

1 **ANTIBACTERIAL POTENTIAL OF AQUEOUS EXTRACT OF NEEM (*Azadirachta indica***
2 **A. Juss) LEAF ON SPERMATOZOA QUALITY IN EXTENDED PORCINE**
3 **SEMEN**

Comment [s1]: Kindly correct Grammatical errors

7
8 **ABSTRACT**

9 The present study was investigated to assess the antibacterial potential of aqueous neem
10 leaves extracts (ANLE) aqueous extract of neem leaf on spermatozoa quality in extended porcine
11 semen. Fresh semen was collected from a mature and intact boar (age, breed, body condition
12 score, health status) using the glove-hand technique. The collected semen samples were as
13 diluted and allotted to six treatments with three replicates per treatment in a completely
14 randomized design and evaluated at 0, 24 and 48 hours of refrigeration at 17 °C. Semen quality
15 parameters such as evaluated are; progressive motility (%), livability (%), morphology (%), pH,
16 acrosome integrity (%), and bacteria load ($\times 10^4$ cfu/mL) were evaluated. At 48 hours, significant
17 difference ($p < 0.05$) in motility was observed across the treatments with Treatment 1 giving the
18 highest mean value (84.67 ± 2.40) and Treatment 6 (100% ANLE) giving the least value
19 (70.00 ± 0.00).
20 At 48 hours, there was significant difference ($p < 0.05$) in liveability across the treatments.
21 Treatment 2 and Treatment 3 (25% ANLE) though with significant difference between the means
22 has given gave the closest mean value (79.67 ± 0.33 and 76.67 ± 0.67 respectively) to Treatment 1
23 (80.00 ± 0.00). At 48 hours, significant difference ($p < 0.05$) in morphology was also observed
24 across the treatments. Treatment 2 and Treatment 3 (25 % ANLE), though with significant
25 difference between the means has given gave the closest mean values (81.33 ± 0.67 and
26 79.33 ± 0.33 respectively) to Treatment 1 (82.00 ± 1.00). At 48 hours, significant difference ($p <$
27 0.05) in acrosome integrity as well as pH was also observed across the treatments. The general
28 trend for bacteria load is a decline as the level of ANLE increases across the treatments at 48
29 hours of refrigeration. These findings suggest that 25 % of aqueous neem leaves extracts
30 (ANLE) can be used in boar semen extension up to 48 hours of storage at (17 °C).

31 Key words: Artificial insemination; aqueous neem leaf extract; bacterial load ; semen
32 quality;

33
34 **1. INTRODUCTION**

35 Microorganisms, especially bacteria constitute a major setback in the productivity of porcine in Nigeria
36 and other tropical countries. Bacterial contamination of extended boar semen has been associated
37 with deleterious effects on semen quality parameters (1) such as reduced motility, increased
38 abnormal spermatozoa structure, agglutination of sperm cell, shortened viability (2) and premature
39 acrosome reaction (2). This bacterial contamination shortens the shelf life of semen doses (3) and
40 reduces fertility, conception rates, and litter size at birth (1).

41 The insemination of contaminated semen may be associated with vulva discharge and return to
42 oestrus (3), embryonic or foetal death, endometritis, systemic infection and/or disease in recipient
43 females or reduced litter size (17), or reduced litter size. The addition of antibiotics to the semen
44 extenders has reduced the bacterial contamination (9). There is a considerable universal interest in
45 reducing the utilization of synthetic antibiotics to curtail the development of antibiotic –resistant strain
46 of bacteria. Extensive use of synthetic antibiotics has resulted in drug resistance for many bacterial
47 species of pigs and other domestic animals such as cattle, sheep and goats.

48 The increase in prevalence of resistant strains of bacteria to synthetic antibiotics necessitates the use
49 of natural antimicrobial alternatives such as aqueous extract of neem (*Azadirachta indica* A. Juss)
50 leaf. Although, there is abundance of scientific data to show the antibacterial potential of neem
51 (*Azadirachta indica* A. Juss) leaf, there is notable dearth of information in terms of previous studies
52 describing the antibacterial potential of aqueous extracts of neem (*Azadirachta indica* A. Juss) leaf
53 on spermatozoa quality in extended boar semen. As a result, the antibacterial potential of ANLE
54 aqueous extracts of *Azadirachta indica* leaves on spermatozoa quality in extended boar semen,

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appropriate inclusion level as well as durations that maintain the quality and viability of extended boar semen was investigated.

2. MATERIALS AND METHODS

2.1 Location of Study

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'N, 3°50'E; 200_m above mean sea level). Preparation of neem extracts and semen analyses were carried out at the Animal Physiology Laboratories of the same institution (reference).

2.2 Preparation of aqueous extracts from fresh neem leaves

The extracts from fresh neem leaves were prepared immediately after sample collection with the following procedure; 1 kg of fresh leaves were collected, washed with distilled water and then chopped into small pieces. These were soaked into 1000 mL of distilled water in overnight, and were then filtered with a cheese cloth. The filtrate was then centrifuged to remove remaining fibre in the extract, thus enhancing visibility of spermatozoa during microscopic evaluation and then stored at 5 °C (reference).

2.3 Preparation of the boar, semen collection and extension

Prior to collection of semen, the boar was thoroughly washed and the pre-putial pouch was cleaned with water by a milking action, to remove urine and other materials that could contaminate semen during collection. Semen was collected using the gloved hand method into a US bag inserted in a collection cup such that the pre and post sperm fractions were separated from the sperm rich fraction (reference).

2.4 Semen evaluation

Semen evaluation was carried out using the following parameters; pH, progressive motility, liveability, morphology, acrosome integrity and microbial load at 0, 24 and 48 hours of preservation (17 °C).

pH

A pH meter (company name and country) was used to measure the hydrogen ion concentrations produced by spermatozoa metabolic activities during the storage period.

Progressive motility

This was assessed by putting a drop of semen on a clean glass slide, covered with a cover slip and examined with microscope under at X400X (microscope model, company name and country). The progressive motility of the spermatozoa were subjectively estimated and rated between 0 and 100 (14). 0 means and 100 means ?? (explain). (reference).

Liveability

This was determined by mixing a drop of semen with drop of a staining solution (eosin-nigrosin) on a clean glass slide gently and a smear developed using the edge of another clean slide, air-dried and examined with a microscope at X400X (1). Detailed procedure and (reference).

Morphology

This was determined following the same method for liveability. Spermatozoa with coiled or double tail, damaged mid-piece and damaged head were considered abnormal (1) (reference).

Acrosome integrity

This was determined following the same method for liveability.

Bacterial load

The pour plate technique was used to determine the microbial load in each sample. From the first dilution, 1mL of the sample was pipetted into other sterile diluents containing 9_mL to obtain 10⁻² dilution. The samples were serially diluted up to 10⁻⁴. 0.1ml of Aappropriate dilution (0.1 mL) was then inoculated into sterile petri dishes and molten plate count agar (PCA) was added and left to solidify. The plate count agar (PCA) was prepared by dissolving 22.5_g into 1000 mL of distilled water and heated in a boiling water bath. The solution was autoclave for 15 minutes at 121 °C. The samples were in three replicates and incubated at 37 °C for 24 hours. The mean counts for triplicate cultures were recorded as the bacterial counts in the sample. The results were expressed as CFU/mL according to America Public Health Association (reference).

2.5 Experimental treatments and design

Comment [s2]: Kindly include what active component in the neem leaf acts as antibacterial substance relation with semen quality profiles and fertility

Comment [s3]: How many animals selected at what basis, how many ejaculates collected, selected and used. Have you estimated the variability between the ejaculates or boars on semen quality parameters? And how to minimized the variability?

Comment [s4]: The period of the experiment?

Comment [s5]: Provide THI value of the experimental station

Comment [s6]: Provide the detailed procedure on extension of semen with ANLE

Comment [s7]: Please show some photos of semen quality parameters

Comment [s8]: Kindly include references. How can utilize the E-N staining for acrosomal integrity, kindly explain???

Comment [s9]: Provide detailed procedure on how did you prepare the ANLE and inclusion in the extender

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

2.6 Statistical analysis

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.

3. RESULTS AND DISCUSSION

3.1 Effect of ANLE on Progressive Motility of Extended Boar Semen

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 3. 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 acceptable normal range.

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 (what?) in ANLE which could be inhibited the growth of microorganism that could detrimentally affect the survival of sperm cells. This is in accordance with findings of (7) and (8) who reported that neem has antibacterial properties that could inhibit the growth of microorganism. Spermatozoa motility between 50 and 70 % is considered as good motility (9). Motility above 60 % is enough for fertilization to take place provided that all other semen parameters are good (12). However, the mean values of spermatozoa motility obtained with the inclusion of ANLE in boar semen were within the acceptable normal range (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 (9). 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.

Table 3: Effect of ANLE on progressive motility of extended boar semen (Mean $\pm$ SD)

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

abcdef: mMean values on the same row with different superscript (a, b, c, d, e and f) are significantly different ($p < 0.05$), SD = Standard Deviation

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Comment [s10]: Kindly include the osmolarity and pH values for all the treatment and control groups

Comment [s11]: Please write how the ANLE minimized the bacterial load and what compound in the ANLE

Comment [s12]: You need to estimate the correlation coefficient between the bacterial load and semen quality parameters for getting better conclusion

Comment [s13]: In statistical analysis, you need to estimate standard error for mean (SEM) for all the table.
You need to recheck the statistical analysis thoroughly

Comment [s14]: What is the relation between the bacterial load and ANLE and semen quality parameters, explain in details

Comment [s15]: Calculate standard error (SEM)

3.2 Effect of ANLE on Liveability of Extended Boar Semen

The result of the effect of ANLE on liveability of extended boar semen at 0, 24 and 48 hours of refrigeration at 17° C is shown in Table 4. At 0 hour, significant difference ($p < 0.05$) in liveability was observed across the treatments with Treatment 1 (positive control) giving the highest mean value (98.00±0.00). Treatment 2 (negative control) and Treatment 3 (25 % ANLE), though with significant difference between the means gave the closest mean value (97.67±0.33 and 97.00±0.00 respectively) to Treatment 1 (positive control) (98.00±0.00). At 24 hours, there was significant difference ($p < 0.05$) in liveability across the treatments with Treatment 1 (positive control) giving the highest mean value. Treatment 2 (negative control), Treatment 3 (25 % ANLE) and Treatment 4 (50 % ANLE), though with significant difference between the means gave the closest mean value (88.67±0.33, 88.33±0.67 and 84.00±0.58 respectively) to Treatment 1 (positive control) (89.33±0.33). At 48 hours, there was significant difference ($p < 0.05$) in liveability across the treatments with Treatment 1 (positive control) giving the highest mean value. Treatment 2 (negative control) and Treatment 3 (25% ANLE), though with significant difference between the means gave the closest mean value (79.67±0.33 and 76.67±0.67 respectively) to Treatment 1 (80.00±0.00). However, all the treatments gave mean values within acceptable normal range. Spermatozoa liveability is also of paramount importance for effective fertilization. The inclusion of ANLE in boar semen had no detrimental effect on spermatozoa liveability at 0, 24 and 48 hours of preservation and this implies that the ANLE possessed active constituents that are capable of enhancing spermatozoa liveability. The mean values of spermatozoa liveability obtained with the inclusion of ANLE in boar semen were within the acceptable normal range throughout the period of preservation. These high percentages of liveability are in agreement with the findings of (9).

Table 4: Effect of ANLE on liveability of extended boar semen (Mean ± SD)

Time (Hours)	ANLE (%)					
	BTS	BTS-A	25	50	75	100
0	98.00 ^e ±0.00	97.67 ^{de} ±0.33	97.00 ^{cd} ±0.00	96.67 ^c ±0.33	94.67 ^b ±0.33	95.00 ^a ±0.00
24	89.33 ^e ±0.33	88.67 ^{de} ±0.33	88.33 ^{cd} ±0.67	84.00 ^c ±0.58	81.33 ^b ±1.33	76.00 ^a ±1.00
48	80.00 ^c ±0.00	79.67 ^c ±0.33	76.67 ^b ±0.67	70.00 ^b ±0.00	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

Comment [s16]: Calculate standard error (SEM)

Comment [s17]: As table 3

3.3 Effect of ANLE on Morphology of Extended Boar Semen

The result of the effect of ANLE on morphology of extended boar semen at 0, 24 and 48 hours of refrigeration at 17° C is shown in Table 5. At 0 hour, there was no significant difference in morphology across the treatment. At 24 hours, there was significant difference ($p < 0.05$) in sperm 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 acceptable normal range. 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 (7) and (8)) 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 (9) who reported that high quality semen contains minimum number (5 to 15 %) of morphologically abnormal spermatozoa whereas low quality semen frequently contains larger number 20 % (or more).

Table 5: 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

Comment [s18]: As similar to table 3

3.4 Effect of ANLE on Acrosome Integrity of Extended Boar Semen

The result of the effect of ANLE on acrosome integrity of extended boar semen at 0, 24 and 48 hours of refrigeration at 17 °C is shown in Table 6. At 0 hour, there was no significant difference in acrosome integrity across the treatments. At 24 hours, significant difference (p < 0.05) in acrosome integrity was observed across the treatments with Treatment 1 (positive control) and Treatment 2 (negative control) giving the same mean values. Treatment 3 (25 % ANLE) and Treatment 4 (50 % ANLE), though with significant difference between the means gave the closest mean values (92.67±0.67 and 90.00±1.00 respectively) to Treatment 1 (94.67±0.33) and Treatment 2 (94.33±0.33). At 48 hours, significant difference (p < 0.05) in acrosome integrity was observed across the treatments with Treatment 1 having the highest mean value (89.33±0.67) and Treatment 6 (100 % ANLE) giving the least value (70.00±0.00). However, all the treatments gave mean values within acceptable normal range (range with reference).

The acrosome is a secretory vesicle that is a sac-like structure below the plasma membrane and covering the anterior nucleus of the sperm head. The integrity of the acrosome is very closely associated with sperm viability because damage to the plasma membrane can trigger a disintegration of the acrosome (12). All the inclusion levels of ANLE in boar semen had a potential of maintaining acrosome integrity by protecting acrosome from undergoing capacitation throughout the period of preservation. The mean values of all the treatments fall within the acceptable normal range and this is justified by the findings of (12) who reported that semen samples with less than 70 % sperm with intact **acrosomes** should be discarded before processing. All treatments maintained acrosome integrity by the inhibition of acrosome reactions. Acrosome reaction is related to spermatozoa fertility and is essential in the process of fertilization. This implies that the active constituents in ANLE are capable of protecting acrosome from undergoing capacitation during refrigeration at 17 °C.

250 **Table 6: Effect of ANLE on acrosome integrity of extended boar semen (Mean $\pm$ SD)**

TIME (HOURS)	ANLE (%)					
	BTS	BTS-A	25	50	75	100
0	98.00 $\pm$ 0.00	98.00 $\pm$ 0.00	98.00 $\pm$ 0.00	98.00 $\pm$ 0.00	98.00 $\pm$ 0.00	98.00 $\pm$ 0.00
24	94.67 ^D $\pm$ 0.33	94.33 ^D $\pm$ 0.33	92.67 ^D $\pm$ 0.67	90.00 ^C $\pm$ 1.00	85.67 ^B $\pm$ 0.33	81.33 ^A $\pm$ 0.88
48	89.33 ^E $\pm$ 0.67	83.67 ^D $\pm$ 0.67	80.33 ^{CD} $\pm$ 0.33	79.67 ^C $\pm$ 0.33	75.33 ^B $\pm$ 2.67	70.00 ^A $\pm$ 0.00

251 abcde: mean values on the same row with different superscript are significantly different (p<0.05), SD
 252 = Standard Deviation
 253

254 3.5 Effect of ANLE on pH of Extended Boar Semen

255 The result of the effect of ANLE on pH of extended boar semen at 0, 24 and 48 hours of refrigeration
 256 at 17°C is shown on Table 7. At 0 and 24 hour, there was no significant difference in pH across the
 257 treatments. At 48 hours, there was significant difference (p < 0.05) in pH across the treatments.
 258 However, all the treatments gave mean values within acceptable normal range.

259 All the inclusion levels of ANLE in boar semen had a potential of maintaining the pH throughout the
 260 period of preservation. It is necessary for pH to be maintained because when the pH of the semen is
 261 declined; the internal pH of the spermatozoa is also reduced leading to a decrease in sperm
 262 metabolism and motility (6). However, the inclusion levels of ANLE in boar semen yielded mean
 263 values of pH that is within the acceptable normal range and this is justified by the findings of (4) who
 264 reported that a pH that is higher than 8 is an indicator of poor quality semen.
 265

266 **Table 7: Effect of ANLE on pH of extended boar semen (Mean $\pm$ SD)**

Time (Hours)	ANLE (%)					
	BTS	BTS-A	25	50	75	100
0	7.00 $\pm$ 0.58	7.00 $\pm$ 0.58	7.00 $\pm$ 0.58	6.97 $\pm$ 0.33	6.87 $\pm$ 0.67	7.03 $\pm$ 0.33
24	7.03 $\pm$ 0.03	7.07 $\pm$ 0.03	7.00 $\pm$ 0.58	6.97 $\pm$ 0.33	6.97 $\pm$ 0.03	7.03 $\pm$ 0.03
48	7.03 $\pm$ 0.03	7.07 $\pm$ 0.03	6.97 $\pm$ 0.03	7.03 $\pm$ 0.33	7.03 $\pm$ 0.03	7.03 $\pm$ 0.03

267

268

269 3.6 Effect of ANLE on Bacteria Load of Extended Boar Semen

270 The result of the effect of ANLE on bacteria load ($\times 10^4$ Cfu/mL) of extended boar semen quality at 0,
 271 24 and 48 hours of refrigeration at 17°C is shown on Table 8. At 0 hour, there was no significant
 272 difference in bacterial load across the treatments. There was no development of bacteria colony
 273 across the treatments. At 24 hours, there was significant difference (p < 0.05) in bacteria load across
 274 the treatments. There was no development of bacteria colony in Treatment 1 (positive control),
 275 Treatment 2 (negative control) and Treatment 3 (25 % ANLE) but the mean values of bacteria load
 276 slightly declined in Treatment 4 (50 % ANLE), Treatment 5 (75 % ANLE) and Treatment 6 (100 %
 277 ANLE) as the level of ANLE increases across the treatments. At 48 hours, significant differences (p <
 278 0.05) were also observed in bacterial load across the treatments with Treatment 1 (positive control),
 279 having the lowest mean value (5.26 $\pm$ 0.14). Treatment 6 (100 % ANLE), though with significant
 280 difference between the means gave the closest mean value (5.65 $\pm$ 0.05) to Treatment 1 (positive

control). The general trend is a decline in bacteria load as the level of ANLE increases across the treatments. Contamination of semen with bacteria is very common in collected boar ejaculates (4). Ejaculates collected from healthy boars are usually contaminated with bacteria containing up to 10^9 microorganisms per mL (1). The presence of bacteria in extended semen creates competition for nutrients and also results in the production of metabolic by-products that may harm the spermatozoa (1). All the inclusion levels of ANLE in boar semen inhibited the development of bacteria colony across the treatments at 0 hour of preservation. At 24 hours, 25 % inclusion level of ANLE in boar semen inhibited the development of bacteria colony but the mean values of bacteria load slightly declined as the inclusion level of ANLE increases across the treatments. At 48 hours of storage, all the inclusion levels of ANLE in boar semen had the potential of reducing the bacteria load as the level of ANLE increases across the treatments. This decrease in bacteria load is probably due to presence of active constituents in ANLE which could inhibited the growth of microorganism that could detrimentally affect the survival of spermatozoa quality. This is justified by the findings of (7) and (8) who reported that neem has antibacterial properties that could inhibit the growth of microorganism.

Table 8: Effect of ANLE on bacteria load ($\times 10^4$ Cfu/mL) of extended boar semen (Mean $\pm$ SD)

Time (Hours)	ANLE (%)					
	BTS	BTS-A	25	50	75	100
0	ND	ND	ND	ND	ND	ND
24	ND	ND	ND	6.50 ^d $\pm$ 0.18	5.69 ^b $\pm$ 0.23	4.09 ^b $\pm$ 0.09
48	5.26 ^a $\pm$ 0.14	6.76 ^c $\pm$ 0.08	6.69 ^c $\pm$ 0.08	6.62 ^c $\pm$ 0.08	6.57 ^c $\pm$ 0.09	5.65 ^b $\pm$ 0.05

abcd: mean values on the same row with different superscript are significantly different ($p < 0.05$), SD = Standard Deviation, ND = No Development

4. CONCLUSION

The study has shown that the inclusion of aqueous neem leaf extracts (ANLE) in boar semen up to 48 hours of refrigeration gave mean values which fall within acceptable range of normal values indicative of good semen quality for all semen quality parameters. This study, therefore, recommended 25 % inclusion level of aqueous neem leaf extracts (ANLE) in boar semen extension up to 48 hours as indicated by observed mean values of all parameters, which fall within acceptable range of normal values indicative of good semen quality.

ETHICAL APPROVAL

The study was carried out with permission from the Nigeria Institute of Animal Science Welfare and Ethics Committee (Act No. 26 of 2007).

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