Philippine Journal of Science
149 (1): 121-131, March 2020
ISSN 0031 - 7683
Date Received: 22 Aug 2019

Coconut Water with Either Tomato Juice or Garlic Extract as Extender Components for Paraoakan Native Chicken Semen at Different Storage Temperatures

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Computer-assisted Semen Analyzer (CASA)- based analysis of extended semen from 18 adult Paraoakan Philippine native chickens using coconut water (CW) with either tomato juice or garlic extract were carried out in this study. Only extended semen samples passing the preliminary quality evaluation were further analyzed. Pooled semen were randomly distributed into four types of semen extender: (1) Ringer's solution (RS), (2) RS + 20% CW + 0.02 g sodium phosphate (SP), (3) RS + 20% CW + 7% tomato juice + 0.04 g SP, and (4) RS + 20% CW + 2% garlic extract + 0.04 g SP and stored at either low (7–10 °C) or room (22–25 °C) temperature. The procedure was done for 13 collection periods, which served as the blocking factor. Results showed that except for garlic-supplemented extender, semen diluted with other types of extender and maintained at low temperature (7–10 °C) demonstrated the longest average shelf life, which is characterized by the period of time observed before sperm motility falls below 20%. Collectively, the addition of 20% CW on RS, with supplementation of 7% tomato juice can maintain sperm motility above 20% for 24 h at low temperature. This can be attributed to the chemical composition of CW and tomato juice that are beneficial to sperm cell metabolism, control of pH, and osmotic pressure and inhibition of microbial growth. Results also showed that CW can be used as an effective and economical partial substitute to RS.

Keywords: coconut water, garlic extract, Paraoakan native chickens, semen extension, tomato juice

INTRODUCTION

Semen extension is a very useful technique in realizing the full potential of artificial insemination (AI) in poultry. Poultry semen has low volume per ejaculation but is very concentrated, containing about six billion spermatozoa per milliliter (Donoghue and Wishart 2000). The fertility capacity and sperm motility of fresh semen *in vitro* usually decrease within an hour after collection (Dumpala *et al.* 2006; Oluwatoba *et al.* 2017). Thus, an extender will facilitate prolonged semen handling by maintaining good sperm motility and viability by inhibiting pathways that are detrimental to semen survival (Oluwatoba *et al.* 2017).

Choosing the right extender is an important prerequisite of processing semen for AI (Peterson *et al.* 2007; Ogbu *et al.* 2014). Numerous extenders have been recommended for poultry semen (Iaffaldano *et al.* 2005; Parker and McDaniel 2006; Dumpala *et al.* 2006; Ogbu *et al.* 2014). A proper semen extender will provide an energy source for the spermatozoa and like the natural medium of the sperm, the seminal plasma will maintain conducive osmolarity and pH levels (Siudzińska and Łukaszewicz 2008; Boucif *et al.* 2011; Udeh and Oghenesode 2011;

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Ogbu *et al.* 2014). Commercial extenders differ in content and complexity. The standard component of extenders is glutamic acid for it is the most significant chemical component of the poultry seminal plasma (Siudzińska and Łukaszewicz 2008; Ogbu *et al.* 2014).

CW, which is widely available locally and also a natural buffer solution, has been used for semen preservation. It has been tested as a substitute for cow's milk or egg yolk as a main component of extenders in goat semen extension (Juma 2017). It has also been used for cryopreservation of semen in goats (Daramola *et al.* 2016), dogs (Cardoso *et al.* 2005), and boars (Luzardo *et al.* 2010). The use of powdered CW has been tested and proven to maintain the fundamental properties of the seminal plasma, thus maintaining stability and longevity of roosters semen (Moreira-Neto *et al.* 2009; Purdy *et al.* 2009; Soares and Guerra 2009; Bongalhardo 2013; Freitas *et al.* 2018). In addition, there are reported CW-based semen extenders formulated for roosters semen (Rashid and Qistina 2015; Khaeruddin and Srimaharani 2019; Rochmi and Sofyan 2019). It is also noteworthy to mention that the study conducted by Puja *et al.* (2018) revealed that dog semen has more than 60% motility up to five days when extended with 20% CW and storage temperature of 4 °C.

Meanwhile, other natural ingredients like tomato juice contain carotenoids that are natural antioxidants (Mangiagalli *et al.* 2007) and the supplementation of 7% tomato juice in semen diluent was found to increase the quality and prolong the shelf life of chicken semen (Al-Daraji 2012b). Other readily available ingredients like garlic contain phenolic and flavonoids fractions, which have shown beneficial effects such as antioxidant properties (Miller *et al.* 2000; Balogun *et al.* 2017). Balogun *et al.* (2016) reported that with the use of garlic extract at 1:4 extraction ratio (20 g garlic to 80 mL double distilled water) and at 2% or 6% inclusion rate during semen extension will yield to a better fertility percentage when used in AI in chicken. This is further supported in their other study (Balogun *et al.* 2017) in roosters, where the use of aqueous garlic extract at a 2% inclusion rate leads to better semen characteristics such as high mass activity, motility, and viability. Unfortunately, the available methods for semen storage are only effective for a short time, usually up to 12 hours (Thurston 1995; Al-Daraji 2012a). Although there are numerous studies about chicken semen extension (Al-Daraji 2012a, 2012b; Ogbu *et al.* 2014; Rashid and Qistina 2015; Balogun *et al.* 2016, 2017; Daramola *et al.* 2016; Okoro *et al.* 2016; Oluwatoba *et al.* 2017; Freitas *et al.* 2018; Ezzat and Habeib 2019; Usman and Sir 2019), researches on the use of natural extracts are still limited.

The Philippine native chicken population constitutes 44.78% of the total chicken inventory (PSA 2018). In rural areas, native chickens are commonly raised by most households (Mananghaya 2017). Traditionally, these native birds are sources of additional income and high-quality protein food. Native chickens are classified as high-grade chicken products that are paid at premium prices with a local niche market (Mananghaya 2017). One of the largest native chicken strains in the Philippines is the Paraoakan, which is predominantly found in Palawan province, has long legs and neck, and is being utilized for its meat and eggs (Tabuada 2019).

Conservation, improvement, and utilization of native animals are among the priority programs in the country today. A critical part of these programs is to generate technologies on how to economically prolong the shelf life of native chicken semen. Therefore, this study demonstrated the use of natural ingredients like tomato juice, garlic extract, and/or CW as a supplement for an RS-based extender for Paraoakan native chicken semen.

MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of the Philippines Los Baños (UPLB) with assigned protocol number CAFS-2018-006. Semen collection was done at the UPLB University Animal Farm in Brgy. Putho-Tuntungin, Los Baños, Laguna, Region IVA, Philippines (14°09'24.4"N, 121°15'06.6"E). All semen processing and evaluation were conducted at the Animal Physiology Laboratory, Institute of Animal Science (IAS), College of Agriculture and Food Science (CAFS), UPLB.

Experimental Design

The experiment was carried out using a 4 x 2 factorial experiment in a randomized complete block design (RCBD) with four types of extender and two storage temperatures. The collection period was used as the blocking factor. The types of extender were: T1 (100% RS); T2 (80% RS + 20% CW + 0.02 g SP); T3 (73% RS + 20% CW + 7% tomato juice + 0.04 g SP); and T4 (78% RS + 20% CW + 2% garlic extract + 0.04 g SP). The storage temperatures used were low temperature with 7–10 °C and room temperature with 22–25 °C.

A total of 102 semen samples with at least 80% total motility and normal morphology collected from 18 sexually mature Paraoakan native chickens (14-mo-old and with an average weight of 2.28 kg) over 13 collections periods were used in this study.

Management and Care of Native Chickens

Eighteen (18) 4-mo-old Paraoakan native cockerels were

purchased from the National Swine and Poultry Research and Development Center, Bureau of Animal Industry, Tiaong, Quezon, Philippines. They were brought and raised to maturity at the University Animal Farm. Roosters were individually caged with a floor space of 2.0 ft^2/bird, in slightly elevated flooring, and housed in an open-sided housing system under normal farm conditions (21–26 °C). Birds were fed with commercial chicken breeder feeds and provided with clean drinking water. Farm's immunization program against Newcastle disease every 6 mo was followed. Cages were cleaned every week with the manual scraping of feces and hosing down of water with disinfectant. Training of cockerels for semen collection was done using dorso-abdominal massage method by massaging the rooster's abdomen and back for 1 min for seven consecutive days. After which, gentle but rapid stroking of the rooster's abdomen and back region towards the tail was done to stimulate protrusion of the papillae and release of semen (Burrows and Quinn 1937). This is then repeated every three days.

Semen Collection

Semen collection was done every three days at 07:00 AM by one trained personnel at the farm and processed by other personnel in the laboratory. Caution was taken to avoid any dirt or fecal contamination of the semen. An interval of 17 h from feeding to collection is done to ensure that there will be no fecal contamination during the collection of semen samples. Removal and plucking of feathers (*i.e.* fluff feathers) at the peri-cloacal region were also done to prevent dirt contamination during collection. Semen collection usually takes 5–10 min for the entire collection period. During semen collection, anti-stress supplements were given to all the experimental animals. Fresh semen samples were first evaluated individually for gross characteristics. Among the parameters observed were color (*i.e.* either watery or creamy via visual appraisal) and volume (*i.e.* using a sterile disposable 1 mL syringe with 0.01 mL calibration). Semen samples from the same-day collection were pooled using a sterile disposable 1 mL syringe until a minimum total volume of 1 mL is reached. This depends on the number of chickens needed to reach at least 1 mL of total semen volume. Immediately, the 1 mL syringe containing the semen sample was placed inside a sterile beaker with sterile cloth and placed inside a clean and disinfected foam-padded icebox and sent to the laboratory for evaluation and processing. The procedure was done for 13 collection periods spread out from May to July 2019.

Semen Processing and Evaluation

Semen samples were pooled to eliminate the effects of individual variability from gamete donors. Afterward, using a conventional hemocytometer slide, sperm concentration was assessed as described by Capitan and Palad (1999). The pooled semen was gently mixed and divided into equal volume and was randomly assigned to each experimental treatment following an optimized dilution rate (Table 1) for CASA (Ceros II, IMV Technologies, China) evaluation. Semen with initial total sperm motility and normal morphology of ≥ 80% were used in this study. For each type of extender, samples were further divided into equal volumes and assigned to two different storage temperatures. About 5–10 µL of extended semen sample (from T1) per batch was used for initial sperm motility (%) evaluation using the Gallus setup/module of CASA with the frame capture speed of 60 Hz and camera exposure of 4 ms. Standard microscope slides were used in analyzing the samples. The slides were placed in a MiniTherm Stage Warmer that maintains samples at 37 °C. Five (5) frames were captured for every analysis, which takes an average time of one minute to finish. Microscopic characteristics such as % motility, % progressive, and % normal morphology were analyzed (*i.e.* from 5 frames) every 4 h using CASA until the observed sperm motility falls below 20%.

Table 1. Optimized dilution rate for Paraoakan native chicken semen based on haemocytometer count for CASA.

Haemocytometer count (sperm count)	Dilution rate (semen: extender)
< 650	1:20
651–780	1:30
781–1500	1:40
1501–1700	1:45
> 1701	1:50

Preparation of Extenders

All extenders were prepared in aseptic procedures. Water from young coconut was filtered through a sterilized white cloth and collected in a sterile flask. Garlic extender was prepared using garlic meat with a ratio of 1:4 garlic to water (*e.g.* 20 g garlic to 80 mL double distilled water) and the mixture was set aside for 72 h and stored in the refrigerator. The mixture was then filtered using a sterilized white cloth. Tomato fruit was thoroughly blended and collected in a 250-mL sterile flask. The blended tomato was wrapped in a sterilized white cloth and tightly squeezed to express the extract. The extract was then filtered further through a sterilized white cloth thrice and collected using a sterile flask. The pH of the extenders was adjusted to 6.9–7.6 using SP based on the optimized condition found conducive for sperm viability (Capitan and Palad 1999). Extenders were prepared freshly every collection period.

Statistical Analyses

All data were analyzed using repeated measures multivariate analysis of variance following a 4 x 2 factorial experiment in RCBD. Adjustment for multiple comparisons of least-square means was done using the Tukey-Kramer test. Level of significance was set at $p \leq 0.05$ All statistical analyses were done using SAS University Edition 2.8 9.4 M6 (SAS/STAT®, SAS Institute Inc., NC, USA).

RESULTS AND DISCUSSION

Gross Characteristics

Semen color and volume from the Paraoakan native chickens were observed (n = 156). Eighty-three (83) ejaculates (53.21%) were watery while 73 ejaculates (46.79%) were creamy. The semen volume ranged from 0.05–0.27 mL with an average of 0.16 mL. These findings conform to previous reports on chicken (Gordon 2004; Peters *et al.* 2008; Getachew 2016). The average volume of semen is between 0.05–0.50 mL in light chicken breeds (*i.e.* egg-type) and 0.1–0.9 mL in meat-type breeds (Mohan *et al.* 2018). Similarly, there was no definite ejaculate volume per rooster observed as semen volume is affected by numerous factors like genetics, nutrition, and environment (Anderson 1945; Saeed and Al-Soudi 1975; Kabir *et al.* 2007; Peters *et al.* 2008; Murugesan *et al.* 2013; Tarif *et al.* 2013; Okoro *et al.* 2016; Getachew 2016). Time and frequency of collection were also found to significantly affect semen volume (Egbunike and Oluyemi 1979; Okoro *et al.* 2016; Riaz *et al.* 2004). Due to the low volume of poultry semen, studies on semen quality were done using pooled semen samples. However, there is a potential impairment of semen quality as a consequence of possible sperm competition interactions (Schneider *et al.* 2017).

Microscopic Characteristics

Sperm concentration in pooled semen of Paroakan native chickens ranged between 4.6–22.1 x 10^9 spermatozoa/mL with an average of 12.8 x 10^9 ± 5.5 x 10^6 spermatozoa/mL, which was higher than the published data for chicken. Ideally, the normal sperm count in chicken ranges from 3– 7 x 10^9 spermatozoa/mL (Getachew 2016). In a study by Usman and Sir (2019) indigenous cockerel sperm concentration was significantly higher compared to Amo breeds. Similar findings were reported by Ajayi *et al.* (2011). A study by Khaeruddin and Srimaharani (2019) showed that sperm concentration of Kampong chickens was 2.65 x 10^9 spermatozoa /mL. Daily production of sperm is about 100 M per gram of testes weight (mature testes weigh around 15–20 g) and this is constant regardless of the collection or mating frequency (Kharayat *et al.* 2018). The significant difference in sperm concentration can involve several factors such as feed intake, body size, genetic makeup, body weight, breed differences, and frequency of semen collection (Malik *et al.* 2013; Usman and Sir 2019). Sperm concentration is an important measure of semen quality and provides information on the extent of dilution necessary to obtain required sperm numbers per insemination dose (Abutu 2015).

As expected, there was a decreasing trend in percentage (%) total motility over time among pooled extended semen samples divided and equally assigned in the four experimental extender treatments and maintained at different storage temperatures (Figure 1). While the study did not monitor the pH through time, the reduction in motility could likely be due to the change in pH. The acidic environment negatively affects sperm motility. During the long storage of spermatozoa, semen pH decreases leading to a reduction in spermatozoa motility. This results from the increase in the production of lactic acid anaerobically over time during sperm metabolic activity (Abutu 2015).

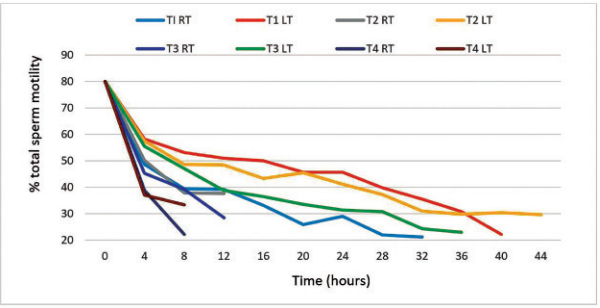

Figure 1. Changes in (%) sperm motility of Paraoakan semen diluted using different treatment extenders and maintained at low or room temperature.

On the other hand, Table 2 shows a significant interaction between the type of semen extender and the holding temperature ($p = 0.0269$). This is with reference to the average time-lapsed before sperm motility drops below 20%. The longest average time was demonstrated by semen, which was extended using any of the three preparations except with garlic-supplemented extender and maintained at low temperature. This observation is even longer than in the report of Rashid and Qistina (2015) using Kampung cockerels with a significantly higher percentage of live sperm in a 6-h observation. On the other hand, higher than 20% sperm motility over time was seen comparable in semen diluted using tomato-supplemented extender maintained at low temperature and RS at room temperature. Meanwhile, short and comparable average time-lapse responses were demonstrated by semen diluted using garlic-supplemented extender maintained at low temperature and in semen diluted using any of the four treatments maintained at room temperature.

Table 2. Least square mean (± SEM) time (h) for the Paraoakan native chicken semen to fall below 20% motility when diluted with different extenders at different storage temperatures.

Temperature	Type of extender				
	100% RS (T1)	80% RS + 20% CW (T2)	73% RS + 20% CW + 7% tomato juice (T3)	78% RS + 20% CW + 2% garlic extract (T4)	Mean for temperature
Room (25–27 °C)	14.77 ± 1.79^{bc}	10.15 ± 1.79^{c}	9.22 ± 1.88^{c}	3.70 ± 2.21^{c}	10.52 ± 1.09
Low (7–10 °C)	29.23 ± 1.79^{a}	23.07 ± 1.79^{a}	21.89 ± 1.88^{ab}	6.91 ± 1.98^{c}	20.47 ± 0.95
Mean for type of extender	22.00 ± 1.24	16.62 ± 1.24	16.32 ± 1.35	7.03 ± 1.87	
				p-values	
Type of extender x temperature				0.0269	
Type of extender				< 0.0001	
Temperature				< 0.0001	

Values with the same superscript are not significantly different ($p > 0.05$).

Extension and storage of semen in temperatures below 15 °C help in reducing sperm metabolic activity. Thus, conserving their energy and delaying the accumulation of metabolic waste products in the medium. More so, low temperature also protects the semen from the detrimental effects of microbial contamination since this temperature is not conducive for most microbial growth (Gadea 2003; Abutu 2015). The same observation on dilution of fowl semen at low temperatures and maintaining it up to 24 h without impairing the viability and fertilizing capacity of the sperm were reported (Siudzińska and Łukaszewicz 2008; Getachew 2016).

Percent Motile and Progressive Sperm

Remarkably, as seen in Table 3, the use of any of the four extenders at different holding temperatures do not affect the sperm motility (%) of Paraoakan native chickens. However, a significant interaction effect (p = 0.0071) between the type of extender and the holding temperature was observed on sperm progressive motility (%) (Table 4) where semen extenders, except with tomato-supplemented, maintained at both storage temperatures were comparable. While semen diluted using RS at room temperature and garlic-supplemented at low temperatures were comparable to RS with CW only and tomato-supplemented maintained at both storage temperatures.

CW as an extender has been used in various studies and shown to successfully preserve the semen of goats (Daramola *et al.* 2016), dogs (Cardoso *et al.* 2005), and boars (Luzardo *et al.* 2010). In bucks, there has been an improvement in sperm viability with CW extenders, and this was attributed to the ability of the CW to harness its potassium content for the survival of the spermatozoa (Daramola *et al.* 2016). In other studies, (Kannan and

Table 3. Least square mean (± SEM) motility (%) of Paraoakan native chicken semen when diluted with different extenders at different storage temperatures and observed before sperm motility drops to 20%.

Temperature	Type of extender				
	100% RS (T1)	80% RS + 20% CW (T2)	73% RS + 20% CW + 7% tomato juice (T3)	78% RS + 20% CW + 2% garlic extract (T4)	Mean for temperature
Room (25–27 °C)	46.46 ± 2.09	49.74 ± 2.09	47.91 ± 2.31	45.14 ± 3.53	47.31 ± 1.30
Low (7–10 °C)	49.36 ± 2.09	47.00 ± 2.09	44.35 ± 2.20	45.45 ± 2.58	46.54 ± 1.13
Mean for type of extender	47.91 ± 1.48	48.37 ± 1.48	46.14 ± 1.61	45.29 ± 2.23	
			p-values		
Type of extender x temperature			0.4266		
Type of extender			0.5781		
Temperature			0.6518		

Values with the same superscript are not significantly different ($p > 0.05$).

Jain 2004; Arabi and Seidaie 2008; Daramola *et al.* 2016), antioxidant compounds such as pyridoxine and vitamin C derived from the addition of CW was attributed to the improved viability parameters in the observed spermatozoa due to its low toxicity and good water solubility. Compounds in CW also serve as protection against oxidative damage due to the increased production of reactive oxygen species or free radicals related to the *in vitro* storage at low temperatures, and especially to the polyunsaturated fatty acids in the cell membrane or to the nucleic acids in the nucleus (Evans and Halliwell 2001; Peruma *et al.* 2011; Daramola *et al.* 2016). Amino acids such as arginine and lysine in CW prolong the period of sperm motility (Novak *et al.* 1960; Rodriguez 2016). The use of CW has also a cryoprotective role, where it helps in the maintenance of osmotic balance and energy provision of sugars to support the survival of the sperm during deep freezing (Koshimoto and Mazur 2002; Aboagla and Terada 2003; Yancey 2005; Naing *et al.* 2010; Juma 2017).

Lycopene in tomato juice, acting as an antioxidant protecting the spermatozoa, has already been reported in various studies (Brzezińska-Ślebodzińska *et al.* 1995; Kessopoulou *et al.* 1995; Geva *et al.* 1996; Al-Daraji 2012b). In this study, a 7% inclusion rate of tomato juice resulted in comparable sperm motility (%) (Table 3). This observation is in agreement with the findings of Al-Daraji (2012b).

Garlic extract also has antioxidant properties and this has shown to increase fertility by reducing lipid peroxidation (Moher *et al.* 2009; Musavi *et al.* 2018). Although the use of garlic extract help in the prevention of oxidant damage, it was also demonstrated that the sperm motility of extended semen decreases as the garlic extract inclusion rate increases (Balogun *et al.* 2016). This can be attributed to its high spermicidal effect as the level of supplementation increases (Okoro *et al.* 2016). In this study, the use of garlic extract at 2% inclusion rate was found to be comparable to the three other treatments in terms of sperm motility (%) (Table 3). Although the use of garlic extract was shown to be inferior to the other treatments with reference to semen shelf life, its potential as a natural extender ingredient may be realized at a lower than 2% inclusion rate. However, reduction in the motility of the sperm in diluted samples can be attributed to various causes like mechanical damage on the sperm during the mixing of the diluents, introduction of toxic substances and/or dilution of seminal components essential in semen survival and protection of sperm changes in the permeability of cell (Baguio and Capitan 2008).

Percent Morphologically Normal Sperm

There was no significant interaction observed between the type of extender and holding temperature on morphologically normal sperm (%) in Paraoakan native chicken semen. Moreover, the average morphologically normal sperm (%) in all the treatments were found to be negligible (Table 5). However, holding temperature significantly affected sperm morphology (%) ($p = 0.0056$) suggesting that low temperature is better than room temperature in maintaining the normal sperm morphology during extension.

Chicken spermatozoa are filiform in shape (Pelaez *et al.* 2011). They have simple acrosome with the mid-piece being cylindrical of distal centriole surrounded by a cover of mitochondria (Abutu 2015). However, in chickens and turkeys, spermatogenesis does not stop after pubertal onset. Nevertheless, it is strongly affected

Table 4. Least square mean (± SEM) progressive sperm (%) of Paraoakan native chicken when diluted with different extenders at different storage temperatures and observed before sperm motility drops to 20%.

Temperature	Type of extender				
	100% RS (T1)	80% RS + 20% CW (T2)	73% RS + 20% CW + 7% tomato juice (T3)	78% RS + 20% CW + 2% garlic extract (T4)	Mean for temperature
Room (25–27 °C)	10.29 ± 1.18^{ab}	12.21 ± 1.18^{ab}	8.69 ± 1.24^{b}	14.85 ± 1.45^{a}	11.44 ± 0.70
Low (7–10 °C)	14.31 ± 1.18^{a}	11.07 ± 1.18^{ab}	7.67 ± 1.24^{b}	10.57 ± 1.30^{ab}	10.56 ± 0.61
Mean for type of extender	12.30 ± 0.80	11.69 ± 0.80	7.99 ± 0.87	12.02 ± 1.20	
				p-values	
Type of extender x temperature				0.0071	
Type of extender				0.0019	
Temperature				0.3412	

Values with the same superscript are not significantly different ($p > 0.05$).

Table 5. Least square mean (± SEM) morphologically normal sperms (%) of Paraoakan native chicken when diluted with different extenders at different storage temperatures and observed before sperm motility drops to 20%.

Temperature	Type of extender				
	100% RS (T1)	80% RS + 20% CW (T2)	73% RS + 20% CW + 7% tomato juice (T3)	78% RS + 20% CW + 2% garlic extract (T4)	Mean for temperature
Room (25–27 °C)	95.78 ± 0.61	94.62 ± 0.61	95.25 ± 0.67	95.32 ± 1.02	95.24 ± 0.38
Low (7–10 °C)	97.33 ± 0.61	96.73 ± 0.61	97.43 ± 0.63	95.13 ± 0.75	96.66 ± 0.33
Mean for type of extender	96.56 ± 0.43	95.68 ± 0.43	96.34 ± 0.47	95.22 ± 0.65	
				p-values	
Type of extender x temperature				0.4256	
Type of extender				0.2479	
Temperature				0.0056	

Values with the same superscript are not significantly different ($p > 0.05$).

by low testosterone and gonadotropin levels, leading to low sperm concentrations (Santiago-Moreno *et al.* 2009) and morphologically abnormal spermatozoa (Wakely and Kosin 1951; Santiago-Moreno *et al.* 2016). During storage, a decrease in live and an increase in morphologically abnormal dead spermatozoa characterized by having bent heads were reported in previous studies on avian (Blesbois *et al.* 1999; Siudzińska and Łukaszewicz 2008; Schneider *et al.* 2017). It was also reported that storage of fowl semen using RS causes an increase in the production of chloride ions, which then increases abnormality in the spermatozoa (Saeki 1960; Hudson *et al.* 2016). In addition, the peculiar cylindrical morphology and lesser cytoplasmic content of avian spermatozoa as compared to mammalian spermatozoa pose a challenge in any preservation techniques (Rashid and Qistina 2015). Even though the normal morphology of the sperm is poorly correlated with fertility, it is unlikely that morphologically abnormal spermatozoa to possess a good fertilizing ability (Etches 1996; Sayed *et al.* 2017).

Economic Analysis of the Treatments

The cost of all the extender preparations is shown in Table 6. It shows that RS with CW was 7.77% cheaper than RS alone while tomato-supplemented and garlic-supplemented extenders were 14.27% and 8.83% cheaper than RS, respectively. The use of CW can help in the preservation of semen quality and in lessening the cost of semen extension. Coconut is cheap and readily available (Rashid and Qistina 2015) and, with the surplus on the supply of tomato (Sarian 2018) and garlic (Bulan 2019), the usage of these extracts can help in generating income for the farmers.

Table 6. Cost-benefit analysis of each treatment.

		Price (Php) per liter			
	Amount (Php) / quantity	100% RS	80% RS + 20% CW	73% RS + 20% CW + 7% tomato juice	78% RS + 20% CW + 2% garlic extract
800 mL RS	Php 179.90/L	179.90	143.92**	131.32**	140.32**
200 mL coconut	Php 20.00/pc	–	20.00	20.00	20.00
Tomato	Php 90.00/kg	–	–	0.90***	–
Garlic	Php 150.00/kg	–	–	–	0.75****
Distilled water	Php 11.00 / 350 mL	–	–	–	0.94****
Processing fee	Php 2.00/L	–	2.00	2.00	2.00
Total		179.90	165.92	154.22	164.01

*Volume of each treatment = 1 L
**800 mL RS
***100 g tomato = 70 mL tomato juice
****7.5 g garlic: 30 mL distilled water

CONCLUSION

This is the first report on the characteristics and semen quality in extended Paraoakan native chicken semen. The gross characteristics (semen color and volume) of Paraoakan native chickens are similar to previously reported on fowls. Our results also showed that CW can be used as an effective and economical partial substitute for RS. Longer and comparable average shelf life was seen in semen extended using T1 (100% RS), T2 (80% RS + 20% CW), or T3 (73% RS + 20% CW + 7% tomato juice) and maintained at low temperature (7–10 °C). However, no difference was noted with reference to motility (%) in semen extended using T1, T2, T3, or T4 (78% RS + 20% CW + 2% garlic extract) and maintained in either room (25–27 °C) or low temperature. Nonetheless, progressive sperm (%) over time was seen as inferior for semen diluted using T3 and maintained in both storage temperatures. The longer and favorable shelf life observed can be due to the complementary chemical composition of natural ingredients like CW and tomato that is favorable for the extension of Paraoakan native chicken semen.

ACKNOWLEDGMENTS

This work was supported by the Department of Agriculture – Bureau of Agricultural Research (DA-BAR) through the DA-BIOTECH program funded project entitled, "Development of cryopreservation prototypes as biotechnological interventions for the conservation of genetic diversity of Philippine native pigs, chickens, and ducks" implemented by the IAS, CAFS, UPLB.

STATEMENT ON CONFLICT OF INTEREST

All authors have no conflict of interest to declare.

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