserve its fertilizing ability (Adewolu et al., 2008). During chilling and shipping processes, it serves as a buffer to protect sperm cells from cold shock and osmotic shock. Extenders are needed to dilute the milt and increase its contact areas, which will increase the rate of fertilization during fish artificial breeding because mature male fish typically produce a small volume of milt that is highly viscous and highly concentrated (Adewolu et al., 2008). Clarias gariepinus is one of the most significant fresh water fish species currently raised both inside and outside of tropical and subtropical environments (Adewolu et al., 2008; Alagbe, 2019). Because of its positive traits, which include its hardy nature, good taste, and capacity for rapid growth among others, this species is well-liked in Nigeria (Olaleye, 2005).

An important alcoholic beverage called palm wine is produced when the sap of a palm tree spontaneously ferments; yeast and bacteria have been implicated in this process. (Onwuka, 2011; Opara et al., 2013). It is the fermented sap of certain varieties of palm trees including raphia palm (Raphia hookery or Raphia. vinifera) (Ali, 2008). Fresh palm wine is a clear, neutral, colorless juice that contains only a small amount of sugar (less than 0.5%), protein, gums, and minerals. (Opara et al., 2013). According to Oyeku et al., (2009), It primarily consists of water, sugar, vitamins, and numerous small amounts of aroma and flavorings. The palm wine plays a significant role in customary practices in traditional African societies, especially the distilled palm wine, a potent gin known by a variety of names in West Africa. (Amoa-Awua et al., 2006). Over ten million people consume palm wine in West Africa (Onwuka, 2011) According to traditional beliefs, palm wine has a diuretic effect and stimulates lactation when consumed by nursing mothers. Due to the concentration of yeast cells in palm wine, it has also been used to increase male potency (Onwuka, 2011). Palm wine has been proven to be beneficial over the years and also studies have shown that palm wine possess the following properties: beneficial microorganisms such as the occurrence of yeast, acetic acid bacteria and lactic acid bacteria, these microorganism have been confirmed to stimulate probiotic functions and also helps in promoting bioactive compounds (Amoa-Awua et al., 2007). Also sources have demonstrated yeast as a valuable source of amino acids and peptides, palm wine have been shown to possess antioxidant properties like vitamin C and polyphenols which are molecules that counteract free radicals and prevent tissue damage caused by these free radicals. Palm wine has also been proven to have biochemical composition which though varies among different locations and may also depend on the species of the palm tree from which the palm wine was sourced, the biochemical composition of palm wine has been reported and it consist of sugars (Bassir, 1962), this ranges from 0.1mg/100ml of maltose and 8.74mg/100ml of sucrose. Other values reported include protein 39.03mg/100ml, free amino acids 59.63 mg/100ml, lipids 62.65mg/100ml and ethanol 3.4mg/100 ml. essential elements like magnesium, zinc, and phosphorus which are part of a healthy diet are found in the drink (Faparusi et al,. 1972a). Since there is abundance of palm wine in Nigeria, This work aimed to investigate the use of freshly harvested palm wine (Raphia vinifera and Elaeis guineensis) as milt extenders and compare with coconut water in terms of milt quality and also compare fresh palm wine (Raphia vinifera and Elaeis guineensis) incorporated with antibiotic with freshly collected milt in terms of fertilization, hatchability and fry survival rates.

LITERATURE REVIEW

Study Area

The research was carried out in the Theriogenology Laboratory, Veterinary Teaching Hospital University of Abuja, Federal Capital Territory, Abuja, Nigeria. Geographically, The Federal Capital Territory (FCT) Abuja is found in the North Central part of Nigeria between the latitude 8.941 and longitude 7.092. The temperature of the area ranges from 30-37˚C yearly with the highest temperature experienced in the month of March and with Mean total rainfall approximately 1,650mm per annum (Balogun, 2001). Abuja experiences three seasons each year: a hot and humid rainy season from April to October; a dry season from October to April; and a brief windy season in between known as the Harmatan season, when the dry and dusty West African trade wind blows through the city, coupled with intense cold. Sometimes the dust storms severely limit visibility and can even block the sun for several days, comparable to a heavy fog. During the rainy season temperatures reach around 86oF (30oC) while nights are relatively warm. In the dry season, daytime temperatures in Abuja can soar as high as 104 oF (40 oC) and evenings can be chilly, with temperatures dropping as low as 54 oF (12 oC) National Population Commission Nigeria (2006).

Experimental Design

The experiment consisted of 5 initial groups in phase 1, comprising of groups A (coconut water/control), B (Oil palm wine), C (Raphia palm wine), D (Raphia palm wine + antibiotics) and E (Oil palm wine + antibiotics) Each of the groups was extended with a fresh catfish milt at dilution rate of 1:9 and each group was shared into 10 different sterile containers, each sterile container contained 1ml each of the extended milt each group was repeated into three and they were stored at 4°C and were observed daily for the following; mass activity, motility and pH.

Three superior diluents were used for spawning in phase 2 of the study after 24hrs of chilled storage and this phase included group A (Coconut water), group B (Oil palm + AB), group C (Raphia palm + AB) and group D (Fresh milt) which served as the control for phase 2 . The work was done during the rainy season.

Identification of Plant and Standardization of the Wines

Raphia palm wine and Oil palm wine were collected from wine tapers in Ihioma, Orlu LGA, Imo state, Nigeria in the month of July, 2023. The trees were identified at the University of Taxonomy department, University of Abuja, Nigeria by a certified taxonomist and assigned with a voucher number AD/09S/2024D. The photographs of the trees from their natural habitats (Fig 1 and Fig 2).

The wines were maintained in cold chain and were used within 48hours of collection. Samples of the wines were submitted to CHESCO for phytochemical analysis. Qualitative phytochemical analysis of the samples was done using a UV visible double beam spectrophotometer (Cecil 750, Cambridge England®).

Fig1. Raphia palm tree (arrow)

Fig 2. Oil palm tree (arrow)

The University of Abuja Institutional Animal Ethics Committee, Nigeria approved this study. Male catfish (Brood stock) of eighteen months of age weighing 1.6kg with length of 56cm and width of 4.6cm was used for milt collection and dilution. Another male catfish of same age and weighing 1.5kg with body length of 62cm and width of 4.2cm was also used for fresh milt collection for spawning/ control group (that is Phase 2). Female catfish, weighing

2.2kg with body length of 65cm and width of 6.0cm was used for spawning and egg stripping to collect the eggs.

Milt Collection

Milt was collected by sacrificing the brood stocks using cervical dislocation method. The milt sac was exteriorized and washed with normal saline. The weight and size were measured using a sensitive scale (Electronic compact® BL20001) and a measuring tape respectively and values were recorded. Milt was extracted into a 5ml sample bottle and maintained at room temperature.

Pre-Dilution Examination

The volume was measured as well as the pH of the milt. The colour of milt (semen) was graded on the scale of creamy to watery The three-color categories of milky, creamy and watery, designated as 1, 2 and 3, respectively were used for scoring the milt as described by Zemjanis, (1970). Milt parameters were examined according to the modified method of Ubah et al., (2020).

A drop of fresh (semen) milt was used; small amount of milt was placed on a warm glass slide with a drop of water added to activate motility and covered with a cover slip. The sample was then viewed under the microscope starting from x4 magnification to observe wave motion and then to x10 magnification to observe individual motility.

METHODOLOGY

Preparation of Milt Extenders and Dilution of Milt

Preparation of Raphia Palm Wine and Oil Palm Wine

Raphia palm wine and Oil palm wine were harvested and collected into a clean container.100ml each was measured and placed into a graduated cylinder.

Preparation of Raphia Palm + Antibiotics and Oil Palm + Antibiotic

Raphia palm wine was harvested and collected into a clean container. 100ml was measured and 0.6ml (100,000 i/u and 200mg) of penicillin/streptomycin injection was made and added, the mixture was placed into a graduated cylinder and the same was done for Oil palm wine.

Preparation of Coconut Water

The coconut was washed with clean water, broken gently with the aid of hammer and the water extracted with a needle and syringe into a clean container, 100ml was measured and 0.6ml (of penicillin/streptomycin injection (100,000 i/u and 200mg) was made and added, both mixture was placed in graduated cylinder,

Milt (Semen) Dilution

The milt collected was diluted at the ratio of 1:9 by taking 1ml of fresh milt into 9ml of each of the extenders (A, B, C, D and E). Each group of the extended milt was distributed into 10 different sterile sample bottles containing 1 ml of the diluted milt each and stored at 4^oC after initial assessment.

Post Dilution Examination

Each of the experimental groups was evaluated. The extended milt was assessed starting from day 0 to day 7 post dilution, day 0 served as the baseline for this research. Parameters that were examined included; mass activity, progressive motility, semen pH. These were carried out as already described.

Spawning

Spawning was carried out 24hrs post dilution with the chilled extenders. Another male catfish was sacrificed to obtain fresh milt which was used as control group for fertilization studies. Macroscopic and microscopic examination was carried out on the freshly collected milt. The night prior (12hrs) to spawning at 9pm, two female catfish (brood stocks) were injected with ovuline® (Ningbo Farmland Training Co Ltd) (0.5ml/kg a GnRH for ovulation induction in fish.

Fertilization of Eggs (Roes) and Estimation of Fertilization Rate

Phase 2 of the experiment which involved fertilization, hatchability and fry survival rate was designed to have four groups in which group A was Coconut water, group B was Oil palm + antibiotics, Group C was Raphia palm + antibiotics these extenders were used basically after 24 hours of chilled storage and Group D was Fresh milt. Eggs were stripped after 12 hours of latency period and a tea spoon full of stripped eggs weighing 7gram estimated to be 3,017 eggs was measured and taken into a bowl and was diluted with normal saline. 1ml of the extended semen was added and mixed thoroughly. Water was also added to permit activation of the eggs. 2ml of the activated eggs was taken and dispensed into a petri dish for determination of fertilization rate between 40 minutes and 120 minutes of mixing the gametes according to the method of Victor *et al.,* (2017). The same was done for Group A (Coconut water), Group B (Oil palm +antibiotics), Group C (Raphia palm + antibiotics) and Group D (Fresh milt). Fertilization rate was determined by mounting representative samples of activated mixture of the egg and milt from each group and their repeated samples on a glass slide, covered with a cover slip and viewed under the microscope at x4 magnification. A total of 100 eggs were counted from each slide and the numbers of the dividing embryos were noted with the aid of a computer screen connected to a light microscope, and photographs were taken. The number of fertilized eggs obtained was expressed as percentages of the total number of eggs counted on the slide as shown below;

$$\text{Fertilization rate (\%)} = \frac{\text{No of Fertilized eggs on the glass slide}}{\text{Total number of eggs counted (100)}} \times 100$$

Estimation of Hatchability Rate

Hatchability rate was estimated according to Arun and Shigeharu (2004) modified method, by counting a total of 100 fertilized eggs into a petri dish and incubated using mini-incubation trough covered with a mesh, this was incubated alongside the rest of the samples in an incubation tank under the same hatchery conditions and water quality. After 24hrs of incubation, the petri dish was observed to identify unhatched embryos with the naked eyes. The unhatched embryos were counted and subtracted from the total number of embryos (100) incubated to estimate the hatchability rate. Hatched larvae were also counted.

$$\text{Hatchability rate (\%)} = \frac{\text{Hatched larvae in the petri dish}}{\text{Total number of fertilized eggs in the petri dish}} \times 100$$

Estimation of Fries Survival Rate

Survival rate was estimated by counting the total numbers of live fries in the main incubation tanks after 7 days and subtracted from the total number of hatched larvae. The total number of hatched larvae was estimated from the total number of eggs activated.

$$\text{Survival rate (\%)} = \frac{\text{Recovered fries}}{\text{Initial number of hatched larvae}} \times 100$$

Water Collection and Estimation of Water Quality

Water samples were collected from the supply tank before incubation and were subjected to analysis to determine the physical and chemical properties of water before incubation, According to the modified method of Ubah *et al.,* (2023).

Statistical Analysis of Data

All data were analyzed using descriptive statistics. Data were expressed as the mean± Standard Error of Mean and the differences among the group were analyzed using one way analysis of variance (ANOVA). Tukey's multiple comparisons Test was used to determine the differences among the test groups. P value P≤0.05 was considered statistically significant. Results were represented in form of tables and figures.

RESULTS

Results showed that the sperm sacs of the male brood stock used for milt extraction and dilution had the following values (1) weight of left sac was 4g, right sac 3g, (2) length of left sac was 4.2cm, right was 5cm, (3) width of left sac was 1.2cm, right was 1.4cm, (4) volume of left sac was 2ml, right was 2.5ml. The sperm sacs of the male brood stock used for fertilization study had the following values (1) weight of left sac was 4g, right sac 5g, (2) length of left sac was 2.5cm, right was 3cm, (3) width of left sac was 1.5cm, right was 1.3cm, (4) volume of left sac was 2ml, right was 1.5ml. Pre dilution parameters of fresh milt of African catfish used for milt parameters analysis showed the following values (1) pH = 7, (2) mass activity = +++, (3) motility = 98% (4) colour = creamy, (5) volume 4.5 ml. Pre spawning parameters of fresh milt of African catfish used for fertilization study showed the following values (1) pH = 7, (2) mass activity = +++, (3) motility = 98% (4) colour = creamy, (5) volume 3.5 ml. Qualitative phytochemical analysis of Palm wine and Raphia wine indicated presence of Saponins, Terpeniods, Flavonoids, Cardiac glycosides, Volatile oil and Glycosides. Alkaloids were present only in oil palm wine (Table 1). Mean (±SEM) daily post dilution pH of catfish milt in coconut water, Oil palm, Raphia palm, Raphia palm + Antibiotics, Oil palm +Antibiotics showed no significant difference among the groups (p>0.05). But it showed an appreciable difference between coconut and the palm wines. Coconut had a value of 7±0.577 from day 0 while the palm wines showed values of 5±0.577, 5±0.577, 5±0.577 and 5±0.577 respectively (Table 2). Mean (±SEM) daily mass activity of catfish milt in Coconut water, Oil palm, Raphia palm, Raphia palm + antibiotics, Oil palm + antibiotics. Showed that the differences between the means were significant across all groups except for Raphia palm + antibiotics versus Oil palm + antibiotics (P < 0.05) (Table 3). Coconut water versus Oil palm + antibiotics, Coconut water versus Raphia palm

+ antibiotics had a lower level of significance (p<0.05) as compared to Coconut water versus Oil palm and Coconut water versus Raphia palm (p< 0.01) This trend continued from day 0 till day 7 (Table 3). Daily sperm motility showed that the differences between the means were significant across all groups. Coconut water versus Oil palm + antibiotics and Coconut water versus Raphia palm + antibiotics had a lower level of significance (p<0.01) compared to Coconut water versus Oil palm and Coconut water versus Raphia palm which had a higher level of significance (p< 0.0001) This trend continued from day 0 till day 7. (Table 4). Fertilization took place in all the groups at different rates (Table 5, Figs 1-4). There were significant differences (p<0.0001) in fertilization rate for Coconut water versus Oil palm + antibiotics, Coconut water versus Raphia palm + antibiotics, while Coconut water versus Fresh milt was not significantly different (p>0.05) (Table 5). There were significant differences (p<0.0001) in hatchability rate for Coconut water versus Oil palm + antibiotics, Coconut water versus Raphia palm + antibiotics and also Oil palm + antibiotics versus Raphia palm + antibiotics were significantly different (p<0.01) while the difference between Coconut water and Fresh milt was not significantly different (p>0.05) (Table 5). There were significant differences (p<0.0001) in fry survival rate for Coconut water versus Oil palm + antibiotics, Coconut water versus Raphia palm + antibiotics and also Oil palm + antibiotics versus Raphia palm + antibiotics were significantly different (p<0.01) while the difference between Coconut water and Fresh milt was not significantly different (p>0.05) (Table 5). Daily water rearing temperature ranged between 37oC and 38oC throughout the rearing period in all the groups. Water quality parameters pre – hatch were revealed that Physical appearance = turbid, Colour = colourless, Odour = Odourless pH = 7.85, Total Dissolved Solids (TDS) mg/L =31.00, Nitrate mg/L = 3.4, Nitrite mg/L = 0.8, Sulphate mg\L = 18.9, Conductivity µS/cm = 46.30, Dissolved oxygen mg\L =8.50 and Turbidity (NTU) =. 0.4, all the parameters were within acceptable range (Ubah et al., 2023).

Table 1: Qualitative Phytochemical Analysis of Palm Wine and Raphia Wine

Parameters	Oil palm wine	Raphia palm wine
Tannins	-	-
Steroids	-	-
Anthraquinones	-	-
Saponins	+++	+++
Phenol	-	-
Alkaloid	+	-
Cardinolides	-	-
Terpeniods	+++	+++
Flavonoids	++	++
Cardiac glycosides	+++	+++
Volatile oil	++	++
Glycosides	++	++
Carbohydrate	-	-
Phlobatannins	-	-

Balsams	-	-

Table 2. Mean (±SEM) Daily Post Dilution Ph of Catfish Milt in Coconut Water, Oil Palm, Raphia Palm, Raphia Palm + Antibiotics, Oil Palm +Antibiotics

Day	Coconut water	Oil palm	Raphia palm	Raphia palm + AB	Oil palm + AB
0	7±0.577	5±0.577	5±0.577	5±0.577	5±0.577
1	7±0.577	5±0.577	5±0.577	5±0.577	5±0.577
2	7±0.577	5±0.577	5±0.577	5±0.577	5±0.577
3	7±0.577	5±0.577	5±0.577	5±0.577	5±0.577
4	7±0.5773	5±0.577	5±0.577	5±0.577	5±0.577
5	7±0.577	5±0.577	5±0.577	5±0.577	5±0.577
6	6±0.577	5±0.577	5±0.577	5±0.577	5±0.577
7	6±0.577	5±0.577	5±0.577	5±0.577	5±0.577

*Values with same superscripts alphabets on the same row (a, b, c) were significantly different (p<0.05). There was no difference in the means of the different groups observed, hence the daily post dilution pH of the extenders were not significantly different (p>0.05)

Table 3. Mean (±SEM) Daily Mass Activity of Catfish Milt in Coconut Water, Oil Palm, Raphia Palm, Raphia Palm + Antibiotics, Oil Palm + Antibiotics

Day	Coconut water	Oil palm	Raphia palm	Raphia palm +AB	Oil palm +AB
0	3 ± 0.33^{a}	$1±0.57^{b}$	$1±0.57^{b}$	$2±0.57^{c}$	$2±0.57^{c}$
1	$3±0.33^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$2±0.57^{c}$	$2±0.57^{c}$
2	$3±0.33^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$2±0.57^{c}$	$2±0.57^{c}$
3	$3±0.33^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$2±0.57^{c}$	$2±0.57^{c}$
4	$3±0.33^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$2±0.57^{c}$	$2±0.57^{c}$
5	$3±0.33^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$2±0.57^{e}$	$2±0.57^{c}$
6	$3±0.33^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$2±0.57^{e}$	$2±0.57^{c}$
7	$3±0.33^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$2±0.57^{c}$	$2±0.57^{c}$

*Values with different superscripts alphabets on the same row (a, b, c, d, e) were significantly different (p<0.05).

Table 4. Mean (±SEM) Daily Sperm Motility (%) of Catfish Milt in Coconut Water, Oil Palm, Raphia Palm, Raphia Palm + Antibiotics, Oil Palm + Antibiotics

Day	Coconut water	Oil palm	Raphia palm	Raphia palm + AB	Oil palm + AB
0	$95±0.57^{a}$	$45±2.88^{b}$	$50±2.88^{b}$	$65±2.88^{c}$	$65±2.88^{c}$
1	$95±0.57^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$65±2.88^{c}$	$65±2.88^{c}$
2	$95±0.57^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$65±2.88^{c}$	$55±2.88^{c}$
3	$95±0.57^{a}$	$0±0.00^{b}$	$0±0.00^{b}$	$65±2.88^{c}$	$55±2.88^{c}$

4	85 ± 2.88^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	65 ± 2.88^{c}	55 ± 2.88^{c}
5	85 ± 2.88^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	55 ± 2.88^{c}	50 ± 2.88^{c}
6	70 ± 2.88^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	55 ± 2.88^{c}	50 ± 2.88^{c}
7	70 ± 2.88^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	55 ± 2.88^{c}	50 ± 2.88^{c}

*Values with different superscripts alphabets on the same row (a, b, c, d, e) are significantly different ($p<0.05$).

Table 5. Mean (±SEM) Fertilization, Hatchability and Fry Survival Rate (%) of Chilled African Catfish Milt Extended in Coconut Water, Oil Palm + Antibiotics, Raphia Palm + Antibiotics And Fresh Milt

Parameters	Coconut water	Oil palm + AB	Raphia palm + AB	Fresh milt
Fertilization rates (%)	99 ± 0.58^{a}	80 ± 1.16^{b}	80 ± 1.16^{b}	99 ± 0.58^{a}
Hatchability rates (%)	85 ± 2.89^{a}	36 ± 2.89^{b}	56 ± 2.88^{c}	95 ± 2.88^{a}
Fry survival rates (%)	98 ± 0.58^{a}	90 ± 0.58^{b}	95 ± 0.58^{c}	98 ± 0.58^{a}

*Values with different superscripts alphabets on the same row (a, b, c, d, e) are significantly different ($p<0.05$).

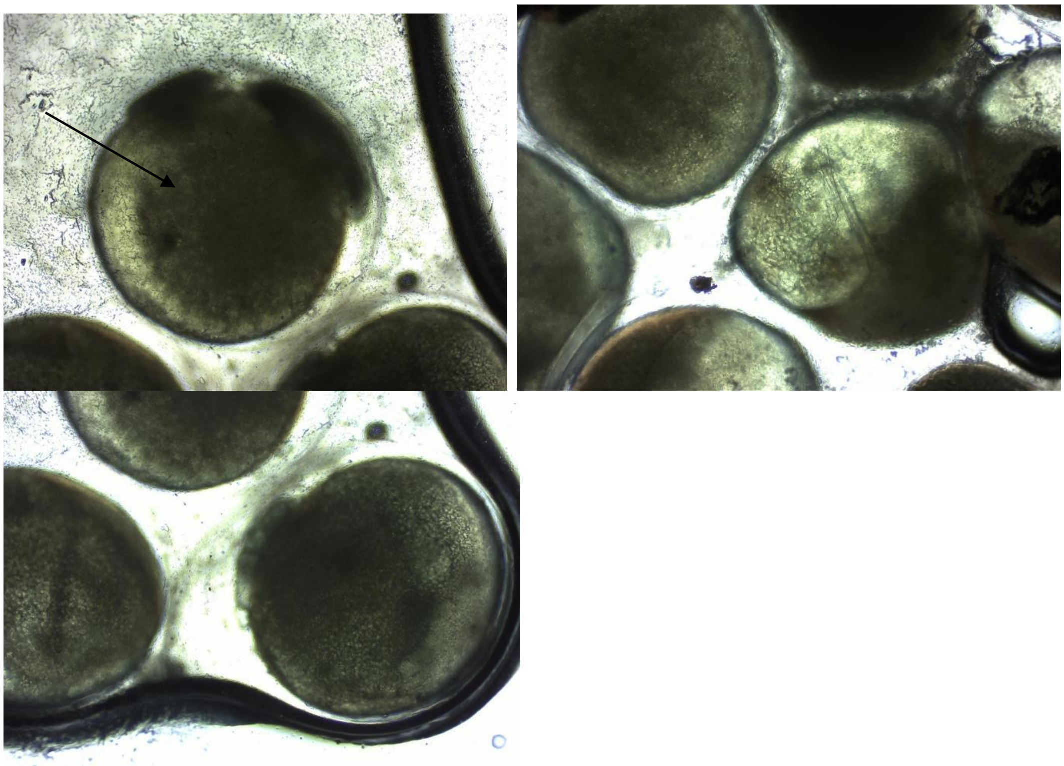

Fig. 3. Photograph of Fertilized Eggs with Embryonic Discs (Arrow) after Fertilization of Eggs With Milt Extended in Coconut Water (X4 Objective)

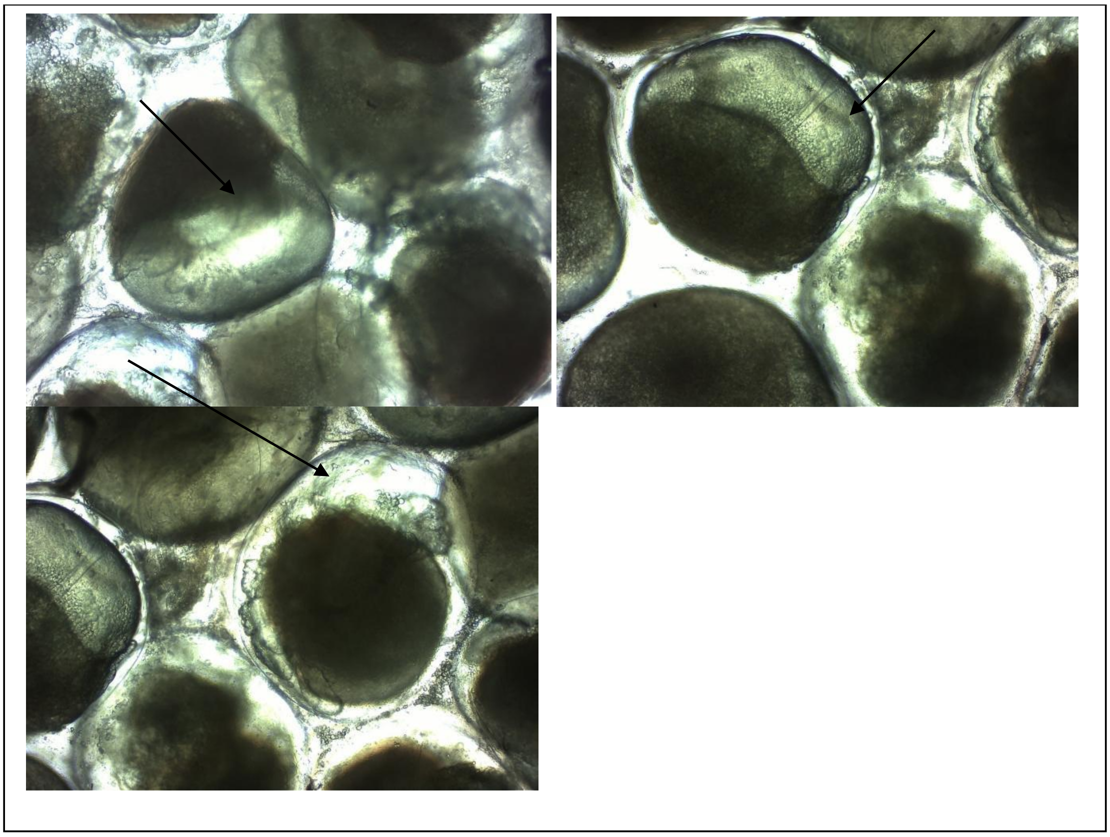

Fig 4. Photograph of Fertilized Eggs With Embryonic Discs (Arrow) After Fertilization With Milt Extended in Oil Palm Wine +Antibiotics (X4 Objective)

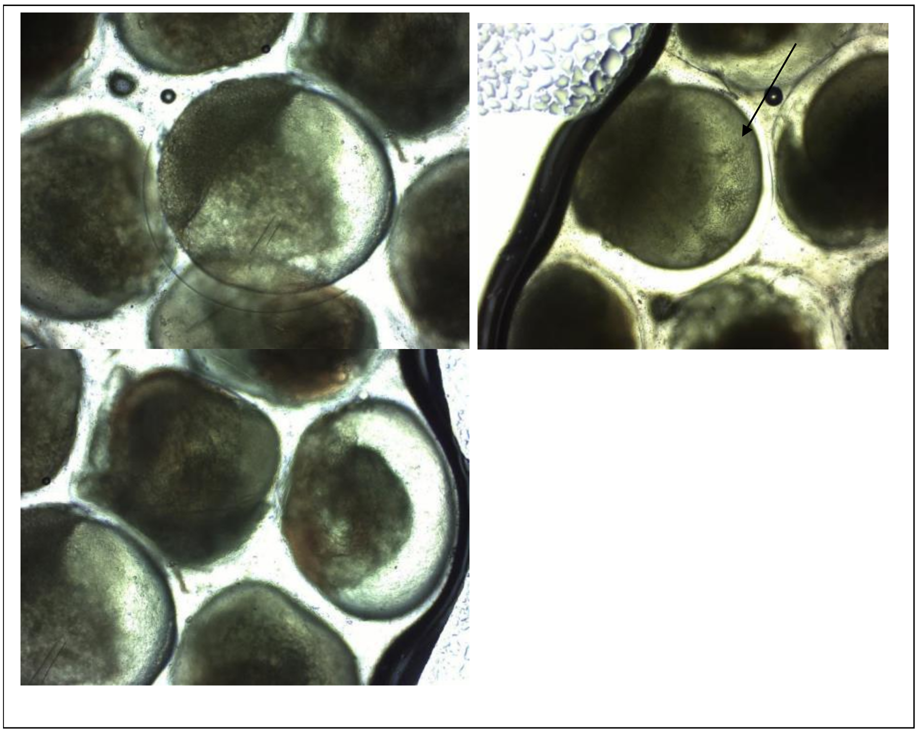

Fig 5. Photograph of Fertilized Eggs With Embryonic Discs (Arrow) After Fertilization of Eggs With Milt Extended in Raphia Palm Wine +Antibiotics (X4 Objective)

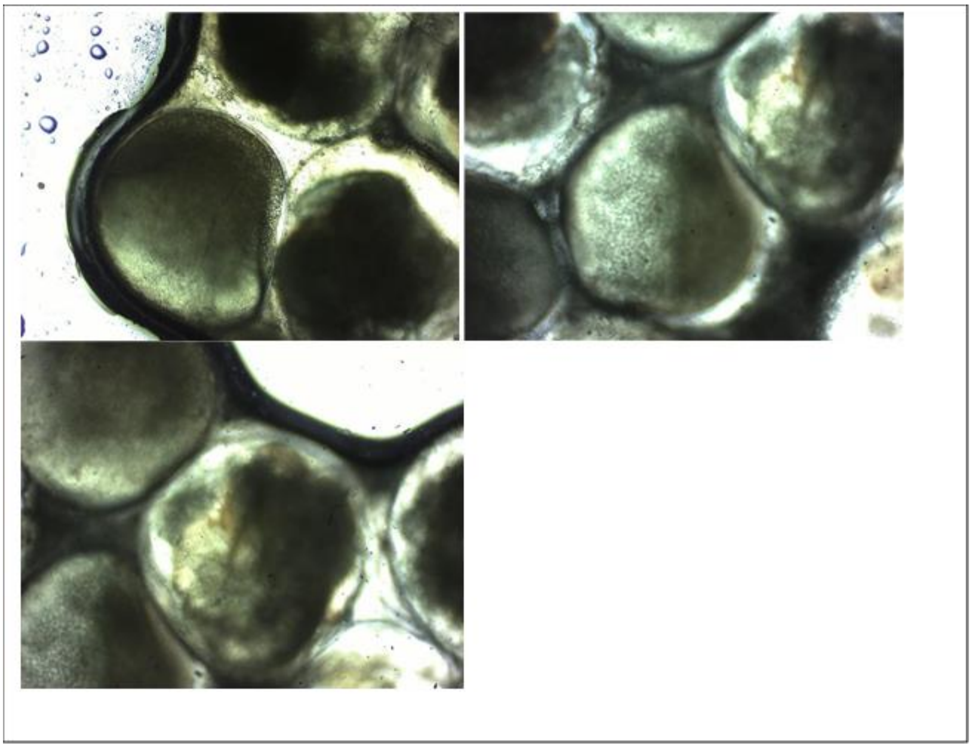

Fig 6. Photograph of Fertilized Eggs With Embryonic Discs (Arrow) After Fertilization of Eggs With Fresh Milt (X4 Objective)

DISCUSSION

The importance and aim of this study was to carry out tests on Raphia palm wine, Oil palm wine, Raphia palm wine+AB, Oil palm wine + AB and Coconut water of which in this contest were all used as extenders and to compare the characteristic performance of catfish milts in terms of semen quality, fertilization rate, hatchability rate and fry survivability, although Coconut water has been used or tested previously by Ubah et al., (2023) and has been proven to be an excellent milt extender, with high fertilization and hatchability percentages recorded. Muchlisin et al., (2010) reported Coconut water as best extender for C. gariepinus spermatozoa. 7gram of eggs estimated to be 3,017 eggs was used to carry out these tests for each of the groups. The milt quality parameters that were observed in this research showed that the test groups differed significantly ($P<0.05$). However regarding this study, Coconut water extender revealed a high percentage mass activity and sperm motility compared to other groups which was in agreement with Muchlisin (2005) and this was followed by Raphia palm + AB, Oil palm+AB. While Raphia palm and Oil palm (without antibiotics) showed poor results.

The high percentage motility observed in sperm cell extended with Coconut water also confirmed or agreed with the report that Coconut water based extender has been found to be effective for cryopreservation of semen (Sule et al., 2007). And this can be attributed to the constituents of Coconut water which includes sugars, amino acids and vitamins (Yong et al., 2009; USDA National Nutrient Database, 2015). Hence it was suitable as a control for phase 1 of the experiment.

Among all the groups, Oil palm and Raphia palm wine without antibiotics showed the lowest motility, this could be due to presence of bacteria responsible for palm wine fermentation, studies have shown that palm wine possess the following properties: beneficial microorganisms such as the occurrence of yeast, acetic acid bacteria and lactic acid bacteria, these microorganism have been confirmed to stimulate probiotic functions and also helps in promoting bioactive compounds (Amoa-Awua et al., 2007). This observation is in agreement with (Kuster and Althouse, 2016) who reported that bacteria presence in sperm cells can cause damages such as agglutination, decreased motility, damage to the acrosome and membrane integrity of boar semen. Other phytochemicals such as cardiac glycosides, saponins etc present in palm wine also have been proven to cause significant reduction in sperm motility of male Wistar rats suggesting an inhibitory effect on fertilizing capacity (Aitken et al., 1984). Saponnins present in Soya milk have been attributed to poor semen motility in Clarias gariepinus (Ubah et al., 2023). Also there was an observable increase in motility of the palm wine groups with antibiotics inclusion (Raphia palm + AB and Oil palm + AB). Research has shown that sperm motility is an important factor to consider in determining the quality and fertilization ability of fish spermatozoa and therefore agreed with the report which stated that sperm motility is the most evaluated criterion for assessing sperm quality due to its relationship with fertility (Rurangwa et al., 2001). The fertilizing capacity across all groups had slight changes and in comparison showed difference in the level of significance, Coconut water and Fresh milt were shown to have the highest percentage of fertilization (99%) which therefore agreed with the report that fresh milt offers better fertility and conception rate (Lanford et al., 1979) while Raphia palm + AB and Oil palm + AB showed (80%) fertilization rate respectively which was significantly lower. This may be a reflection of the lower sperm motility observed in their extended milt. Hatchability rate observed amongst the groups were 85% in Coconut water, 56% in Raphia palm + AB, 36% in Oil palm + AB and 95% in Fresh milt. In comparison between groups it was reported that all groups showed a difference in the level of significance ($P<0.05$) except Coconut water versus Fresh milt. Hatchability rate was significantly lower in the palm wines with antibiotics, but the most interesting was that Oil palm wine was significantly lower than Raphia palm wine. This may be related to the phytochemical compositions of the wines. Alkaloids are present in Oil pal wine but not in Raphia palm wine. Alkaloids may be detrimental to hatchability of C gariepinus.

In terms of fry survivability Coconut water and Fresh milt showed the highest percentage (98%) followed by Raphia palm + AB (95%) Oil palm + AB (90%). This observation may reflect hatchability pattern, because poor quality hatch will affect neonatal performance. The difference in the hatchability rate and fry survival rate between Coconut water and the palm wine groups can be as a result of the protective effects and the ability of Coconut water extender to efficiently harness its potassium content for survival of the spermatozoa. Abnormal level of potassium have been reported to be correlated with infertility in humans, the addition of potassium to semen extenders has been shown to improve the motility of stallion and human sperm (Padilla et al., 1991 and Karow et al., 1992) It has been proven that Coconut water contains a good amount of potassium (Yong et al., 2009; USDA National Nutrient Database, 2015). The water quality parameters showed that the water parameters were within acceptable range and were suitable for hatching and fry rearing.

It was concluded that both Oil palm wine and Raphia palm wine without antibiotics cannot be applied for use as milt extenders. The addition of antibiotics increased the potential of both Oil palm wine and Raphia palm wine to be used as milt extenders. Raphia palm wine seemed to have a better potential to be used as a milt extender probably due to the absence of alkaloids. It was recommended that both Oil and Raphia palm wine with the addition of antibiotics can be used for ultrashort term preservation of African catfish milt (Clarias gariepinus. More studies should be focused on the modification of the Oil and Raphia palm wine extenders in order to achieve their full potentials as milt extenders.

CONCLUSIONS AND RECOMMENDATIONS

It was concluded that the addition of antibiotics increased the potential of both palm wines to be used as milt extenders.

FURTHER STUDY

It was recommended to further modify the palm wines to explore their potentials as milt extenders.

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