Research Article

The Potential of Lontar Fruit (*Borassus flabellifer*) Fiber Extract as a Natural Bioactive Compound to Enhance the Quality of Boar Semen

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Abstract | Sperm quality degradation during storage poses a substantial challenge to the effective implementation of artificial insemination technology in the swine industry. Innovative efforts grounded in natural ingredients are imperative to enhance the storage and quality of sperm. The objective of this study was to investigate the capacity of Lontar fruit (*Borassus flabellifer*) fiber extract (LFFE) as a natural bioactive compound to enhance the quality of pig sperm in Tris and Beltsville Thawing Solution (BTS) diluents. Fresh semen from boars that met national quality standards was diluted using Tris or BTS diluents, each with LFFE added at levels of 0%, 3%, 6%, 9%, and 12%. The parameters that were observed included sperm motility, viability, abnormalities, and plasma membrane integrity (PMI) during storage periods of up to 60 hours. Incorporation of 9% LFFE markedly improved sperm motility (48.0% in Tris; 47.6% in BTS), viability (60.1% and 56.2%), and plasma membrane integrity (60.4% and 56.1%), while reducing abnormalities throughout the 60-hour storage period. Among diluents, Tris with 9% LFFE maintained motility slightly longer, but both diluents benefited similarly from LFFE supplementation. These findings suggest that LFFE has the potential to serve as an effective natural additive, enhancing the storage life and quality of boar sperm. Consequently, LFFE has the potential to enhance the efficiency of artificial insemination programs.

Keywords | Beltsville thawing solution, Boar sperm, Lontar fruit fiber extract, Sperm quality

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INTRODUCTION

Sperm preservation represents a pivotal technology in contemporary livestock farming, particularly within the context of artificial insemination programs for swine (Waberski *et al.*, 2019). This technique has been demonstrated to facilitate broader genetic distribution, enhanced reproductive efficiency, and diminished costs associated with the maintenance of breeding stock. However, the efficacy of this technology is contingent upon the effectiveness of the sperm diluent employed during storage. In the domain of boar sperm preservation, the utilization of dilution agents such as Tris and Beltsville Thawing Solution (BTS) has emerged as the prevailing

standard of practice. This phenomenon is primarily attributed to the efficacy of these agents in preserving the viability and motility of sperm (Namula *et al*., 2019; Vinansius *et al*., 2019). Notwithstanding, the primary challenge in sperm storage is increased oxidative stress, which can result in membrane integrity impairment, reduced motility, and diminished sperm fertility (Dutta *et al*., 2019). To address this challenge, the addition of antioxidant supplements is necessary to help stabilize the physiological conditions of sperm during storage.

Although Tris and BTS diluents have been widely used in sperm preservation, their effectiveness is still limited due to the accumulation of reactive oxygen species (ROS), which contribute to a decline in sperm quality over time (Chianese and Pierantoni, 2021; Mislei *et al*., 2020). This oxidative stress can cause membrane lipid damage, protein denaturation (Jakubczyk *et al*., 2020), and DNA fragmentation, (Hamilton and Assumpção, 2020) which ultimately have a negative impact on sperm fertility. Various synthetic antioxidants have been used to address this issue, but their effectiveness remains highly variable. Therefore, this study proposes the use of a natural material, namely the extract of lontar fruit fiber (*Borassus flabellifer*), as an alternative antioxidant supplement in sperm diluents to improve pig sperm quality during storage.

Borassus flabellifer, commonly known as the palmyra palm, contains significant amounts of phenolic compounds and flavonoids, which exhibit strong antioxidant properties. These compounds are crucial in neutralizing reactive oxygen species (ROS) and reducing oxidative stress, which can damage sperm cells. These include gallic acid, coumarin, and quercetin, which are present in the male flower ethanolic extract of *B. flabellifer* (Tunit *et al*., 2022). The extract contains high levels of flavonoids, such as quercetin and epigallocatechin gallate (EGCG), which contribute to its antioxidant activity (Kurpios *et al*., 2021; Tunit *et al*., 2022). Studies have shown that *B. flabellifer* extracts can protect cells from oxidative stress by maintaining cellular redox balance and mitochondrial function (Tusskorn *et al*., 2021). This is particularly important for sperm cells, which are highly susceptible to oxidative damage.

Tris and BTS diluents have been demonstrated to be effective in maintaining sperm viability and motility. However, both are less effective in counteracting the accumulation of reactive oxygen species (ROS), which causes oxidative stress, plasma membrane damage, decreased motility, and sperm DNA fragmentation. A substantial body of research has previously investigated a variety of natural compounds.

The distinguishing features and added value of this study are attributable to its innovative approach to utilizing local natural materials to enhance reproductive technology (Hamed *et al*., 2019; Luo *et al*., 2020; Ahmed *et al*., 2024). The utilization of lontar fruit fibre extract as a supplement in Tris and BTS diluents provides a safer and more sustainable natural solution in comparison to synthetic antioxidants. Furthermore, this study makes a substantial contribution to the field of reproductive biotechnology and supports the utilization of abundant yet underutilized biological resources (Mahayasa *et al*., 2024). Consequently, the findings of this study are anticipated to offer substantial scientific contributions and implications for enhancing the efficiency of the livestock industry through the optimization of sperm preservation technology.

The objective of this study is to compare the effectiveness of Lontar fruit fiber extract with conventional diluents in maintaining sperm viability, motility, and membrane integrity. Furthermore, this study will ascertain the optimal concentration of Lontar fruit fiber extract that provides the most efficacious protective effect on sperm during storage.

MATERIALS AND METHODS

Experimental design and ethical approval

All procedures were approved by the Animal Ethics Commission at the Faculty of Animal Husbandry, Marine and Fisheries, Nusa Cendana University (075/1.KT/KEPPKP/IV/2024). Animal handling, semen collection, and laboratory analyses complied with institutional and national guidelines for the ethical use of animals in research. The experiment was conducted using a completely randomized factorial design (2 × 5). Factor A was the semen diluent (Tris–fructose–citric acid [Tris] vs. Beltsville Thawing Solution [BTS]), and Factor B was the concentration of Lontar fruit fiber extract (LFFE; 0, 3, 6, 9, and 12% w/v). Each treatment combination was replicated five times, resulting in a total of 50 ejaculates (experimental units) collected from three clinically healthy 3-year-old Landrace boars maintained under standard husbandry conditions. Only ejaculates meeting the inclusion criteria (>80% progressive motility and <20% morphological abnormalities, in line with the Indonesian National Standard for artificial insemination) were used in the experiment.

Preparation of lontar fruit fiber extract (LFFE)

Ripe *Borassus flabellifer* fruit fibers (dark yellow) were trimmed, homogenized, and macerated (using ethanol 70%) in distilled water (1:10, w/v) for 72 h at room temperature with intermittent agitation. The suspension was filtered through cloth or filter paper, and the filtrate was dried at 40 °C in an incubator to obtain LFFE powder. Working solutions at the desired concentrations were

freshly prepared on the day of use.

Semen dilution and preservation

Qualified ejaculates were diluted with the assigned extender at a 1:3 semen-to-diluent ratio according to treatment allocation. Diluted samples were stored at 15–20 °C, following short-term boar semen preservation protocols. Sperm quality assessments were carried out at 0 h and at 12-h intervals up to 96 h.

Semen quality evaluation

Sperm motility was assessed under a light microscope at 400× magnification from eight randomly selected microscopic fields. Viability was evaluated using the eosin–nigrosin staining technique, in which at least 200 sperm cells per sample were observed; viable sperm excluded the stain, while non-viable cells appeared pink (Alfian *et al*., 2025). Morphological assessment was carried out on stained smears by examining a minimum of 200 sperm cells per sample, with abnormalities recorded in the head, midpiece, tail, or in the presence of cytoplasmic droplets (Nirmala *et al*., 2025). Plasma membrane integrity was evaluated using the Hypo-Osmotic Swelling Test (HOST), where intact membranes were indicated by characteristic tail swelling in a hypotonic solution (Diansyah *et al*., 2025). In addition, sperm concentration was measured at baseline using a hemocytometer or an equivalent validated method to confirm sample quality prior to dilution.

Statistical analysis

Data were analyzed using linear mixed-effects models, with diluent, LFFE concentration, storage time, and their interactions as fixed effects. Ejaculate (nested within boar) was included as a random effect to account for repeated measures. Model assumptions were verified, and data were transformed when necessary. Tukey's test was used for post-hoc multiple comparisons. Results are expressed as mean ± standard deviation (SD), with significance set at $p < 0.05$. Statistical analyses were performed in SPSS v25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Sperm motility

As shown in Table 1, at 0 hours of storage, no significant differences ($P > 0.05$) in sperm motility were observed among treatments. However, from 12 hours onward, significant differences emerged ($P < 0.05$). The highest motility was consistently recorded in treatments supplemented with 9% LFFE (P4 and P9), with values of 48.00% and 47.60% at 60 hours, respectively. These were significantly higher than the control (P1, 20.00%) and other treatments. Lower or higher LFFE concentrations showed reduced motility, indicating 9% as the most effective level for maintaining sperm motility during storage.

Sperm viability

The incorporation of LFFE into Tris t and BTS diluent at varying concentrations (0%, 3%, 6%, 9%, and 12%) did not demonstrate a substantial impact ($P > 0.05$) on sperm motility at hour 0 of storage (following dilution). However, beginning at the 12th hour of storage, a substantial increase ($P<0.05$) in sperm motility was observed in the treatment with 9% LFFE addition, both in Tris and BTS diluent, in comparison to the other treatments (Table 2).

Table 1: Percentage of boar sperm motility during storage up to 60 hours in Tris and BTS extenders supplemented with different levels of LFFE.

Treatment	Storage times (hours)					
	0	12	24	36	48	60
P1	84.00±2.00^{a}	70.00±7.07^{bc}	60.00±5.00^{c}	49.00±6.52^{c}	33.00±4.47^{e}	20.00±3.54^{g}
P2	83.00±2.00^{a}	73.00±2.74^{abc}	61.00±2.24^{c}	52.00±2.74^{c}	38.00±2.74^{d}	26.00±4.18^{ef}
P3	83.00±2.00^{a}	73.00±2.74^{abc}	63.00±2.74^{bc}	51.00±2.24^{c}	40.00±3.54^{cd}	29.00±4.18d^{e}
P4	83.00±2.00^{a}	78.00±2.74^{a}	73.00±2.74^{a}	68.00±2.74^{a}	61.00±5.48^{a}	48.00±2.74^{a}
P5	83.00±2.00^{a}	75.00±0.00^{ab}	68.00±2.74^{ab}	60.00±0.00^{b}	50.00±0.00^{b}	40.00±0.00^{b}
P6	83.00±2.00^{a}	69.00±5.48^{c}	55.00±3.54^{d}	42.00±2.74^{d}	33.00±2.74^{e}	23.00±2.74^{fg}
P7	83.00±2.00^{a}	71.00±2.24^{bc}	62.00±2.74^{c}	52.00±2.74^{c}	40.80±1.10^{cd}	30.00±0.00^{cde}
P8	83.00±2.00^{a}	70.00±3.54^{bc}	62.00±4.47^{c}	52.00±4.47^{c}	43.00±2.74^{c}	33.00±2.74^{cd}
P9	83.00±2.00^{a}	78.00±2.74^{a}	73.00±2.74^{a}	63.00±2.74^{b}	58.00±2.74^{a}	47.60±2.51^{a}
P10	83.00±2.00^{a}	75.00±5.00^{ab}	65.00±7.07^{bc}	54.00±5.48^{c}	44.00±5.48^{c}	34.00±5.48^{c}
P-value	1.000	0.003	0.00	0.00	0.00	0.00

Note: Different superscript letters within the same column indicate significant differences among treatments ($p < 0.05$, Tukey's test). Tris treatments are represented by P1–P5, and BTS treatments by P6–P10, each corresponding to different levels of LFFE supplementation.

Table 2: Percentage of boar sperm viability during storage up to 60 hours in Tris and BTS extenders supplemented with different levels of LFFE.

Treatment	Storage time (hours)					
	0	12	24	36	48	60
P1	89.54±2.37^{a}	79.63±3.43^{bcde}	66.37±4.87^{c}	56.68±5.97^{de}	39.11±4.44^{e}	30.22±4.77^{f}
P2	89.44±2.55^{a}	77.99±2.64^{cde}	67.03±4.08^{c}	58.30±2.56^{d}	46.62±2.89^{cd}	35.42±4.00^{def}
P3	89.00±2.03^{a}	82.08±1.18^{abcd}	69.73±3.54^{bc}	59.09±1.90^{cd}	48.24±4.80^{cd}	39.01±5.44^{cde}
P4	90.40±1.67^{a}	86.46±1.76^{a}	80.30±1.28^{a}	77.90±1.20^{a}	68.73±6.85^{a}	60.06±3.50^{a}
P5	89.32±2.10^{a}	83.44±0.78^{abc}	77.21±3.04^{a}	66.66±7.24^{bc}	59.64±2.66^{b}	47.05±3.36^{b}
P6	89.60±2.50^{a}	75.93±7.19^{e}	63.67±3.94^{c}	50.31±4.98^{e}	42.74±4.73^{de}	34.58±4.45^{ef}
P7	89.37±2.63^{a}	78.53±3.99^{cde}	70.05±4.66^{bc}	59.19±3.58^{cd}	48.19±2.56^{cd}	38.19±3.98^{cde}
P8	88.85±2.20^{a}	76.82±5.12^{de}	68.90±8.94^{bc}	57.83±7.53^{d}	50.60±3.33^{c}	42.66±6.00^{bc}
P9	90.03±1.92^{a}	85.17±3.29^{ab}	80.65±4.10^{a}	71.08±4.43^{ab}	66.52±4.81^{a}	56.15±4.03^{a}
P10	89.46±1.85^{a}	82.31±5.22^{abcd}	74.71±7.18^{ab}	64.18±9.56^{bcd}	52.41±9.97^{c}	41.85±8.74^{bcd}
P-value	0.991	0.001	0.00	0.00	0.00	0.00

Note: Different superscript letters within the same column indicate significant differences among treatments ($p < 0.05$, Tukey's test). Tris treatments are represented by P1–P5, and BTS treatments by P6–P10, each corresponding to different levels of LFFE supplementation.

Table 3: Percentage of boar sperm abnormality during storage up to 60 hours in Tris and BTS extenders supplemented with different levels of LFFE.

Treatment	Storage time (hours)					
	0	12	24	36	48	60
P1	4.02±0.43^{a}	4.26±0.37^{ab}	4.31±0.29^{a}	4.54±0.35^{a}	4.81±0.31^{a}	5.09±0.28^{a}
P2	3.97±0.48^{a}	4.21±0.56^{ab}	4.49±0.59^{a}	4.93±0.65^{a}	5.06±0.60^{a}	5.29±0.61^{a}
P3	3.92±0.59^{a}	4.08±0.60^{ab}	4.36±0.59^{a}	4.57±0.57^{a}	4.82±0.50^{a}	5.01±0.53^{a}
P4	3.91±0.60^{a}	4.02±0.55^{a}	4.24±0.60^{a}	4.71±0.72^{a}	5.01±0.63^{a}	5.31±0.69^{a}
P5	3.93±0.54^{a}	4.15±0.63^{ab}	4.43±0.70^{a}	4.82±0.77^{a}	5.12±0.72^{a}	6.46±0.48^{b}
P6	3.93±0.57^{a}	5.11±0.58^{b}	6.11±0.80^{b}	7.72±0.49^{cd}	9.25±0.54^{d}	10.96±0.80^{d}
P7	4.01±0.64^{a}	4.88±0.86^{ab}	6.39±1.24^{b}	8.18±1.19^{d}	8.76±0.72^{cd}	10.89±0.49^{d}
P8	4.05±0.62^{a}	4.94±0.88^{ab}	5.78±0.80^{b}	7.03±0.94^{bc}	8.02±1.01^{bc}	9.73±0.98^{c}
P9	4.15±0.56^{a}	4.72±0.95^{ab}	5.52±0.83^{b}	6.51±1.36^{b}	7.40±0.59^{b}	7.50±1.51^{b}
P10	4.25±0.72^{a}	5.09±0.83^{b}	5.92±0.45^{b}	6.50±0.41^{b}	7.22±0.38^{b}	9.27±1.05^{c}
P-value	0.995	0.070	0.00	0.00	0.00	0.00

Note: Different superscript letters within the same row indicate significant differences among treatments ($p < 0.05$, Tukey's test). Tris treatments are represented by P1–P5, and BTS treatments by P6–P10, each corresponding to different levels of LFFE supplementation.

This increase in sperm motility persisted until the 24th, 36th, 48th, and 60th hours of storage. At the 60th hour, the highest sperm motility was recorded in the group with 9% LFFE addition, at 48.00% for Tris diluent and 47.60% for BTS diluent. This was significantly different ($P<0.05$) compared to the control group and other treatment groups with different LFFE concentrations.

Sperm abnormality

A similar pattern was also observed in sperm viability. Abnormality in Table 3 showed no statistically significant differences ($P > 0.05$) were observed between the treatments at 0 hours of storage. However, beginning at 12 hours of storage, the incorporation of 9% LFFE demonstrated a substantial enhancement in sperm viability ($P<0.05$) in comparison to alternative treatments, across both Tris and BTS dilutions.

At the 60th hour of storage, the highest sperm viability was achieved by the treatment group with 9% LFFE addition, at 60.06% in Tris diluent and 56.15% in BTS diluent. The values obtained in this study differed significantly ($P<0.05$) from the control group and the treatment groups with other LFFE concentrations.

Plasma membrane integrity

The evaluation of sperm plasma membrane integrity (PMI) yielded results that were consistent with those of other parameters. No statistically significant differences ($P > 0.05$) were observed at 0 hours of storage (Table 4).

Table 4: Percentage of boar sperm plasma membrane integrity during storage up to 60 hours in Tris and BTS extenders supplemented with different levels of LFFE.

Treatment	Storage time (Hours)					
	0	12	24	36	48	60
P1	87.85±2.38^{a}	79.16±3.57^{cd}	66.83±5.09^{de}	57.39±5.47^{de}	32.78±16.43^{e}	32.54±2.02^{e}
P2	88.05±2.86^{a}	78.34±2.49^{cd}	69.38±6.08^{cde}	58.63±2.58^{d}	47.05±3.44^{d}	35.84±4.20^{de}
P3	88.46±2.96^{a}	80.94±2.28^{bcd}	70.02±3.57^{cde}	61.71±4.47^{d}	50.72±5.02^{d}	39.99±5.06^{cd}
P4	88.43±2.53^{a}	85.88±1.46^{a}	80.22±1.06^{a}	78.21±1.75^{a}	70.46±5.40^{a}	60.39±3.09^{a}
P5	88.50±2.72^{a}	81.36±0.87^{abc}	77.54±2.44^{ab}	69.38±6.25^{bc}	61.14±1.53^{bc}	47.48±2.74^{b}
P6	86.96±2.03^{a}	76.28±5.42^{d}	64.95±2.69^{e}	50.83±3.87^{e}	45.19±3.26^{d}	36.36±4.88^{de}
P7	87.25±2.46^{a}	79.77±3.42^{bcd}	71.43±4.26^{bcde}	60.03±3.54^{d}	49.33±1.77^{d}	40.86±3.43^{cd}
P8	88.33±2.57^{a}	78.92±3.78^{cd}	72.81±8.14^{bcde}	59.85±6.14^{d}	52.34±2.79^{cd}	45.03±5.10^{bc}
P9	89.06±3.11^{a}	84.31±2.13^{ab}	80.93±2.98^{a}	71.58±3.83^{ab}	67.28±4.34^{ab}	56.07±3.71^{a}
P10	87.85±2.38^{a}	79.16±3.57^{cd}	66.83±5.09^{de}	57.39±5.47^{de}	32.78±16.43^{e}	32.54±2.02^{e}
P-value	0.977	0.002	0.00	0.00	0.00	0.00

Note: Different superscript letters within the same column indicate significant differences among treatments ($p < 0.05$, Tukey's test). Tris treatments are represented by P1–P5, and BTS treatments by P6–P10, each corresponding to different levels of LFFE supplementation.

However, beginning at 12 hours of storage, a substantial increase ($P<0.05$) in MPU was observed in the group with 9% LFFE addition, both in Tris and BTS diluents.

At the 60th hour of storage, the highest percentage of sperm with PMI was recorded in the 9% LFFE group, at 60.39% for Tris diluent and 56.07% for BTS diluent. The values obtained in this study differed significantly ($P < 0.05$) from the control group and the treatment groups with other LFFE concentrations.

DISCUSSION

Lontar fruit fibre extract is a natural source that is abundant in bioactive compounds. These bioactive compounds have the potential to improve sperm quality during storage. As indicated by the findings of preceding studies, the lontar fruit (*Borassus flabellifer*) contains a variety of significant compounds, including phenolic compounds, flavonoids, vitamin C (Lenggu *et al*., 2020), and dietary fibre with comparatively high antioxidant activity. These compounds neutralize ROS, reduce oxidative damage, and maintain cellular health, making *B. flabellifer* a promising natural agent for enhancing sperm quality and reproductive health (Dzigbor *et al*., 2025). Excessive ROS concentrations have been demonstrated to trigger a series of deleterious effects, including plasma membrane damage, lipid peroxidation, and mitochondrial dysfunction (Darmawan, 2007). These effects can culminate in reduced sperm motility and viability. Consequently, the inherent antioxidant properties of LFFE function as a biological shield, thereby preserving the structural and functional integrity of sperm cells. The present study demonstrates that the incorporation of 9% LFFE, both in Tris and BTS diluent, results in enhanced motility, viability, IPM, and a reduction in sperm abnormalities.

In addition to its antioxidant content, Lontar fruit is a source of complex carbohydrates, including polysaccharides and water-soluble fiber. These carbohydrates have the potential to serve as an additional energy source for sperm during storage (Aimanah, 2018). Sperm are known to require substantial energy to maintain optimal motility, membrane integrity, and fertilization capacity. This energy requirement is well-documented and well-established. This phenomenon is particularly evident under conditions of long-term storage, which can result in a decline in intracellular energy reserves. The complex carbohydrates present in LFFE, notably in the form of simple sugars resulting from the hydrolysis of dietary fiber, can function as an alternative metabolic substrate. This, in turn, supports mitochondrial activity and extends the progressive motility of sperm during storage (Hine *et al*., 2014).

The combined support of antioxidant compounds and carbohydrates in LFFE is a key factor explaining why the addition of LFFE at an optimal level of 9% significantly improves the quality of boar sperm after 60 hours of storage. Consequently, LFFE functions not only as a protective compound against oxidative stress but also as an essential natural energy source for the maintenance of sperm physiological function. These findings underscore the efficacy and applicability of LFFE as a natural additive in semen diluent formulations, thereby opening avenues for the innovative utilization of locally sourced products to enhance the efficiency of livestock reproductive technology.

The findings of this study suggest that both Tris and BTS

diluent possess the capacity to preserve the integrity of boar sperm during storage, particularly when employed in conjunction with the incorporation of LFFE at a concentration of 9%. However, studies have demonstrated that the utilization of Tris diluent generally results in sperm of superior quality when compared to BTS. This is evidenced by parameters such as motility, viability, the presence of abnormalities, and the integrity of the sperm plasma membrane, particularly following 60 hours of storage. The findings of this study are consistent with the fundamental characteristics of each diluent, which have been extensively documented in the extant literature. Tris is a tris (hydroxymethyl) aminomethane-based diluent with excellent buffering capacity. Research findings have demonstrated that this compound can maintain pH stability within the semen environment during storage (Blegur *et al*., 2020; Hine and Nalley, 2025). As is well-established, pH stability is imperative for maintaining plasma membrane integrity, as well as for ensuring the optimal level of enzymatic activity and ion balance in sperm. Consequently, this results in a physiological slowing down of sperm quality degradation during storage.

In contrast, BTS has gained significant popularity in the swine artificial insemination industry as a practical diluent, primarily due to its ease of use and its ability to maintain sperm quality during short to medium-term storage (Nalley *et al*., 2024). However, the buffering capacity and membrane protection of BTS are comparatively limited. The discrepancy in effectiveness between the two types of extenders becomes increasingly evident under storage conditions that induce oxidative stress, where the contribution of LFFE bioactive compounds functions as an ancillary protective factor. Consequently, while the use of LFFE has been demonstrated to enhance sperm quality in both types of diluents, the optimization of Tris diluent usage is still advised for boar semen storage applications, particularly in scenarios involving storage periods exceeding 48 hours. The combination of Tris diluent with LFFE at optimal levels could serve as a potential strategy to enhance the efficiency of boar artificial insemination programs through improved sperm quality during storage.

The findings of this study carry substantial practical ramifications for the advancement of swine reproductive technology, particularly regarding enhancing the efficiency and success of artificial insemination programs. A significant challenge in implementing artificial insemination in swine agriculture pertains to the temporal limitations imposed on the storage of fresh semen, which can result in a decline in sperm quality (Hine and Nalley, 2025). This decline encompasses parameters such as motility, viability, PMI, and an increase in abnormalities, thereby exerting a direct influence on the success rates of fertilization. The results of this study indicate that the addition of 9% Lontar fruit fiber extract to Tris or BTS diluents can significantly maintain boar sperm quality for up to 60 hours of storage. This finding indicates the possibility of LFFE functioning as an effective natural additive. It demonstrates the capacity to prolong the storage life of fresh semen while decreasing the necessity for synthetic additives commonly employed in the boar farming industry.

In addition, the utilization of local materials, such as LFFE, possesses the capacity to furnish a more economical and accessible solution for farmers, particularly in tropical regions such as Indonesia. In this country, the Lontar palm (*Borassus flabellifer*) is widely prevalent but has not yet been fully utilized to its full potential. The employment of LFFE in semen diluents offers a multifaceted strategy for addressing contemporary agricultural challenges. Firstly, the product under consideration provides an alternative nutritional supplement that has the potential to enhance sperm performance. Secondly, the practice aligns with the principles of sustainability that have emerged in modern farming systems. It achieves this alignment by leveraging the local biological potential to improve production efficiency. Consequently, the findings of this study provide a significant scientific contribution to the understanding of the mechanism of action of natural bioactive compounds on sperm quality. Moreover, the results of this study have a substantial impact on the promotion of innovative, cost-effective, and environmentally friendly reproductive technologies in the swine farming industry.

Even though the present study provides relevant information regarding the prospective use of LFFE as a natural additive to enhance the quality of boar sperm in both Tris and BTS diluent, it is imperative to acknowledge the presence of several methodological and technical limitations. A notable limitation of the present study is the absence of phytochemical analysis, which is a critical component of evaluating bioactive compounds in LFFE associated with improved sperm quality.

However, a more comprehensive understanding of the chemical composition of LFFE, including the types and concentrations of flavonoids, phenolics, polysaccharides, and other antioxidants, is crucial to elucidating the biological mechanisms underlying the protective effects of LFFE on sperm cells. Absent such data, the interpretation of the results remains general and cannot be fully attributed to specific active compounds contained in LFFE. Furthermore, the study's design was constrained to the assessment of sperm quality parameters in vitro, including motility, viability, abnormalities, and PMI. It did not encompass the implementation of fertility assessments in the field, such as the measurement of pregnancy success

rates or birth rates after artificial insemination utilizing semen that had undergone treatment with LFFE. However, while in vitro sperm quality parameters are indeed significant, they do not always directly correlate with actual fertility outcomes in the field. Consequently, to ensure the practical effectiveness of LFFE use in artificial insemination programs, further testing at the farm level involving real fertility parameters is required.

A notable constraint of the present study is its scope, which is currently confined to a single species, specifically boars, and utilizes semen from a particular breed. The potential of LFFE as a natural semen diluent additive in other livestock species, such as cattle, goats, or sheep, as well as under various environmental conditions, has not yet been explored. Furthermore, the interaction between LFFE and other diluents beyond Tris and BTS remains to be investigated. This presents a potential avenue for further research, which could lead to the development of more applicable, comprehensive, and specific recommendations tailored to the diverse range of farm conditions.

In consideration of the findings and the constraints that have been identified, the present study suggests several avenues for future research. These avenues include the potential of LFFE as a natural semen diluent additive. A primary recommendation is the necessity of a comprehensive phytochemical characterization of LFFE, with the aim of identifying the types, levels, and stability of bioactive compounds, including phenolics, flavonoids, polysaccharides, vitamin C, and other antioxidant compounds (Prasad *et al*., 2022, 2023; Bahera and Nayak, 2022). These bioactive compounds are suspected to contribute to the enhancement of sperm quality. This analysis can be conducted using high-performance liquid chromatography (HPLC) or spectrophotometry. It is imperative to ensure that the resulting data is not only descriptive but also capable of explaining the molecular mechanisms underlying the positive effects of LFFE.

Moreover, further experimentation is necessary to assess the repercussions of implementing LFFE in semen diluents on real fertility parameters in the field. The following parameters are of relevance in this context: artificial insemination success rates, pregnancy rates, live births, and the quality of offspring produced. Such evaluations are imperative to ascertain that the observed enhancements in sperm quality in vitro genuinely exert a favourable influence on reproductive efficiency at the livestock population level. This recommendation assumes particular significance considering the study's findings, which indicate that Tris diluent augmented with 9% LFFE can preserve sperm quality for a duration of up to 60 hours of storage. To ensure the validity of this assertion, it is imperative that the findings be substantiated through field testing.

Furthermore, additional exploration of LFFE's potential in other livestock species, including cattle, goats, and sheep, is imperative to assess the consistency of this compound's efficacy across diverse sperm types, which possess varying physiological characteristics. These studies are of particular importance because each species exhibits different sensitivities to storage stress and to additive components in diluents. In addition, research initiatives that concentrate on the incorporation of LFFE with other natural additives, including honey, herbal extracts, and micronutrient supplements, are justified. The objective of these research endeavours is to formulate a semen diluent that is more optimal, efficient, and environmentally friendly.

It is imperative that further research be conducted to assess the feasibility of implementing LFFE in more stable and pragmatic processed forms, such as dry extracts or microcapsules, to facilitate its on-farm application. In addition, future work should include on-farm AI trials, multi-breed semen testing, and antioxidant mechanism assays. Collectively, these efforts would advance LFFE-based innovations that not only provide scientific benefits but also enhance reproductive technology efficiency and support sustainable livestock production systems utilizing local resources.

CONCLUSION

Supplementation of 9% LFFE effectively preserves sperm motility, viability, and PMI up to 60 h. LFFE is a potential natural additive for semen storage. These results suggest that LFFE has the potential to act as a natural additive, thereby enhancing the storage life and quality of boar semen. It is recommended that Lontar fruit fiber extract be used as a natural additive in boar semen diluents, particularly at a level of 9% in Tris diluent. Further research is necessary to test its effectiveness on field pregnancy success rates and its potential application in other livestock species.

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NOVELTY STATEMENT

This study is the first to systematically evaluate the

use of Lontar fruit (Borassus flabellifer) fiber extract (LFFE) as a natural bioactive additive in both Tris and Beltsville Thawing Solution (BTS) extenders for boar semen preservation. The findings demonstrate that supplementation with 9% LFFE significantly improves sperm motility, viability, and plasma membrane integrity while decreasing abnormalities during storage for up to 60 hours. This represents a novel, eco-friendly approach, utilizing an abundant, underexploited local biological resource to enhance swine reproductive technology and artificial insemination success.

AUTHOR'S CONTRIBUTION

WMN, TMH, KU and PK: Conceived and designed the experiment. WMN, TMH, KU and PK: Performed the experimental procedures. WMN, ARR, NMPS, and YYB: Supervised and coordinated the research and provided clinical data. ABL and ANB: Statistical analysis was conducted. RMJR, AR, AMD, and AB: The initial draft of the manuscript was prepared. All authors critically reviewed and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

No generative AI or AI-assisted technology was utilized in the design, data collection, analysis, or initial preparation of this manuscript. All experimental design, data processing, and preliminary drafting were conducted exclusively by the authors without the involvement of generative AI. However, DeepL and Quillbot, both language-support software tools, were used to improve the linguistic quality of the manuscript. These tools were used for editing, grammar correction, and paraphrasing to enhance readability, while ensuring that all original ideas scientific interpretations remain the authors' own.

Conflict of interest

The authors have declared no conflict of interests.

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