

POTENTIAL OF *Telfairia occidentalis* LEAF EXTRACT AS BOAR SEMEN EXTENDER

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Abstract: The preservation of semen for Artificial insemination (AI) required the use of extender, which served as a buffer media to keep the sperm cell in good condition for fertilization. In this study, fluted pumpkin *Telfairia occidentalis* leaf extract (TOLE) potential as an unconventional boar semen extender was compared to Beltsville thawing solution (BTS), a conventional semen extender over a 48hr storage period. Aqueous solution of TOLE was prepared at three (3) concentration levels (25, 50 and 75%). Fresh ejaculates from boar were extended at (1:4) in TOLE solutions and BTS extender in a completely randomized design; treatments were replicated four (4) times. Extended semen was stored at 17⁰C and evaluated for semen qualities which include pH, dead/live ratio (%) and sperm cell concentration at time intervals (0, 12, 24, 36 and 48) hrs. Extended semen was significantly ($P < 0.05$) affected by semen extenders and storage periods for pH, and dead/live ratio (%) but sperm cell concentration ($10^3/\text{cm}^3$) not ($P > 0.05$) affected. The pH values for BTS was significantly ($P < 0.05$) higher as compared to TOLE extenders. 75% TOLE extender had highest value (43.89%), and BTS had lowest value (12.15%) for dead/live ratio significantly ($P < 0.05$). The pH and dead/live ratio (%) values increased over storage time in all extender. The optimum performance of TOLE as compared to BTS extender was recorded at 50% concentration of TOLE extender. TOLE showed a potential as boar semen extender, but further studies are required to validate and improve its application.

Keywords: Semen extender, boar, pH, *Telfairia occidentalis*, beltsville thawing solution

Introduction

Swine farming in sub-Saharan Africa is faced with challenges which include nutrition, health management and breeding. The need to sustain existence

of good breeds has led to importation of pigs from other continents a procedure since replaced by artificial insemination (AI). An important aspect of AI that has facilitated its wide application is storage of semen for subsequent use.

Artificial insemination (AI) techniques enhance production rates and carcass homogeneity as well as the application of new management systems; hence have increased in the last decade. The advantage of AI is that the genetic potential of the best boar can be transferred to a large number of sows, leading to genetic improvement at a large scale (*Kaeoket et al., 2010*). An important aspect of AI that has facilitated its wide application is storage of semen for subsequent use. The seminal plasma supplies the necessary nutrients for the high metabolic demands of sperm transport through the female genital tract. In the ejaculate, this high metabolic activity can only be maintained over a limited period (*Rijsselaere et al., 2012*). As temperature declines, the proportion of spermatozoa that maintain normal membrane integrity, ultrastructure and biochemical components decreases (*Johnson et al., 2006*).

The important traits for quantitative and qualitative evaluation of ejaculate include: volume of ejaculate, concentration of sperm, motility, percentage of abnormal spermatozoa, total number of spermatozoa and number of dead/live spermatozoa (*Savić et al., 2015, 2017*). Evaluation of sperm cell concentration, volume and percent of live spermatozoa is very important for the determination of maximal dilution of sperm suitable for artificial insemination or for a number of sows which can be inseminated (*Savić et al., 2017, Ivelina 2017*).

The fertilizing ability of stored semen is achieved by using a semen extender, which is a liquid diluent added to the semen (*Savić et al., 2017*). This ensures that the functional characteristics of the sperm cells are maintained such that a higher conception rate is achieved (*Gadea 2003*). Factors affecting the properties of an extender include pH, ionic strength, ions and osmotic pressure of the medium. Anti-microbial substances are also commonly included in diluents (*López Rodríguez et al., 2012*). A number of extenders have been developed which decrease the metabolic activity of spermatozoa using an environment high in CO₂ at ambient temperature (*Gadea 2003; Johnson et al., 2006; Boonkusol et al., 2010*). Presently, Beltsville-TS (BTS) is one of the most widely used semen extender for both short and long term storage. However, with the increase in AI practices, sub-Saharan African countries are faced with the challenges of semen extenders availability, due to the high cost through importation. Therefore, there is need to find an alternative diluent for short term storage of boar semen.

Telfairia occidentalis (*T. Occidentalis*) (family *cucurbitaceous*) is a tropical vine grown in West and Central Africa. It is a popular vegetable in Nigeria, commonly called “Ugwu” and highly reputed in traditional medicine (*Agatemor 2006; Fasuyi and Nonyerem 2007; Kayode and Kayode 2011*). The plant produces luxuriant edible green leaves, which are rich in minerals, (*Salman et al., 2008; Oboh et al., 2010*), and vitamins (*Kayode and Kayode 2011*). The leaf also contains

phytochemicals and has some antiplasmodial properties (*Salman et al., 2008; Oboh et al., 2010; Kayode and Kayode 2011*). Despite the need for affordable semen extenders in Nigeria and the proven rich nutritional profile of *T. Occidentalis*, studies on the usage of *T. Occidentalis* as a potential semen extender are lacking in Nigeria pig production industry. Therefore, the broad objective of this research is to investigate the potential of *T. Occidentalis* as a short-term extender for boar semen.

Materials and methods

Location of study

The study was conducted at the Piggery unit, Teaching and Research Farm of the University of Ibadan, Nigeria. All analyses and examinations were carried out at the University Physiology Laboratory.

Processing of aqueous Telfairia occidentalis leaf extracts (TOLE)

Fresh leaves were harvested from matured stems at onset of flower emergence. Leaves were rinsed off debris with distilled water. *T. Occidentalis* leaves (350 g) were homogenized with distilled water using a homogenizer and the homogenate filtered with a Whatman filter paper (*Oboh et al., 2010*). Proximate analyses content were determined using the standard protocols (AOAC, 1995). The filtrate was then concentrated to one tenth of its original volume and stored at 4°C in a refrigerator using an airtight plastic jar. At the point of application as semen extender, a serial dilution was carried out to have 25%, 50% and 70% concentrations.

Experimental animal and semen collection

Four seven-month-old boars were housed in individual pens, under the same environmental conditions, and fed a standard commercial diet to meet their nutrient requirements. Prior to semen collection, males were trained to mount a dummy sow and semen was collected using the hand gloved method. Ejaculates were collected once a week over the entire 6 weeks' training period. On the 7th week, ejaculates were collected using the gloved-hand technique (*Malo, 2010*) into a thermos pre-warmed to 37 °C; lined with a disposable plastic bag (IMV International Corp., Maple Grove, MN); and covered with a disposable milk filter (IMV International, Eden Prairie, MN) . All ejaculates were pooled together and the volume of semen was estimated by weighing the ejaculate.

Extended semen analysis

Each ejaculate was diluted (1: 4) semen to extender respectively at room temperature ($22^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$). Evaluation of semen was carried out at time intervals (0, 12, 24, 36 and 48) hrs. The 0 hr evaluation was carried out at 10mins post semen addition to extenders. Dead/live (D/L) ratio was evaluated by using the eosin-nigrosin stain described by (*Lukaszewicz et al.*, 2011). The pH was determined using the pH meter (Model pH 25) by dipping the pH electrode into the semen and the readings shown on digital scale were taken after 5mins. The concentration of sperm cells present in extended samples was determined by using the improved Neuber haemocytometer as described by (*Bonato et al.*, 2014).

Table 1. Proximate composition (%) of *T. Occidentalis* leaves extract (TOLE) (w/v)

Parameters	Percentage (%)
Moisture	78
Crude protein	20
Crude fibre	13
Crude fat	8
Ash	10
Nitrogen free extracts	65

Statistical analysis

A two way analysis of variance (ANOVA) was performed using the fixed effect model. Bonferroni was used to test for the significance ($P < 0.05$) of variance for all recorded and calculated data between different treatments, main effect of factors (semen extenders and storage period were considered) using model:

$$Y_{ijk} = \mu + T_i + S_j + e_{ijk}$$

Where Y_{ij} = Individual observation

μ = General mean

T_i = Fixed effect of semen extender ($i = 1 \dots 4$)

S_j = Fixed effect of storage period ($j = 0 \dots 48$)

e_{ijk} = Expected error

Results and Discussion

The results of the proximate composition, as presented in Table 1, showed that aqueous extract of *T. Occidentalis* leaf meal has a potential protein and carbohydrate source comparable with other conventional plant protein and energy sources (*Salman et al.*, 2008). The relatively rich nutrient profile of the leaf extract

is complemented with early age of the leaves as at the time of harvesting (*Fasuyi and Nonyerem 2007; Salman et al., 2008*).

Table 2. Composition of BTS extender used in this study

Composition	Volume (g/l)
Glucose	37.75
Sodium citrate	6.0
EDTA (ml/l)	1.25
Sodium bicarbonate	1.25
Potassium chloride	0.75
Penicillin	1.50

Where: EDTA- Ethylene diethyl tetra acetate

In Table 3, the effect of extenders on pH, dead/live (D/L) %, and sperm cell concentration ($10^3/\text{cm}^3$) were presented. The pH and D/L (%) parameters were significantly ($P < 0.05$) affected by semen extenders.

Table 3. Main Effect of semen extenders on extended boar semen

Treatments	Parameters		
	pH	D/L ratio (%)	Sperm Conc. ($10^3/\text{cm}^3$)
BTS	7.78 ^a	10.15 ^d	250.48
25% TOLE	6.95 ^b	39.03 ^{ab}	252.10
50% TOLE	6.83 ^c	24.24 ^c	253.7
75% TOLE	6.30 ^d	43.89 ^a	250.20
SEM	0.02	0.80	1.52

^{abcd} Means along the same column with different superscripts are significantly ($P < 0.05$) different using Bonferroni as post hoc analysis

The pH of extended boar semen in BTS extender had the highest value (7.78) which was significantly ($P < 0.05$) different to the values obtained for TOLE extenders. A unique trend observed among the TOLE semen extenders was that pH decreases as inclusion level increase. However, 75% TOLE had the lowest pH value (6.30) which was significantly ($P < 0.05$) different to 50% (6.83) and 25% (6.95) inclusion levels. The D/L ratio (%) was significantly ($P < 0.05$) affected by semen extenders as shown in Table 3. As reported for D/L ratio (%), TOLE (75%) had the highest value (43.89%) which was significantly ($P < 0.05$) different as

compared to other extenders. BTS had value (10.15%), which was significantly lower ($P < 0.05$) as compared to TOLE extenders. The sperm cell concentration (10^3) was not affected by the semen extenders during storage.

As shown in table 4, pH decreased significantly ($P < 0.05$) over storage time in all TOLE extender treatments, while BTS showed an earlier increase in pH before a decrease. The dead/live ratio (%) increased significantly ($P < 0.05$) over storage time for all semen extenders. The sperm cell concentration (10^3) was not affected by storage period as reported in table 4.

Table 4. Effect of storage period on extended semen quality

pH	Period (Hours)					
Extender	0	12	24	36	48	SEM
BTS	7.20 ^c	9.00 ^a	8.93 ^a	8.93 ^a	8.60 ^b	0.02
25% TOLE	6.85 ^d	7.90 ^c	8.72 ^a	8.65 ^a	8.35 ^{ab}	0.05
50% TOLE	6.63 ^d	7.71 ^c	8.47 ^a	8.32 ^a	8.11 ^{ab}	0.11
75% TOLE	6.25 ^c	6.62 ^{bc}	6.74 ^b	6.90 ^{ab}	7.05 ^a	0.12
Dead/Live (%)						
BTS	6.12 ^d	10.83 ^{cd}	12.83 ^c	13.32 ^b	17.67 ^a	0.18
25% TOLE	16.62 ^e	25.39 ^d	43.58 ^c	52.52 ^b	60.04 ^a	0.13
50% TOLE	10.50 ^{de}	15.59 ^d	24.64 ^c	31.78 ^b	38.70 ^a	0.12
75% TOLE	18.16 ^e	28.07 ^d	47.45 ^c	55.78 ^b	67.04 ^a	0.15
Sperm cell conc. ($10^3/\text{cm}^3$)						
BTS	252.01	252.05	251.10	249.11	248.15	1.18
25% TOLE	253.11	253.01	252.10	251.10	251.21	1.13
50% TOLE	255.21	255.13	253.15	253.01	252.03	1.12
75% TOLE	251.26	251.15	250.04	249.31	249.25	1.15

^{abcde} Means along the same column with different superscripts are significantly ($P < 0.05$) different using Bonferroni as post hoc analysis

The initial pH of TOLE was 6.20, BTS 7.52, while that of fresh boar ejaculate used in this study was 7.40. Therefore, the initial variation between the pH values of the extended semen in TOLE and BTS can be attributed to difference in initial pH of extenders. The high content of carbohydrate in the form of NFE may be responsible for the low pH in TOLE, while bicarbonate in BTS is responsible for the relatively alkaline pH. The earlier increase in pH of BTS extended semen can be attributed to instability of the extender. According to *Kaeoket et al. (2010)*, the pH does not become stable from the start of dilution in water and that different extenders show a different pattern of pH change over time. In this study, the presence of a bicarbonate buffering system in the BTS extender explained earlier pH-rise. This increases the CO_2 in the media to reduce metabolic

activities of sperm cell during storage (Vyt *et al.*, 2004, 2007). The results of pH in this study are consistent with study of Kaeoket *et al.* (2010) who reported that the pH values of the extended semen increase with increased storage time. As observed in this study, the presence of glucose in each extender caused a considerable reduction of intracellular pH during storage. This intracellular pH reduction obviously reduced sperm cell metabolism and increase sperm cells survive during storage (Johnson *et al.*, 2006). However, high content of glucose in form of NFE found in TOLE might have resulted into intracellular acidosis, which caused sperm cell distortion and resulted into dead sperm cells with increase concentration (Bonato *et al.*, 2014).

As shown in table 4, the values of D/L ratio (%) increased in all extenders over time. The quantity of living sperm cell is a function of dead/live ratio. The high values of dead/live ratio of about 50% recorded in TOLE extenders may be attributed to bacterial infection. In most cases, the testicular tissue and accessory glands of the boar are bacteria-free, and bacterial contamination of the ejaculate occurs during the semen collection process (Martin Rillo *et al.*, 1998). BTS used in this study contained an antibiotic (penicillin), which inhibits bacteria proliferation. The extender component (glucose) and the temperature at which semen doses are stored (15-16°C) promote the growth of most Gram negative bacteria such as *Escherichia coli*, *Salmonella spp* and *Pseudomonas spp* (Gadea 2003).

In extended semen, bacterial contamination leads to a series of alterations including reduced sperm motility, sperm agglutination, an increased proportion of altered acrosomes, dead sperm cells and pH lowering to acidic levels 5.7 - 6.4 (Althouse *et al.*, 2000). However, the reduction in dead/live ratio for semen extender (TOLE 50%) as compared to other concentration can be attributed to presence of phytochemicals in the leaf extract in required proportion. TOLE was reported to show inhibitory effect to some microbes which include *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus spp* and *Salmonella typhi* (Kayode and Kayode, 2011). The high concentration of these phytochemicals in TOLE 75% may be toxic for the sperm cells and resulted to high dead/live ratio, while low concentration of phytochemicals at TOLE 25% can enhance bacteria proliferation and high dead/live ratio recorded as compared to TOLE 50%.

Sperm cell concentration was not significantly ($P < 0.05$) affected by extender treatments. Although the value decreased slightly over time as shown in table 4. An equal concentration of sperm cell was randomly extended for each treatment at the onset of storage. The slight decrease in values of sperm cell over time may be attributed to dissolution of dead sperm cells in extended media (Martin Rillo *et al.*, 1998)

Conclusion

The BTS extender, a commercial short – term diluent used in this study performed better as compared to TOLE extenders. TOLE 50% concentration had the optimum performance within the TOLE concentrations. The study shows that TOLE has potential to serve as short – term semen extender, with best result in 50% dilution. However, further studies are required to effectively validate TOLE application as short term extender with increase in storage time and evaluation of other semen quality parameters.

Potencijal ekstrakta listova *Telfairia occidentalis* kao ekstendera semena nerastova

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Rezime

Čuvanje semena za veštačku inseminaciju (AI) zahteva korišćenje ekstendera, koji je služio kao puferski medijum za zaštitu i očuvanje ćelija sperme u dobrom stanju za oplodnju. U ovoj studiji, poreden je potencijal ekstrakta lista *Telfairia occidentalis* (TOLE) kao nekonvencionalnog ekstendera semena nerastova sa rastvorom za otapanje Beltsville (BTS), konvencionalnim ekstenderom semena tokom perioda od 48 časova skladištenja. Vodeni rastvor TOLE-a je pripremljen u tri (3) nivoa koncentracije (25, 50 i 75%). Sveži ejakulati nerastova su tretirani u (1: 4) TOLE rastvorima i BTS ekstenderu u potpuno slučajnom dizajnu; tretmani su ponovljeni četiri (4) puta. Tretirano seme je skladišteno na temperaturi od 17⁰C i ocenjivano je njegov kvalitet odn. pH, odnos mrtvih/živih (%) i koncentracija spermatozoida u vremenskim intervalima (0, 12, 24, 36 i 48h). Tretirano seme je bilo značajno ($P < 0,05$) pod uticajem ekstendera semena i perioda skladištenja za pH, a procenat/udeo mrtvih/živih (%), ali i koncentracija spermatozoidnih ćelija (10³) nisu bile pod uticajem faktora ($P > 0,05$). Vrednosti pH za BTS su značajno ($P < 0,05$) veće u poređenju sa TOLE ekstenderima. TOLE ekstender u koncentraciji od 75% je imao najveću vrednost (43,89%), a BTS je imao najmanju vrednost (12,15%) za odnos mrtvih/živi što je statistički signifikantno ($P < 0,05$). Vrednosti pH i odnos mrtvih/živih (%) povećane su tokom vremena čuvanja u svim ekstenderima. Optimalne performanse TOLE-a u poređenju sa BTS ekstenderom zabeležene su u koncentraciji TOLE-a od 50%. TOLE je pokazao potencijal kao ekstender semena nerastova, ali su potrebne dalje studije za validaciju i poboljšanje njegove primene.

Ključne reči: ekstender semena, nerast, pH, *Telfairia occidentalis*, rastvor za otapanje Beltsville

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