APRW -46

Antibacterial Potential of Aqueous Extract of Neem (*Azadirachta indica* A. Juss) Leaf on Spermatozoa Quality in Extended Porcine Semen

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Abstract
This study investigated the antibacterial potential of aqueous extract of neem leaf on spermatozoa quality in extended porcine semen . Fresh semen was collected from a mature and intact boar using the glove-hand technique.The collected semen was diluted and allotted to six treatments with three replicates per treatment and evaluated at 0, 24 and 48 hours of refrigeration at 17 ^{0}C. Semen quality parameters evaluated were; progressive motility (%), morphology (%),The inclusion of aqueous neem leaf extracts (ANLE) in boar semen up to 48 hours of refrigeration gave mean values which fall within an acceptable range of normal values indicative of good semen quality for all semen quality parameters. At 48 hours, significant difference ($p<0.05$) in motility was observed across the treatments with Treatment 1 giving the highest mean value (84.67 ± 2.40) and Treatment 6 (100% ANLE) giving the least value (70.00 ± 0.00). At 48 hours, significant difference ($p<0.05$) in morphology was also observed across the treatments with Treatment 1 having the highest mean value. Treatment 2 and Treatment 3 (25 % ANLE), though with a significant difference between the means gave the closest mean values (81.33 ± 0.67 and 79.33 ± 0.33 respectively) to Treatment 1 (82.00 ± 1.00). These findings suggest that 25 % of ANLE can be used in boar semen extension up to 48 hours of storage (17 °C).

Keywords: Artificial insemination, antibacteria, aqueous neem leaf extract, motility, morphology

Introduction

Microorganisms, especially bacteria constitute a major setback in the productivity of porcine in Nigeria and other tropical countries. Althouse *et al.*, (2008) reported that bacterial contamination of extended boar semen has been associated with deleterious effects on semen quality such as reduced motility, abnormal spermatozoa structure, agglutination of sperm cell, shortened viability and premature acrosome reaction. This shortens shelf life of semen doses and reduces fertility, conception rates, and litter size at birth.

The increase in prevalence of resistant strains of bacteria to synthetic antibiotics necessitates the use of natural antimicrobial alternatives such as aqueous extract of neem (*Azadirachta indica* A. Juss) leaf. Although, there is an abundance of scientific data to show the antibacterial potential of (*Azadirachta indica* A. Juss) leaf, there is notable dearth of literature on the antibacterial potential of aqueous extracts of (*Azadirachta indica* A. Juss) leaf on spermatozoa quality in extended boar semen.

As a result, the antibacterial potential of aqueous extracts of *Azadirachta indica* leaves on spermatozoa quality in extended boar semen, appropriate inclusion level as well as durations that maintain the quality and viability of extended boar semen was investigated.

Materials and Methods

Semen collection was done at the Piggery Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, South Western part of Nigeria (7°20^{1}N, 3°50^{1}E; 200m above sea level). Preparation of neem extracts and semen analyses were carried out at the Animal Physiology Laboratories of the same institution. The extracts from fresh neem leaves were prepared immediately after sample collection following; 1 kg of fresh leaves was collected, washed with distilled water and then chopped into small pieces. These were soaked into 1000 ml of distilled water overnight, and were then filtered with a cheese cloth. The filtrate was then centrifuged to remove remaining fibre in the extract, thus enhancing the visibility of spermatozoa during the microscopic evaluation and then stored at 5 °C.

Semen evaluation was carried out using the following parameters; progressive motility, and morphology, at 0, 24 and 48 hours of preservation (17 °C). This was assessed by putting a drop of semen on a clean glass slide, covered with a coverslip and examined with microscope under at X400. The progressive motility of the spermatozoa were subjectively estimated and rated between 0 and 100 (Khan *et al.,* 2015). This was determined

following the same method for liveability. Spermatozoa with coiled or double tail, damaged mid-piece and damaged head were considered abnormal (Althouse *et al.,* 2008). A completely randomized design was utilized for the study, such that diluted semen was allotted to six treatments with three replicates per treatment and evaluated at 0, 24 and 48 hours:

Treatment 1 (Positive control): Semen + Beltsville Thawing Solution (BTS) Extender
Treatment 2 (Negative control): Semen + BTS without antibiotics (BTS-A)
Treatment 3: Semen + BTS-A + 25 % aqueous neem leaf extract (ANLE)
Treatment 4: Semen + BTS-A + 50 % ANLE
Treatment 5: Semen + BTS-A + 75 % ANLE
Treatment 6: Semen + BTS-A + 100 % ANLE

The composition of BTS used for dilution of collected semen is presented in Table 1

Table 1: Composition of Beltsville thawing solution (100 mL)

Composition	Quantity(g)
Glucose	37.10
EDTA	1.25
Sodium bicarbonate	1.25
Potassium chloride	0.75
Penicillin	10.10
Streptomycin	10.10

EDTA = Ethylediaminetetraacetic acid; **Source**: Pursel *et al.* (1975)

Data collected were subjected to one-way analysis of variance of the Statistical Analysis System (SAS, 2003) programme. The treatment means where significant ($p<0.05$) were separated using the Duncan's Multiple Range Test of the same software.

Results and Discussion

The result of the effect of ANLE on progressive motility of extended boar semen at 0, 24 and 48 hours of refrigeration at 17 ˚C is shown in Table 1. At 0 hour, there was no significant difference in motility across the treatments. At 24 hours, significant difference ($p<0.05$) in motility was observed across the treatments with Treatment 1 (positive control) giving the highest mean value (97.00 ± 0.33) and Treatment 6 (100% ANLE) giving the least value (70.00 ± 0.00). At 48 hours, significant difference ($p<0.05$) in motility was observed across the treatments with Treatment 1 (positive control) giving the highest mean value (84.67 ± 2.40) and Treatment 6 (100% ANLE) giving the least value (70.00 ± 0.00). However, all the treatments gave mean values within an acceptable range.

The result of the effect of ANLE on the morphology of extended boar semen at 0, 24 and 48 hours of refrigeration at 17 ˚C is shown in Table 2. At 0 hour, there was no significant difference in morphology across the treatment. At 24 hours, there was a significant difference ($p<0.05$) in morphology across the treatments with Treatment 1 (positive control) and Treatment 2 (negative control) giving the same mean value (90.00 ± 0.00 and 90.00±0.00 respectively). Treatment 3 (25 % ANLE), Treatment 4 (50 % ANLE), although with significant difference between the means gave the closest mean value (88.33 ± 0.67 and 86.00 ± 0.58 respectively) to Treatment 1 (90.00 ± 0.00) and Treatment 2 (90.00 ± 0.00). At 48 hours, significant difference ($p<0.05$) in morphology was observed across the treatments with Treatment 1 (positive control) having the highest mean value. Treatment 2 (negative control) and Treatment 3 (25 % ANLE), though with significant difference between the means gave the closest mean values (81.33 ± 0.67 and 79.33 ± 0.33 respectively) to Treatment 1 (82.00±1.00). However, all the treatments gave mean values within normal range

Table 1: Effect of ANLE on progressive motility of extended boar semen (Mean ± SD)

Time (Hours)	ANLE (%)					
	(BTS)	(BTS-A)	25	50	75	100
0	98.00±0.00	98.00±0.00	98.00±0.00	98.00±0.00	98.00±0.00	98.00±0.00
24	97.00^{f}±0.33	82.00^{e}±1.53	79.00^{d}±0.58	75.00^{c}±0.00	70.00^{b}±0.00	70.00^{b}±0.00
48	84.67^{c}±2.40	76.00^{b}±2.00	76.67^{b}±0.67	70.00^{b}±0.00	70.00^{a}±0.00	70.00^{a}±0.00

abcdef: mean values on the same row with different superscript are significantly different ($p<0.05$), SD = Standard Deviation

Table 2: Effect of ANLE on morphology of extended boar semen (Mean ± SD)

Time (hours)	ANLE (%)					
	BTS	BTS-A	25	50	75	100
0	98.00^{b}±0.00	98.00^{b}±0.00	98.00^{b}±0.00	98.00^{b}±0.00	97.00^{a}±0.00	96.67^{a}±0.33
24	90.00^{e}±0.00	90.00^{e}±0.00	88.33^{d}±0.67	86.00^{c}±0.58	84.33^{b}±0.67	80.00^{a}±0.00
48	82.00^{c}±1.00	81.33^{c}±0.67	79.33^{c}±0.33	76.33^{b}±1.67	68.67^{a}±0.67	68.00^{a}±0.00

abcde: mean values on the same row with different superscript are significantly different ($p<0.05$), SD = Standard Deviation

Considering spermatozoa progressive motility which is a vital feature for passage through the cervix, utero-tubal junction and even more essential, through the cumulus, the inclusion of ANLE in boar semen had no detrimental effect on sperm motility at 0, 24 and 48 hours of preservation and this is probably due to presence of active constituents in ANLE which could inhibit the growth of microorganism that could detrimentally affect the survival of sperm cells. This is in accordance with findings of Hashmat *et al.* (2012) and Hishmanshu *et al.* (2016) who reported that neem has antibacterial properties that could inhibit the growth of the microorganism. Spermatozoa motility between 50 and 70 % is considered as good motility (Johnson *et al.,* 2000). Motility above 60 % is enough for fertilization to take place provided that all other semen parameters are good. However, the mean values of spermatozoa motility obtained with the inclusion of ANLE in boar semen were within the acceptable normal range throughout the period of preservation. These high percentages of motile spermatozoa indicate that the spermatozoa have not been damaged by the process of dilution and storage and this is justified by the report of khan *et al.,* (2015). This implies that the extension of boar semen with ANLE is capable of enhancing the movement of spermatozoa through the reproductive tract of gilt or sow for effective fertilization.

Morphological abnormalities of spermatozoa can have detrimental effects on fertilization and embryonic development. The inclusion of ANLE in boar semen was also found not to be detrimental to spermatozoa morphology throughout the periods of preservation. The lower morphological abnormalities recorded at these hours of storage could also be as a result of the presence of active constituents in ANLE which are responsible for inhibiting growth of microorganism and this is corroborated by the findings of Hashmat *et al.* (2012) and Hishmanshu *et al.* (2016) who reported that neem has an antibacterial properties that could inhibit the growth of microorganism. This finding is also in compliance with the study of Johnson *et al.* (2000) who reported that high-quality semen contains a minimum number (5 to 15 %) of morphologically abnormal spermatozoa whereas low-quality semen frequently contains the larger number 20 % (or more).

Conclusion

The findings in this study suggest that 25% of aqueous neem leaf extracts (ANLE) can be used in boar semen extension up to 48 hours as indicated by observed mean values of all parameters, which fall within range of normal values indicative of good semen quality.

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