



Use of coconut milk as an extender for cock semen

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Abstract

The study was carried out to evaluate the effects of heated and unheated coconut milk (HCM, and UHCM, respectively) as extenders for indigenous cock semen and to compare the effects of these diluents to sodium citrate (Na-citrate), and physiological saline (saline) on semen quality parameters including storage time under ambient conditions. A total of 65 chickens: 50 hens (55 weeks of age and actively in lay) and 15 cocks (~2 years old, 1.8-2.0 kg body weight ($P<0.05$)) held in individual cages were used for the study which lasted for 5 wk. There were 5 treatments (10 hens and 3 cocks each) namely raw semen (T1), Na-citrate (T2), HCM (T3), UHCM (T4) and saline (T5). There were significant ($P<0.05$) main effects of dilution, diluent type and storage time and interaction effect of diluent type \times storage time on the traits measured. SPM and TMS were highest for semen diluted in Na-citrate, HCM and saline compared to UHCM. Semen diluted in Na-citrate and HCM gave highest fertility compared to saline and UHCM. Hatchability was highest for semen diluted in Na-citrate followed by HCM. Undiluted semen and semen diluted in UHCM differed significantly ($P<0.05$) in their effects on SPM, fertility and hatchability of eggs. SPM was 0.00% by 80 min post dilution in UHCM extender but 52.9 ± 1.69 , 40.5 ± 0.90 , and $40.2\pm 0.77\%$ for Na-citrate, HCM, and saline, respectively at 100 min. Heated coconut milk could therefore be used as diluent for semen proposed for short duration (≤ 100 min).

Keywords: Ambient conditions; fertility; hatchability; diluent; sperm motility; storage time

To cite this article: Ogbu CC, SOC Ugwu and CV Ezebili, 2014. Use of coconut milk as an extender for cock semen. Res. Opin. Anim. Vet. Sci., 4(10): 571-577.

Introduction

Artificial insemination (AI) plays significant roles in poultry breeding. To achieve rapid genetic progress in poultry breeding, proven sires (cocks) are selected and mated with equally high performing females (hens). In unselected and unimproved stocks, outstanding males are usually not many. Semen extension enables more females to be inseminated using semen of a proven cock. Semen extension is particularly important in poultry because of the low ejaculate volume characteristic of this specie (Dumpala et al., 2006). Semen dilution thus enables the more efficient use of semen. From reproductive and genetic perspectives, the cock is the most important individual animal in a poultry breeding project and influences more progenies than the hen. The cock semen is hence a critical input in any poultry improvement programme. Therefore, efforts

to improve the Nigerian indigenous chicken must incorporate artificial insemination to increase the selection intensity for males and to achieve greater genetic progress per unit effort.

Choice of semen extender is an important aspect of semen processing for AI (Peterson et al., 2007). Many extenders have been proposed for poultry semen (Iaffaldano et al., 2005; Parker and McDaniel, 2006; Dumpala et al., 2006). These extenders differ in content and complexity. For all animals, an appropriate extender performs similar functions as the seminal plasma – the natural medium for sperm (Siudzinska and Lukaszewicz, 2008; Boucif et al., 2011; Udeh and Oghenesode, 2011). Glutamic acid is believed to be the most important chemical constituent of avian seminal plasma and it has become a standard component of extenders (Siudzinska and Lukaszewicz, 2008).

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The preparation of commercially recommended semen extenders requires considerable technical skill in terms of choice of ingredients, precision in measurement and adjustment of pH and osmolarity of solution. Commercial extenders are also not readily available to farmers based in rural areas of Nigeria. There arises then the need to evaluate the efficacy of some readily available natural products such as coconut milk as a possible extender to maintain cock semen quality (under ambient conditions) for the time duration needed to achieve insemination in small flocks characteristic of back yard poultry production. Such material (s) would enable rural farmers and village extension workers to extend semen for immediate use.

Coconut milk (the liquid extract from fresh coconut meat or kernel) is obtained when shredded coconut meat is soaked in coconut water (or ordinary water) and the mixture filtered (squeezed) through appropriate gauze. This product has been shown to contain energy compounds such as sugars (glucose, fructose, lactose, and sucrose), fatty acids, proteins and amino acids (glutamic acid, glutamine, alanine, arginine, lysine, leucine, proline, etc), minerals and trace elements (Na^+ , Ca^{2+} , K^+ , Mg^{2+} , Cl^- , Fe^{3+} , P, N_2 , Co^{2+} , Zn^{2+} , PO_4^- , HCO_3^- etc), vitamins (ascorbic acid, folic acid, pyridoxine, nicotinic acid, biotin, pantothenic acid), antioxidant enzymes (catalase, peroxidase, etc), phospholipids, and phytohormones (cytokinins) for example kinetin reputed to have antistress, anti ageing, anti-carcinogenic and anti-thrombotic effects (Abara et al., 2007; DebMandal and Mandal, 2011; Solangi and Iqbal, 2011; Ibe et al., 2013). Coconut water and milk have antiviral, antibacterial, antifungal, antiparasitic, and antioxidant properties among other medicinal and health benefits (Manisha and Shamapada, 2011). Coconut milk has been employed in various proportions and in varying combinations in semen extenders for purposes of storage (hours to days) under controlled and ambient conditions (Sule et al., 2007). However, to our knowledge, no report has evaluated the effect of heating on its efficacy as sole extender for chicken semen under ambient conditions. The present study was hence undertaken to evaluate the effects of heated and unheated coconut milk, sodium citrate and normal saline on sperm motility of Nigerian indigenous cock semen for a keeping time (post dilution duration) of 100 min. and the fertility and hatchability of eggs laid by hens inseminated with raw semen and semen extended with these diluents.

Materials and Methods

The study was carried out at the local chicken research farm of the Department of Animal Science, University of Nigeria, Nsukka. A total of 65 indigenous chickens (50 hens and 15 cocks) were used for the

study. The hens were about 55 weeks of age and actively in lay while the cocks were about 2 years old and weighed 2.0 kg on average. The birds were randomly allotted to one of 5 treatments (10 hens and 3 cocks per treatment) namely control or no diluent (T1), sodium citrate (T2), heated coconut milk (T3), unheated coconut milk (T4) and physiological saline (T5) in a Completely Randomized Design. All experimental birds were held in individual cages for semen collection, insemination and egg production. The experiment lasted for 5 weeks.

Preparation of semen extenders

- Sodium citrate: 2.90 g of sodium citrate was dissolved in 100 ml of freshly prepared distilled water and centrifuged to obtain clear supernatant. The solution was adjusted to PH of 7.0 using buffer tablet and a PH meter. This was stored in the refrigerator at 4°C until use.
- Normal saline: 0.85 g of sodium chloride was dissolved in 100 ml of freshly prepared distilled water in a sterilized measuring cylinder to obtain a 0.85% solution. The solution was stirred thoroughly and stored in a refrigerator until use.
- Coconut milk: The preparation of coconut milk extender followed a simple but aseptic procedure. The meat of freshly harvested coconut (*Cocos nucifera*) was thoroughly blended and collected in a 250 ml conical flask. The water from the coconut was added to the blend and the mixture allowed standing for about 1 h. Thereafter, the mixture was wrapped in a heat sterilised white cloth and tightly squeezed to express the milk. The milk was filtered through sterilized white clothes thrice to get rid of all residues and the liquid collected in a sterilised flask. The recovered milk was then divided into two portions. One portion was stored in a refrigerator at 4°C until use. This is the unheated coconut milk (UHCM). The second portion was heated in boiling water to a temperature of 90°C, allowed to cool, filtered through sterilized white cloth and stored in the refrigerator until use. This is heated coconut milk (HCM).

Semen collection, dilution and evaluation

Semen was collected from cocks belonging to each treatment by the dorso-abdominal massage method. Semen samples were collected twice per week from each cock. Individual cock semen was assessed for semen quality but semen was pooled together within each treatment for storage and insemination. In each week, one set of semen samples was assessed without dilution while the other set was diluted (0.1 ml of semen: 1.0 ml of diluent) before assessment. This was to keep up with time requirements. Sets of semen

samples were alternated for the assessments to eliminate positional effect. Semen samples were evaluated for volume of ejaculate (VOE), sperm concentration (SPC), total sperm in ejaculate (TSE), percent sperm motility (% SPM) and total motile sperm (TMS). Volume of ejaculate (VOE) was obtained by reading from a tuberculin syringe calibrated to 0.01 ml. Sperm concentration (SPC) was determined using haemocytometer and microscope at x 400 magnification and recorded in $n \times 10^6/\text{ml}$. For SPM, a drop of semen was placed on a microscope slide, covered with a cover slip and examined under x 400 magnification. Motility was subjectively scored and recorded in percentages. Total sperm in ejaculate (TSE) was obtained by multiplying sperm concentration (SPC) by the volume of ejaculate and recorded in $n \times 10^9$. Total motile sperm in ejaculate (TMS) was obtained by multiplying TSE by SPM and recorded in $n \times 10^9$. Diluted semen was also evaluated for SPM and TMS at 0, 20, 40, 60, 80 and 100 min post dilution (p.d.).

Insemination

Hens belonging to each treatment were inseminated with fresh semen pooled from cocks belonging to that treatment either without dilution (T1) or diluted with the appropriate diluent (T2 to T5). An insemination dose of 0.01 ml/bird for undiluted semen (T1) and 0.40 ml/bird for diluted semen (T2 to T5) was used (Bratte and Ibe, 1989; Das et al., 2004). Insemination was done twice per week for each treatment between 1400 and 1600 h on the day of insemination. Eggs were collected daily, stored at room temperature and incubated after six days. Five batches of incubation and hatches were obtained from each treatment and used to evaluate fertility and hatchability. Table 1 contains the volume of pooled semen and insemination dose for each treatment.

Data collection and analysis

Data were collected on semen quality traits namely: volume of ejaculate (VOE), sperm concentration (SPC), total sperm in ejaculate (TSE), percentage sperm motility (SPM), and total motile sperm (TMS). Fertility was calculated as the percentage of set eggs with embryo at 11 days of incubation while hatchability was calculated as percentage of fertile eggs that hatched (hatchability-1) and percentage of set eggs that hatched (hatchability-2). Eggs that did not hatch after the 23rd day of incubation were broken to confirm fertility status and hatchability values. Data collected were subjected to analysis of variance in a completely randomized design using the GLM of Genstat computer software (Genstat Discovery Edition 3, 2009). The statistical model was:

$$X_{ijk} = \mu + D_i + T_j + (DT)_{ij} + e_{ijk}$$

Where, X_{ijk} is an observation, μ is common mean, D_i and T_j are effects of diluent and storage time post dilution, respectively; $(DT)_{ij}$ is interaction effect of diluent and storage time while e_{ijk} is residual. Preliminary analysis showed that batch effect was not significant ($P < 0.05$) for fertility and hatchability and was therefore removed from the model. Significant means were separated using the least square difference.

Results

The mean solar radiation for the experimental site ranged from 6.0 to 40.0 MJ/m²/day (mean, 28.6 MJ/m²/day) during the experimental period while mean ambient temperature (AT) and relative humidity ranged from 21.0 to 28.0°C and 64.5 to 67.2%, respectively. Table 1 presents the volume of pooled ejaculate for each treatment as well as the insemination dose.

Over the experimental period, volume of pooled semen per collection ranged from 0.50 to 0.70 ml (mean, 0.65 ml) for T1, 0.20 to 0.60 ml (mean, 0.51 ml) for T2, 0.30 to 0.65 ml (mean, 0.44 ml) for T3, 0.25 to 0.60 ml (mean, 0.43 ml) for T4 and 0.20 to 0.60 ml (mean, 0.46 ml) for T5. Mean ejaculate volume per bird ranged from 0.14 ml to 0.22 ml across treatments.

The pre-dilution semen characteristics (VOE, SPC, TSE, SPM and TMS) of cocks belonging to the different treatments are presented in Table 2.

There were no significant differences ($P < 0.05$) between treatments in pre-dilution semen characteristics. Semen volume ranged from 0.17 to 0.21 ml per cock across treatments while SPC and TSE ranged from 146.90 ± 3.00 to $156.20 \pm 2.71 \times 10^6$ and 26.85 ± 2.24 to $31.63 \pm 1.06 \times 10^9$ respectively. Total motile sperm (TMS) ranged from $15.24 \pm 1.36 \times 10^9$ to $18.47 \pm 1.60 \times 10^9$ while SPM ranged from 51.83 ± 2.45 to $58.80 \pm 1.31\%$.

Table 3 presents the effects of dilution and diluent type on TMS, SPM, fertility and hatchability of eggs laid by hens inseminated with semen from the different treatments.

There were significant ($P < 0.05$) differences in SPM, TMS, fertility and hatchability of eggs between treatments. Percent motile sperm (SPM) and TMS were similar in semen diluted in sodium citrate, HCM and saline but significantly ($P < 0.05$) lower in semen diluted in UHCM. The highest fertility of 72.10 ± 1.55 and 66.90 ± 2.33 were recorded in eggs of hens inseminated with semen diluted in sodium citrate and HCM, respectively. These were followed by those hens inseminated using semen diluted in saline (52.50 ± 1.58). Hens inseminated using semen diluted in UHCM laid the least percent fertile eggs. Hatchability of fertile eggs incubated (Hatchability-1) varied significantly ($P < 0.05$). Eggs fertilized with semen diluted in sodium citrate had the highest hatchability of 76.50 ± 1.20

Table 1: Volume of pooled ejaculate and insemination dose according to treatment

Ejaculation	Week 1		Week 2		Week 3		Week 4		Week 5	
	1	2	1	2	1	2	1	2	1	2
Control										
POV	0.70	0.60	0.70	0.70	0.65	0.65	0.70	0.50	0.70	0.60
ISD	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Na-citrate										
POV	0.40	0.50	0.55	0.60	0.50	0.55	0.60	0.50	0.40	0.50
ISD	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Heated coconut milk (HCM)										
POV	0.65	0.65	0.30	0.40	0.40	0.40	0.50	0.40	0.30	0.40
ISD	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Unheated coconut milk (UHCM)										
POV	0.45	0.25	0.40	0.50	0.60	0.44	0.40	0.50	0.40	0.40
ISD	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Saline (0.98%)										
POV	0.50	0.20	0.45	0.55	0.40	0.60	0.50	0.40	0.45	0.55
ISD	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

POV: pooled volume of ejaculate; ISD: insemination dose.

Table 2: Pre-dilution semen characteristics (mean±SE) of cocks assigned to different treatments

Parameter	Treatment					
	Control	Na-Citrate	HCM	UHCM	Normal saline	P Value
VOE (ml)	0.21±0.01	0.20±0.01	0.17±0.01	0.18±0.01	0.17±0.01	0.10
SPC (x10 ⁶ /ml)	146.90±3.00	156.20±2.71	154.93±2.55	155.33±2.63	154.93±2.61	0.10
TSE (x10 ⁹)	31.63±1.73	31.70±2.14	26.85±2.24	27.22±2.25	26.97±2.41	0.24
SPM (%)	51.83±2.45	57.17±1.92	56.50±1.56	58.80±1.31	58.00±1.56	0.06
TMS (x10 ⁹)	16.69±1.29	18.47±1.60	15.24±1.36	16.13±1.45	15.61±1.51	0.55

VOE: volume of ejaculate; SPC: sperm concentration; TSC: total sperm count; TMS: total motile sperm; SPM: sperm motility; Na-citrate: sodium citrate; HCM: heated cocoanut milk; UHCM: unheated cocoanut milk.

Table 3: Effects of dilution and diluent type on sperm motility, fertility and hatchability of chicken eggs

Treatment	Trait				
	Na-citrate	HCM	UHCM	Saline	Control
SPM (%)	71.60±1.59 ^a	65.55±1.63 ^a	30.85±2.39 ^c	68.60±1.02 ^a	43.40±5.10 ^b
TMS (x 10 ⁹)	18.33±1.38 ^a	15.78±1.80 ^a	8.09±1.03 ^b	19.88±2.37 ^a	12.40±1.84 ^b
Fertility (%)	72.10±1.55 ^a	66.90±2.33 ^a	34.90±3.14 ^c	52.50±1.58 ^b	51.80±1.87 ^b
Hatchability-1	76.50±1.20 ^a	70.50±1.35 ^b	51.40±1.16 ^d	65.60±2.40 ^c	54.00±2.09 ^d
Hatchability-2	64.20±1.20 ^a	62.40±1.52 ^a	30.90±0.85 ^c	45.20±2.37 ^b	48.80±1.20 ^b

a, b, c, d: means on the same row with different superscripts are significantly ($P \leq 0.05$); TMS: total motile sperm; Na-citrate: sodium citrate; HCM: heated cocoanut milk; UHCM: unheated cocoanut milk; hatchability-1, 2: hatchability as percentage of fertile and total eggs incubated, respectively.

compared to 70.50±1.35 for HCM, 65.60±2.40 for saline and 51.40±1.16 for UHCM. Hatchability of all eggs incubated (Hatchability-2) did not vary statistically ($P < 0.05$) between eggs laid by hens inseminated using semen diluted in sodium citrate and HCM but was significantly ($P < 0.05$) lower under saline diluent and lowest under UHCM diluent (45.20±2.37 and 30.90±0.85, respectively). There were significant ($P < 0.05$) effects of dilution on all the traits measured. Semen diluted in sodium citrate, HCM and saline surpassed undiluted neat semen in SPM, TMS and hatchability-1. Undiluted semen and semen diluted in UHCM differed significantly in SPM (43.40±5.10 vs 30.85±2.39), fertility (51.80±1.87 vs 34.90±3.14) and hatchability-2 (48.80±1.20 vs 30.90±0.85) but were similar in TMS and hatchability-1. Undiluted neat

semen and semen diluted in saline had equivalent values for fertility and hatchability-2.

The effect of storage time (min) on spermatozoa motility (SPM) of diluted semen is presented in Fig. 1 while the effects of interaction of diluent type and storage time on sperm motility (SPM) is presented in Fig. 2. There was highly significant ($P < 0.00$) effect of storage time (min) on SPM (Fig. 1). Percent motile spermatozoa (SPM) decreased linearly with storage time from 82.13% immediately pd. (0 min) to 79.23, 64.37, 56.41, 50.57 and 39.74% at 20, 40, 60, 80 and 100 min pd, respectively. For each diluent, significant differences ($P < 0.05$) were observed in percent SPM at different storage times. Sperm motility decreased significantly ($P < 0.05$) with increase in storage time being generally significantly ($P < 0.05$) highest at 0 and 20 min pd than at subsequent time periods.

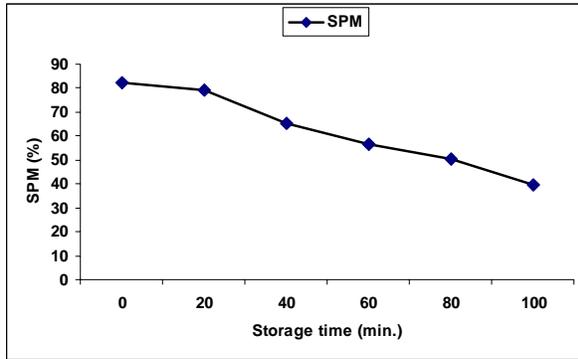


Fig. 1: Changes in sperm motility (SPM) with storage time (irrespective of storage medium) for ambient temperatures: 21-28°C.

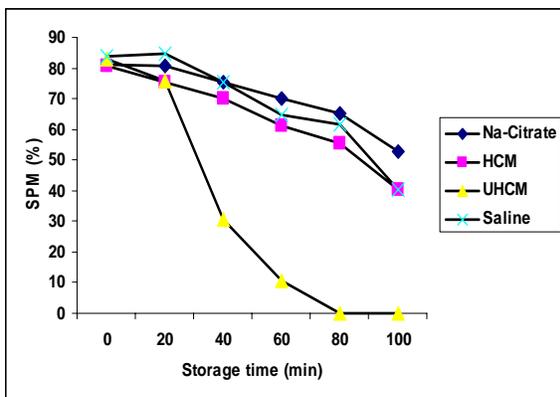


Fig. 2: Trend in sperm motility (SPM) with storage time for semen diluted in different diluents (interaction effect of diluent x storage time on SPM). Range of ambient temperature: 21-28°C. HCM: heated coconut milk, UHCM: unheated coconut milk, Saline: normal saline.

The least percent SPM was therefore observed at 100 min pd for semen diluted with sodium citrate buffer, HCM and saline (52.9 ± 1.69 , 40.5 ± 0.90 and 40.2 ± 0.77 , respectively) but from 80 min for UHCM (0.00 ± 0.00). Reduction in SPM with storage time was very rapid in semen diluted with UHCM compared to other diluents. For instance SPM decreased to 30.5 ± 0.77 (~60% reduction), $10.5 \pm 0.91\%$ (~86% reduction) and 0.00 ± 0.00 (100% reduction) at 40, 60, and 100 min., respectively for this diluent from its value of $75.7 \pm 1.82\%$ at 20 min. pd.

Discussion

Poultry species in general are reported to yield low volume of ejaculate (Das, 2002; Das et al., 2004; Dumpala et al., 2006). The observed pooled ejaculate volume (POV) of 0.20 to 0.70 across treatments (mean range: 0.14-0.22 per bird, Table 1) falls within the

range commonly reported for domestic chickens using the dorso-abdominal massage method (Machebe and Ezekwe, 2005; Siudzinska and Lukaszewicz, 2008; Elagib et al., 2012). The insignificant differences in pre-dilution semen characteristics of cocks assigned to different treatments (Table 2) were expected. Cocks of the same breed, age and similar body weights were randomly assigned to treatments thereby minimizing differences between treatments at the onset of the study. In addition, the same evaluator was used for all semen samples thereby minimizing the effect of subjectivity. Semen characteristics are influenced by breed, age, body weight and a number of environmental factors including the evaluator (Machebe and Ezekwe 2005; Nwachukwu et al., 2006).

The significant differences observed in percent sperm motility (SPM), TMS, fertility, hatchability-1 and hatchability-2 among different diluents (Table 3) reflect the relative impact of the diluents on sperm viability. Unheated coconut milk (UHCM) was the poorest semen extender probably on account of its high viscosity which impeded sperm motility (Sule et al., 2007; Okukpe et al., 2012). The poor performance of UHCM could also be due to presence of microbial contaminants which may have affected the sperm directly or indirectly by depleting the energy substrates in the medium (Ugwu and Igboeli, 2009). There was no significant difference between sodium citrate, HCM and saline in SPM and TMS and between sodium citrate and HCM in fertility and hatchability-2 probably due to the effect of heating. Sule et al. (2007) however reported that sperm motility in buck semen stored for 2 or 3 h in semen extenders was inversely proportional to the concentration of coconut milk in the extenders. We assessed diluted semen for SPM for a total keeping time of 100 min. (<2hr) pd in the present study and we used heated coconut milk instead of raw coconut as employed by Sule et al. (2007). Furthermore, species variation in sperm survival in semen extenders is well established (Peterson et al., 2007). The present study involved cock semen while the report mentioned above involved buck semen. The heating of coconut milk followed by sieving may have reduced its viscosity, and improved its shelf life by reduction of bacterial contamination, preservation of nutrient content, and protection of spermatozoa from immediate microbial attack and oxidative damage. Heating of coconut milk has been shown to improve its shelf life and increase the amount of lauric acid which has antimicrobial effects as well as antioxidant properties (Nevin and Rajamohan, 2004; Raghavendra and Raghavaroa, 2010). Sodium citrate and HCM extenders were superior to saline extender in fertility and hatchability parameters even though the three extenders had equivalent SPM and TMS. The reason for the poor performance of saline extender in fertility and

hatchability values could be due to two main factors (1) lack of energy substrate in the saline extender (Parker and McDaniel, 2006). Sperm cells need energy substrates to generate the ATP needed for metabolic activities, sustained progressive motility, ascension of the female genital tract to the infundibulum and for the penetration of the zona pellucida to effect fertilization of the egg. (2) diluting semen with saline has been reported to cause hyper excitation followed by exhaustion (Packer and McDaniel, 2006) and this reduces their motility subsequently (Packer and McDaniel, 2006) and the fertilizing ability *in vivo*. The above finding indicates that spermatozoa motility *in vitro* may not predict accurately sperm fertilizing ability. Peterson et al. (2007) reported a lack of correlation between membrane integrity (another sperm quality trait) and fertilizing ability while Parker and McDaniel (2006) reported that sperm quality index (which includes SPM) is predictive of fertility and hatchability only at low semen dilutions. Heated coconut milk was superior to UHCM in the studied parameters indicating that heating improved the positive qualities of coconut milk as a semen extender. The observed significant differences between diluted and raw semen in semen quality, fertility and hatchability indicate the importance of semen dilution in enhancing semen quality during storage while the inferior values obtained for some diluents compared to raw semen in some of the parameters point to the need for care in choice of diluents for any particular species or breed and for the particular semen operation proposed.

The rapid loss of sperm motility with storage time (Fig. 1) reflects the tendency of chicken semen to lose viability when exposed to the high tropical ambient temperatures. Reports of inverse relationship between semen quality and storage time abound in literature (Adeyemo et al., 2007; Peterson et al., 2007; Udeh and Oghenesode, 2011; Miskeje et al., 2013). For instance, Peterson et al. (2007) found declining percent motile spermatozoa overtime for semen stored at 4°C and 18°C. In the present study, more than 50% loss in motility was observed over all diluent types by 100 min post dilution probably on account of the high ambient temperature. Loss of motility was most rapid overtime in semen diluted in UHCM (Fig. 2) probably on account of the aforementioned reasons. Effect of heating may have been responsible for the retention of up to 60% motility by 80 min post extension in semen extended in HCM. Heating has been shown to prolong the shelf life of coconut milk (Raghavendra and Raghavaroa, 2010).

Conclusion

From the results presented HCM maintained a level of sperm motility, fertility and hatchability comparable

to that of the best diluent (sodium citrate) in the present study. The use of HCM to extend cock semen proposed for immediate insemination is therefore advocated. Further studies are required to evaluate this medium for longer duration of preservation of semen.

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