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Full length Paper

Antioxidative effects of Ethanolic Garlic (*Allium sativum*) extract on the quality of extended Boar Semen

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ABSTRACT

This study investigated the antioxidative effects of ethanolic *Allium sativum* extract (EASE) on extended boar semen stored at 17°C for 72 hours. Semen was collected using the gloved-hand technique and extended with Beltsville Thawing Solution (BTS) supplemented with 0 (control), 50, 75, 100, and 125 µg/mL of EASE. Parameters assessed at 0, 24, 48, and 72 hours included sperm liveability, normal morphology, pH, lipid peroxidation (as malondialdehyde levels), and plasma membrane integrity using standardized assays such as eosin-nigrosin staining and hypo-osmotic swelling (HOS) test. Results showed that EASE significantly improved liveability, preserved membrane integrity, and reduced lipid peroxidation in a dose- and time-dependent manner. The 100 µg/mL concentration showed the most consistent antioxidative effect over time. These findings suggest that *Allium sativum* extract could be an effective natural antioxidant additive for boar semen extenders. This research supports the application of plant-derived antioxidants in reproductive biotechnology, particularly in swine artificial insemination programs.

Keywords: *Allium sativum*, antioxidative, artificial insemination, biotechnology, boar semen, hypo-osmotic, lipid peroxidation, liveability.

INTRODUCTION

Artificial insemination (AI) is a cornerstone of reproductive biotechnology in swine production, offering a means to disseminate elite genetics efficiently and with reduced disease transmission risks (Lule et al., 2022). Boar semen processing and storage, however, present unique challenges due to spermatozoa susceptibility to oxidative stress (OS), especially during preservation. Reactive oxygen species (ROS), though physiologically necessary at low concentrations for processes such as capacitation and acrosome reaction, become deleterious when in excess, compromising sperm viability, motility, and fertilization potential (Pena et al., 2020).

A major contributor to sperm oxidative stress is lipid peroxidation, driven by the high content of polyunsaturated fatty acids in sperm membranes, particularly docosahexaenoic acid (DHA), rendering them vulnerable to ROS-induced damage (Martí et al., 2021). Traditional extenders like Beltsville Thawing Solution (BTS) offer limited protection, prompting the search for natural antioxidant supplements.

Garlic (*Allium sativum*) has been extensively documented for its potent antioxidative phytochemicals, including allicin, S-allyl cysteine, and diallyl sulfides (El-Saber Batiha et al., 2020). These compounds modulate oxidative processes by enhancing cellular enzymatic defenses and scavenging free radicals, potentially improving sperm longevity during storage (Balamurugan et al., 2021). Phytogenic additives in semen extenders are increasingly considered safer and more sustainable alternatives to synthetic antioxidants, which may have cytotoxic effects at higher concentrations (Mujahid et al., 2020).

This study aimed to evaluate the antioxidative capacity of ethanolic garlic extract incorporated into BTS extender on the quality and fertilizing potential of extended porcine semen.

Justification of Study

High total antioxidant capacity in seminal plasma is strongly correlated with enhanced sperm viability and fertility, largely due to reduced oxidative damage to sperm membranes (Mostafa et al., 2021). Concerns over the cytotoxic effects and cost of synthetic antioxidants have prompted research into safer, plant-based alternatives. Garlic, with its bioactive sulfur compounds, represents a promising phytotherapeutic agent for combating oxidative stress in preserved semen. Evaluating its efficacy in boar semen preservation aligns with current biotechnological trends in sustainable animal reproduction.

MATERIALS AND METHODS

Experimental Site

Semen collection and preliminary evaluations were conducted at the Piggery Unit of the Teaching and Research Farm, University of Ibadan, Nigeria. Laboratory analyses were performed at the Animal Physiology and Bioclimatology Laboratories, Department of Animal Science, University of Ibadan (latitude 7°20′N, longitude 3°50′E, and elevation 200–300 m above sea level).

Experimental Design

A Completely Randomized Design (CRD) was employed. Fresh boar semen was diluted using Beltsville Thawing Solution (BTS) and supplemented with varying concentrations of ethanolic *Allium sativum* extract (EASE). Five treatment groups were prepared, each with three replicates:

T1: BTS only (Control)
T2: BTS + 50 µg/mL EASE
T3: BTS + 75 µg/mL EASE
T4: BTS + 100 µg/mL EASE
T5: BTS + 125 µg/mL EASE

Assessments were performed at 0, 24, 48, and 72 hours post-extension.

Animal and Semen Collection

A mature, healthy boar was maintained under standard housing and feeding conditions. Semen was collected using the gloved-hand technique as described by Paudel et al. (2020), employing a dummy sow. Semen was collected into a pre-warmed (37°C) thermos lined with a disposable filter. The semen volume was measured using a digital balance.

Preparation of Garlic Extract

Fresh garlic cloves (150 g) were peeled, sliced, and oven-dried at 45°C for 7 days. The dried material was ground and sieved (80 mesh). Ethanolic extraction was performed using a Soxhlet extractor with absolute ethanol. The solvent was evaporated using a rotary evaporator, followed by incubation at 45°C to remove residual ethanol. The dried extract was reconstituted with 135 mL of dimethyl sulfoxide (DMSO) to a working concentration using a measuring cylinder and stored at 4°C.

Semen Extension

BTS was prepared following Henning et al. (2021), with the following composition per liter:
Glucose – 37.0 g
Sodium citrate – 6.0 g
Potassium chloride – 0.75 g
EDTA – 1.25 g
Sodium bicarbonate – 1.25 g
Penicillin – 1.1 g
Streptomycin – 1.1 g
Semen was diluted at a 1:3 semen-to-extender ratio for all treatments.

Semen Evaluation Procedures

Evaluations were conducted at each time point using the following standardized protocols:

Sperm Liveability: Determined by eosin-nigrosin staining (Akhter et al., 2021). Live sperm remain unstained; dead sperm take up the dye.

Sperm Morphology: Assessed under ×400 magnification. Abnormal forms (e.g., coiled tails, detached heads) were recorded.

pH: Measured with a calibrated digital pH meter (Mettler Toledo).

Lipid Peroxidation: Quantified by thiobarbituric acid reactive substances (TBARS) assay following Kumar et al., 2021. Malondialdehyde (MDA) levels were measured as markers of lipid oxidation.

Plasma Membrane Integrity (PMI): Evaluated using the Hypo-Osmotic Swelling (HOS) test (Mehdipour et al., 2020). Sperm were incubated in hypo-osmotic solution and examined for tail swelling under × 400 magnifications.

Table 1. Baseline Characteristics of Fresh Boar Semen

Parameters	Value
Volume (ml)	242.8
pH	7.24
Progressive motility (%)	95
Mass activity	++++
Normal Spermatozoa (%)	95
Temperature[1] (°C)	32.5

++++ represents Very strong wave motion or excellent mass motility.

Table 2. Liveability (%) of Extended Boar Semen Across Storage Time

Time (Hours)	T1 (Control)	T2 (50µg)	T3 (75µg)	T4 (100µg)	T5 (125µg)
0	85.00a	82.50b	83.00b	82.80b	82.60b
24	75.00c	78.50b	80.00ab	82.00a	80.50ab
48	65.20c	69.00bc	70.10b	72.50a	71.30ab
72	55.10d	60.40c	63.10bc	66.50a	65.00ab

(Each bar represents mean ± SEM; bars with different letters differ significantly at $P < 0.05$).

Statistical Analysis

Data were analyzed using One-Way Analysis of Variance (ANOVA) in SAS software version 9.3 (SAS Institute, 2011). Means were separated using Duncan's Multiple Range Test at $P < 0.05$.

RESULTS

Pre-Extension Semen Quality

The initial quality of boar semen was assessed before extender treatments. All parameters were within the acceptable range for artificial insemination use (Table 1).

Effect of EASE on Sperm Liveability

Liveability decreased over time across all treatments. However, EASE-treated groups, particularly T4 (100 µg/mL), preserved sperm viability significantly better than the control (Table 2).

Effect on Sperm Morphology (Normal Spermatozoa)

EASE preserved sperm morphology, with T4 and T5 performing best at 48 and 72 hours (Table 3).

Effect on Semen pH

EASE treatment helped maintain pH stability. Control group (T1) showed a marked decline over time, while T4 and T5 maintained more optimal values (Table 4).

Lipid Peroxidation (MDA Levels)

Malondialdehyde (MDA) levels, an indicator of oxidative damage, were significantly lower in EASE-treated groups. T4 consistently recorded the lowest values, suggesting better oxidative protection (Table 5).

Plasma Membrane Integrity (PMI)

Hypo-osmotic swelling test results showed that T4 and T5 significantly maintained PMI across storage periods, indicating improved cell membrane protection (Table 6).

DISCUSSION

The findings from this study demonstrate that ethanolic *Allium sativum* extract (EASE) significantly enhanced the quality and stability of extended boar semen during 72-hour storage at 17°C. The antioxidant properties of garlic were evident through improved sperm liveability, morphology, pH stability, reduced lipid peroxidation, and preserved plasma membrane integrity.

Sperm Liveability and Morphology

Liveability and normal morphology declined in all groups with time, as expected due to sperm aging. However,

Table 3. Percentage of Morphologically Normal Spermatozoa.

Time (Hours)	T1	T2	T3	T4	T5
0	94.5a	94.0a	94.3a	94.2a	94.1a
24	88.0b	91.5a	90.0ab	89.8ab	89.5ab
48	80.2c	84.0b	86.5a	87.2a	86.0a
72	74.0d	78.0c	81.0b	83.0a	82.5a

Table 4. Effect of EASE on Semen pH Over Time.

Time (Hours)	T1	T2	T3	T4	T5	SEM
0	7.27ab	7.26ab	7.24b	7.23b	7.33a	0.01
24	7.16b	7.29ab	7.20b	7.38a	7.20b	0.01
48	7.12b	7.15b	7.15b	7.21a	7.13b	0.01
72	7.09b	7.13a	7.16a	7.14a	7.14a	0.01

Table 5. Lipid Peroxidation (MDA, nmol/mL) Over Storage Time.

Time (Hours)	T1	T2	T3	T4	T5
0	2.10a	2.00a	1.95a	1.92a	1.90a
24	3.85a	3.10b	2.80b	2.55c	2.60c
48	5.20a	4.00b	3.50c	3.00d	3.10d
72	6.45a	5.20b	4.60c	3.80d	4.00d

Table 6. Plasma Membrane Integrity (%) Over Storage Time.

Time (Hours)	T1	T2	T3	T4	T5
0	91.0a	91.2a	91.1a	91.3a	91.4a
24	83.5b	85.0b	87.0a	88.5a	87.8a
48	75.2c	77.5b	79.8a	81.0a	80.5a
72	66.0d	69.0c	71.5b	74.0a	73.2a

EASE-treated groups—especially T4 (100 µg/mL)—retained higher viability and morphological normalcy. This aligns with the report of Rashidi et al. (2021), who observed that garlic supplementation maintained sperm viability and morphology in ram semen by reducing oxidative degradation.

pH Stability

The slight alkalinization seen in T4 and T5 at 24–48 hours is favourable for sperm motility and survival. According to Althouse et al. (2020), maintenance of neutral to slightly alkaline pH in extenders helps mitigate acidification from sperm metabolism during storage. This indicates EASE may have buffering or metabolic stabilizing effects.

Lipid Peroxidation

EASE supplementation significantly reduced MDA levels, indicating protection against lipid peroxidation. This result corroborates the work of Ahmed et al. (2020), who reported that garlic extract reduced ROS-induced oxidative damage in cryopreserved goat sperm. Garlic's antioxidant compounds, such as allicin and S-allyl cysteine are known to scavenge free radicals and enhance the activity of endogenous antioxidant enzymes like glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) (El-Sayed and El-Masry, 2022).

Plasma Membrane Integrity

Sperm plasma membrane is rich in polyunsaturated fatty

acids and thus highly susceptible to oxidative damage. The improved plasma membrane integrity observed in EASE-treated samples is consistent with the hypothesis that garlic's organosulfur compounds protect lipid membranes by preventing ROS propagation, as also observed by Wang et al. (2020) in bovine semen studies using phytogenic antioxidants.

CONCLUSION

These findings support the potential of *Allium sativum* extract as a viable phytogenic additive to semen extenders. It is particularly advantageous as it provides a natural alternative to synthetic antioxidants, whose long-term safety and metabolic effects remain uncertain.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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