Original Article

A preliminary study of *Lasia spinosa* Thw. feed supplementation improving semen quality of subfertile purebred boars

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Abstract

Poor semen quality is a significant issue among imported purebred boars in Thailand, affecting both semen quality itself and reducing productivity at AI stations. The present study investigated the effects of dietary supplementation with *Lasia spinosa* Thw. (LST) on improving boar semen quality. A group of boars experiencing poor semen quality were monitored, and three were selected due to their persistent low semen profiles for the supplementation experiment. Dried LST powder was prepared in small bags at a dosage of 4 g per 100 kg body weight and added to the regular feed for daily oral administration over an 8-week experiment period. Semen was collected from the boars one week before treatment initiation and at the end of the supplementation period. Each sample was assessed for volume, sperm concentration, motility, viability, acrosome integrity, mitochondrial activity, and morphology. Dietary supplementation with LST significantly improved semen volume (P = 0.03), sperm motility (P = 0.03), viability (P = 0.03), and acrosome integrity (P = 0.03). The percentage of normal sperm head morphology remained unchanged. However, both major and minor sperm defects were significantly reduced (P = 0.03). In conclusion, LST powder supplementation enhances semen quality in subfertile boars and is a feasible intervention for swine farms.

Keywords: Boar, *Lasia spinosa* Thw., semen quality, subfertility

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Received March 16, 2024
Accepted June 10, 2024

Introduction

The boar station serves as the central unit for producing chilled semen used in artificial insemination (AI) on farms (Tretipskul *et al*., 2010). The low semen quality of boars in AI stations is a pressing issue (Kaewma *et al*., 2021), adversely affecting profitability and increasing the boar culling rate. This problem can arise from infectious or non-infectious issues (Maes *et al*., 2016). Non-infectious diseases are caused by poor nutrition, improper management, and especially severe climate change (Vanderhaeghe *et al*., 2013). The heat stress and humidity in tropical countries, such as Thailand can have extreme effects on boar health and semen production, impairing semen quality by inducing germ cell damage (Li *et al*., 2015) and reducing testosterone levels (Murase *et al*., 2007).

Lasia spinosa Thw. (LST) is a local herb, which can grow in tropical and subtropical regions of South Asia and Southeast Asia (Akter *et al*., 2023). This plant has been shown to improve growth and meat quality in broilers, and enhance reproductive performance in roosters and buffaloes (Hong *et al*., 2022; Suthikrai *et al*., 2007; Zhang *et al*., 2022). Its pharmacological activities have also been reported to include anti-bacterial, anti-inflammatory, antioxidant, anti-hyperlipidemic, and other disease-preventive factors (Kankanamge *et al*., 2017). Additionally, Jintana *et al.* (2013) demonstrated that the herb contains natural phytoestrogens and phytoandrogens. Supplementation with LST has been proven to provide reproductive support in swamp buffaloes by regulating blood testosterone (Suthikrai *et al*., 2007). Moreover, it increases testicular weight and sperm production in male rats (Kaewamatawong *et al*., 2013). Therefore, the present study aimed to evaluate the ability of LST supplementation to improve semen characteristics in subfertile boars.

Materials and Methods

Animals and experimental plan: The study was conducted at the AI center of a private facility in Ratchaburi Province, in western Thailand. From March to October 2019 (summer to the rainy season), twenty-two imported boars were monitored for low semen production and poor semen characteristics. These boars had an age between 24 to 32 months and an average weight of 252 ± 18.63 kg. They were housed under the same environmental conditions (no exceeding 28°C). The animals were fed 2.0 kg of commercial feed containing 14.0% protein twice a day.

During the experimental period from October to December 2019 (winter season), three purebred boars (Berkshire, Duroc, and Landrace) were selected for a detailed study due to their persistent low semen profiles (Rozeboom, 2009). All boars were tested negative for reproductive infectious disease. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC), Chulalongkorn University (Approval No. 2131019).

LST powder was prepared from the under-soiled trunk and root part of the herb. The herb was sun-dried, chopped, and ground into a fine powder (Suthikrai *et al*., 2007). Each boar received a daily dose of 4 g per 100 kg body weight, calculated and pre-packaged for 8 weeks (Mitchaothai *et al*., 2012). The powder was added to the regular feed during the morning meal.

Semen collection and evaluation: Semen from three purebred boars with poor semen quality were collected in two batches, with a five-week interval between them, resulting in a total of six semen samples. The gloved-hand technique was used for collection both before and after LST supplementation. Notably, semen collection is performed weekly on these boars as part of the routine semen production at this AI center. After collection, fresh semen from each boar was preserved in a commercial semen extender and cooled in a styrofoam box maintained at 16°C for transportation.

The semen parameters were determined as follows: the semen volume (mL), the sperm concentration (SpermaCue (Minitube®, USA)), sperm motility (computer-assisted semen analysis (SCA® CASA system, MICROPTIC S.L, Barcelona, Spain)) (Kaewma *et al*., 2021), sperm viability (SYBR-14/Ethidium Homodimer-1 staining technique (Fertilight®, Sperm Viability Kit, Molecular Probes Europe, Leiden, The Netherlands)) (Axnér *et al*., 2004; Kupthammasan *et al*., 2022), acrosome integrity (FITC-PNA staining (Sigma-Aldrich®, USA)) (Chanapiwat *et al*., 2009), mitochondrial activity (JC-1 staining (Molecular Probe Inc., USA)) (Huo *et al*., 2002), and sperm morphology (Giemsa's staining foe head (Mansur *et al*., 2018) and formal saline fixing for tail evaluation).

For sperm morphology, both major and minor defective characteristics of the sperm head and tail were classified (Chenoweth, 2005; Kondracki *et al*., 2020).

Statistical analysis: Data analysis was conducted using the R programming language (version 4.1.2) and R studio (version 2022.07.0+548). The data are presented as the sample mean ± standard deviation (SD). The normality of the data was checked using the Shapiro-Wilk normality test. All changes in semen parameters were tested using a paired *t*-test for data with a normal distribution. For data that did not follow a normal distribution, the Wilcoxon signed-rank test was considered. The significance level was set at a *P*-value of 0.05.

Results and Discussion

In the experiment, LST improved some parameters of semen quality in purebred boars with a history of poor semen production. A previous study (Mitchaothai *et al*., 2012) showed that supplementation with LST powder at the same dosage as our study could increase blood testosterone levels but their semen quality results were not improved. In contrast to our study, their experiment was performed on boars with normal semen quality. Therefore, the testosterone concentration was expected to be the main point of semen quality improvement. Joshi and Raeside (1973) revealed that the synergistic action of testosterone and estrogens increased the secretory activity of the male accessory glands. Moreover, exogenous testosterone administration has been correlated with libido,

ejaculated semen volume, and the percentage of normal spermatozoa (Bilskis *et al*., 2012).

Semen volume was improved 12% (23.33 mL) from 171.67 ± 21.09 to 195.00 ± 20.49 mL ($P < 0.05$) (Table 1), which is a critical parameter used to assess semen quality for processing. Previous studies have identified various factors influencing semen volume, including the frequency of semen collection (Frangež *et al*., 2005), the semen collection procedure, and the boar's health status (Ax *et al*., 2000). In addition, factors such as the breed, season, environmental conditions (especially high atmospheric temperature) (Smital, 2009; Smital *et al*., 2004; Trudeau and Sanford, 1986), and blood testosterone levels (Park and Yi, 2002) have been reported to impact semen volume. The main fraction of semen volume consists of clear seminal plasma (spermatozoa-free fluid), and the gel form, separated from the semen collection procedure (Queensland, 2022). There is evidence suggesting that the increase in boar semen volume may mainly originate from the seminal vesicle gland (Leal *et al*., 2018). Importantly, the lack of blood testosterone has been linked to lower semen volume and electrolyte concentration imbalances (Murase *et al*., 2007). Moreover, the increase in seminal plasma in the semen fraction has been positively associated with sperm motility, acrosome, and plasma membrane stability (Leal *et al*., 2018). This may contribute to improvement of these parameters following LST supplementation.

Table 1 Average fresh semen quality of boars with poor semen quality before and after *Lasia spinosa* Thw. supplementation.

Parameters	Before treatment	After treatment	*P*-value
	Mean ± SD	Mean ± SD	
Semen volume (mL)	171.67 ± 21.09	195.00 ± 20.49	0.03*
Concentration (×10⁶ spz./mL)	167.00 ± 183.58	171.67 ± 158.72	1.00
Sperm production (×10⁹ spz.)	27.69 ± 33.42	34.47 ± 35.65	0.14
Sperm motility (%)	40.13 ± 26.03	76.63 ± 5.74	0.03*
Sperm viability (%)	38.83 ± 20.60	60.83 ± 1.61	0.03*
Acrosome integrity (%)	52.17 ± 23.82	68.17 ± 4.65	0.03*
Mitochondrial activity (%)	71.00 ± 21.66	71.83 ± 12.50	1.00
Sperm head morphology (%)			
Normal head	90.87 ± 3.38	90.93 ± 2.69	1.00
Narrow head	0.93 ± 1.62	2.60 ± 1.11	0.13
Narrow at base	0.73 ± 1.10	3.20 ± 1.22	0.13
Pear-shaped head	0.80 ± 0.72	0.80 ± 1.04	0.58
Variable size head	0.80 ± 0.60	1.13 ± 0.81	0.53
Abnormal contour	0.47 ± 0.42	1.20 ± 0.87	0.03*
Abaxial head	0.20 ± 0.35	0.07 ± 0.12	0.58
Loosing head	5.20 ± 5.56	0.07 ± 0.12	0.03*
Sperm tail morphology (%)			
Normal tail	21.33 ± 5.20	26.33 ± 10.02	0.03*
Proximal droplet	49.17 ± 14.29	49.33 ± 13.29	1.00
Distal droplet	1.83 ± 0.29	1.83 ± 0.76	1.00
Simple bent tail	4.83 ± 4.01	3.50 ± 1.32	0.53
Coil tail	17.83 ± 17.90	18.17 ± 14.49	1.00
Acrosome defect	0.17 ± 0.29	0	0.35
Loosing tail	4.83 ± 4.31	0.83 ± 1.04	0.03*

spz. is spermatozoa.
*Values within rows are significantly different ($P<0.05$).

Also, LST supplementation demonstrated the ability to improve both major (6.73 ± 4.46 and 4.07 ± 2.00%, $P < 0.05$) and minor defects (6.93 ± 4.71 and 3.8 ± 1.91%, $P < 0.05$) of the sperm head (Table 2). Enhancing spermatogenesis, which is associated with testosterone levels, may play a key role in improving these parameters (Pinart and Puigmulé, 2013). However, it is our opinion that the herb may also influence other pathways within testicular tissue to promote spermatogenesis. This study was not able to deeply describe the effects of LST on these pathways. Hence, further study is required, for example, the analysis of gene expression levels in the testicular tissue of supplemented boars. Regarding tail characteristics, testosterone levels have been implicated (Bilskis *et al*., 2012). In this study, LST supplementation could reduce these defects. The reduction may be influenced by testosterone, which is crucial for sperm maturation within epididymis.

The present study found that LST supplementation could enhance semen volume, sperm motility (40.13 ± 26.03 and 76.63 ± 5.74%; $P < 0.05$), sperm viability (38.83 ± 20.60 and 60.83 ± 1.61%; $P < 0.05$), acrosome integrity (52.17 ± 23.82 and 68.17 ± 4.65%; $P < 0.05$), and some characteristics of sperm morphology in boars with poor semen quality. This suggests that testosterone insufficiency may be one of the causes of poor semen quality, potentially influenced by factors such as season- and/or photoperiod-related testosterone levels (Murase *et al*., 2007). This plant has been proven to play a key role in maintaining testosterone levels and may offer other benefits that require further study to fully understand its advantages for semen and sperm function. The issue observed with the boars in this study could be attributed to environmental factors, seasonal changes, and/or photoperiod variations, to which some imported purebred boars in the AI center could not adapt themselves while residing in Thailand. Moreover, our observations of other boars in the same center revealed that some of them were able to produce semen that exceeded the quality standard. This

suggests that individual variation or other factors may also play a role in semen quality.

Table 2 Major and minor sperm defects of boars with poor semen quality before and after *Lasia spinosa* Thw. supplementation.

Sperm defects	Before treatment	After treatment	*P*-value
	Mean ± SD	Mean ± SD	
Major defects			
Sperm head (%)	6.73 ± 4.46	4.07 ± 2.00	0.03*
Sperm tail (%)	67.17 ± 9.36	67.5 ± 10.21	0.03*
Minor defects			
Sperm head (%)	6.93 ± 4.71	3.8 ± 1.91	0.03*
Sperm tail (%)	6.67 ± 4.25	5.33 ± 1.53	0.03*

*Values within rows are significantly different (P<0.05).

In conclusion, the present study demonstrates that *Lasia spinosa* Thw. supplementation can improve boar semen volume and have a positive impact on sperm motility, sperm viability, acrosome integrity, and some morphological characteristics in imported purebred boars.

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