

Enhancement of Semen Quality of Crossbred Boars from Different Breeds via Dietary Supplementation with Grape Pomace (*Vitis vinifera*)

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Abstract

The semen quality of crossbred boars can be improved through antioxidants supplementation, one of which is grape pomace supplementation. Grape pomace is a by-product of the wine industry. The antioxidant content of grape pomace has been widely studied and is known to suppress free radicals, thereby providing positive effects on reproductive quality. The study aimed to evaluate the improvement of semen quality in crossbred boars of different breeds through dietary supplementation of grape pomace (*Vitis vinifera*). The experiment was conducted using a completely randomized design (CRD) with three treatments based on different crossbred boar breeds: Landrace crossbred (B1), Duroc crossbred (B2), and Yorkshire crossbred (B3), within ages ranging from 1 to 1.5 years. Semen collection was performed five times for each boar using the hand massage method. All treatments received dietary supplementation of grape pomace at 5% of the ration, administered orally for a defined experimental period. The observed variables included macroscopic parameters (semen volume, color, odor, consistency, and pH) and microscopic parameters (spermatozoa concentration, motility, and viability). The results showed that grape pomace supplementation significantly affected semen quality ($P < 0.05$), particularly in Yorkshire crossbred boars (B3), which exhibited the best overall performance.

Keyword: Crossbred Boars; Semen Quality; Grape Pomace; Antioxidants; Free Radical

1. Introduction

Low boar semen quality can result in fertilization failure and extended farrowing intervals, ultimately leading to a reduction in pig population. This issue can be addressed through artificial insemination (AI) using high-quality semen obtained from superior boars [1]. A decline in semen quality is often associated with the presence of free radicals, including superoxide anion, hydroxyl, and peroxy radicals, which induce lipid peroxidation. Lipid peroxidation adversely affects membrane integrity, fluidity, structure, and cellular function, ultimately causing spermatozoa death and a consequent reduction in semen quality [2]. Therefore, strategies to improve boar semen quality are required and can be achieved through oral antioxidant supplementation, which inhibits free radical activity in semen by converting reactive compounds into more stable forms [3].

The role of natural antioxidants is considered safer and more effective than synthetic antioxidants in suppressing free radicals [4]. In general, natural antioxidant compounds can be derived from plants or fruits [5], one example is grape pomace (*Vitis vinifera*). Grape pomace is abundantly generated as a by-product in the wine industry and is often underutilized, despite its potential as an alternative source of antioxidants for livestock [6]. The polyphenolic compounds contained in grape pomace exhibit biological effects by scavenging free radicals through mechanisms involving reduction and electron donation or transfer [7,8,9].

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Previous research [10] reported that supplementation of grape pomace (*Vitis vinifera*) at 4% in the diet of crossbred boars improved fresh semen characteristics by increasing motility percentage, reducing lipid peroxidation, and decreasing spermatozoa abnormalities through the action of polyphenols. Similarly, another study [11] found that supplementation of grape pomace at 5% in the diet of rams subjected to oxidative stress due to housing confinement produced positive effects by enhancing spermatozoa quality, which was associated with a reduction in malondialdehyde (MDA) levels resulting from lipid peroxidation and free radicals.

This study aimed to evaluate the effect of dietary supplementation with grape pomace (*Vitis vinifera*) on the semen quality of crossbred boars and to identify which breed exhibits the highest semen quality under these conditions.

2. Materials and Methods

The study was conducted over a period of three months, involving semen sample collection from crossbred boars located in Demulih Village, Susut, Bangli Regency, Bali, and subsequent evaluation of spermatozoa quality at the Animal Reproduction Laboratory, Faculty of Animal Husbandry, Udayana University, Jimbaran, Badung Regency, Bali.

The experiment was conducted using a completely randomized design with three treatments based on different crossbred boar breeds: Landrace crossbred (B1), Duroc crossbred (B2), and Yorkshire crossbred (B3), within ages ranging from 1 to 1.5 years, and actively used for semen collection for artificial insemination. The boars were housed individually in pens measuring 220 cm in length and 190 cm in width. Each pen was equipped with feed throughs and automatic water nipples.

All boars were fed a commercial feed (type 556, PT. Charoen Pokphand) supplemented with rice and corn bran. Additionally, each boar received grape pomace (*Vitis vinifera*) at 5% of total feed weight. The grape pomace was prepared by collecting and sorting pomace (including skins, seeds, residual pulp, and selected fruit residues) from the wine industry. The collected pomace was dried until to 9-10% moisture [12] and finely ground for easier incorporation into the feed [11].

Semen was collected from each boar five times at three-day intervals in the afternoon using the massage method. A total of 15 semen samples were obtained for evaluation. Semen quality was assessed macroscopically (volume, viscosity, color, odor, and pH) and microscopically (spermatozoa motility, viability, and concentration).

Data were analyzed using parametric analysis of variance (ANOVA) with SPSS version 29 (2022). When significant differences were observed among treatment means ($P < 0.05$), multiple comparison tests were performed using the Least Significant Difference (LSD) method [13].

3. Results and Discussion

Fresh semen from the three crossbred boars (B1, B2, and B3) was collected in five replicates and evaluated for macroscopic and microscopic characteristic. The study aimed to identify the boar breed with the highest sperm quality following dietary supplementation with 5% grape pomace (*Vitis vinifera*). Results from the three treatments are presented in Table 1.

The supplementation of grape pomace at 5% in the diet had positive effect on semen quality parameters of crossbred boars from different breeds. This was evidenced by mean values for B1, B2, and B3 meeting exceeding established standards. Furthermore, all measured semen quality parameters differed significantly among treatments ($P < 0.05$). The three breeds evaluated-crossbred Landrace (B1), crossbred Duroc (B2), and crossbred Yorkshire (B3)-exhibited varied responses to grape pomace supplementation.

Table 1 Semen quality parameters of different crossbred boars supplemented with 5% grape pomace.

Parameter	Average observation results			SEM	Standard
	B1	B2	B3		
Sperm Volume (ml)	232±30.54 ^b	239±17.46 ^b	192±14.83 ^a	7.64	150-200 [14]
Color	Cloudy white	Milky white	Milky white	-	Milky white [15]
Odor	Typical semen	Typical semen	Typical semen	-	Typical semen [16]
Consistency	Watery	Watery	Watery	-	Watery [16]
pH	7.7±0.0 ^b	7.8±0.11 ^b	7.5±0.13 ^a	0.05	7.3-7.8 [14]
Spermatozoa Concentration (10 ⁶ /ml)	204.8±29.05 ^c	327.2±34.95 ^b	442.2±20.6 ^a	26.80	200-300 [14]
Spermatozoa Motility (%)	65.4±3.65 ^b	76.0±4.18 ^a	80.0±0.0 ^a	1.82	50-80 [14]
Spermatozoa Viability (%)	76.8±4.09 ^a	80.2±3.42 ^{ab}	85.0±5.00 ^b	1.35	70-90 [14]

SEM= Standard Error of the Treatment Means; Values with the same superscript letters in the same row are not significantly different (P>0.05); Crossbred Landrace (B1); Crossbred Duroc (B2); Crossbred Yorkshire (B3).

3.1. Macroscopic sperm evaluation

Regarding semen volume, supplementation with grape pomace resulted in significant differences among treatments (P<0.05). The highest mean semen volume was observed in B2 (crossbred Duroc) averaging 239 ± 17.46 mL, followed by B1 (crossbred Landrace), while B3 (crossbred Yorkshire) exhibited the lowest mean volume. These results indicate that B2 responded more favorably to grape pomace supplementation in terms of semen volume compared to the other breeds. The increase in semen volume may be attributed to enhanced testicular function or increased secretion from accessory sex glands [10]. Additionally, grape pomace contains antioxidant compounds, particularly high levels of polyphenols found in grape seeds and skins, such as flavonoids, anthocyanins, quercetin, tannins, and resveratrol [6]. Previous research [17] reported that resveratrol acts as a natural phytoalexin with antioxidant properties and positive effects on the reproductive system by increasing testosterone levels, which are positively correlated with sperm production.

Semen color varied among treatments in response to grape pomace supplementation. A positive response was observed in B3 (crossbred Yorkshire) and B2 (crossbred Duroc), both exhibiting a milky white semen color that met established standards. In contrast, B1 (crossbred Landrace) showed a cloudy white color. These findings suggest that B3 had the highest spermatozoa concentration, resulting in a more intense semen color, whereas B1, which had the lowest spermatozoa concentration, produced a cloudy appearance.

Semen odor was consistent across all treatments and aligned with established standards, characterized by a typical semen odor with a slightly fishy or pungent smell. This indicates that grape pomace supplementation did not adversely affect semen odor and may reflect a healthy reproductive tract. The characteristic strong fishy or pungent odor, commonly referred to as boar taint, is influenced by the steroid androstenone, which is produced by Leydig cells in the testes alongside testosterone and functions as a pheromone [18].

Semen consistency was also uniform across all treatments and complied with the established standard, characterized by a watery consistency. Watery semen consistency in boars facilitates freer semen flow, enhancing sperm motility and increasing the likelihood of successful fertilization [19].

Semen pH (potential of hydrogen) showed significant differences among treatments (P < 0.05) following dietary supplementation with 5% grape pomace. The highest mean pH value (more alkaline) was observed in B2 (crossbred Duroc) at 7.8 ± 0.11, whereas the lowest pH (more acidic) was observed in B3 (crossbred Yorkshire) at 7.5 ± 0.13. These variations may be attributed to the effects of grape pomace supplementation, which contains vitamin C at 26.25 mg AAE/g ± 0.01, as well as physiological differences among the animals. Vitamin C content in grapes has the potential to lower semen pH, making it more acidic. Previous studies [20] have reported that dietary vitamin C can influence the pH of body fluids, including seminal plasma, resulting in a more acidic environment.

3.2. Microscopic sperm evaluation

Dietary supplementation with 5% grape pomace significantly affected sperm concentration in crossbred boars ($P < 0.05$). B3 (crossbred Yorkshire) showed the highest mean sperm concentration (442.2×10^6 cells/mL ± 20.36), whereas B1 (crossbred Landrace) had the lowest (204.8×10^6 cells/mL ± 29.05), indicating a better response of B3 to grape pomace supplementation. The increase in sperm concentration may be attributed to the zinc (Zn) content in grape pomace, approximately 25.00 mg/g, which can enhance spermatogenesis through stimulation of Leydig cells, without affecting ejaculate volume or the percentage of abnormal sperm [21].

Sperm motility was also significantly influenced by grape pomace supplementation ($P < 0.05$). B3 exhibited the highest mean motility ($80.0\% \pm 0.00$), whereas B1 had the lowest ($65.4\% \pm 3.65$). Mean motility was associated with semen pH, with higher motility and sperm concentration in B2 corresponding to the lowest pH (more acidic) among treatments. The lower motility observed in B1 may be due to lipid peroxidation, which can impair sperm metabolism and reduce energy availability, leading to decreased motility [22].

Sperm viability differed significantly among treatments ($P < 0.05$), with B3 showing the highest mean viability ($85.0\% \pm 5.00$) and B1 the lowest. The observed viability may be influenced by antioxidant minerals provided by grape pomace, including selenium (Se, 0.20 g/kg), copper (Cu, 0.049 g/kg), zinc (Zn, 0.025 g/kg), and manganese (Mn, 0.013 g/kg). Selenium protects cells from oxidative damage in conjunction with enzymatic antioxidants such as glutathione peroxidase (GPx), whereas Cu, Zn, and Mn contribute to superoxide dismutase (SOD) activity, which serves as the first line of defense against reactive oxygen species (ROS), thereby maintaining optimal sperm viability [23].

4. Conclusion

Based on the results of this study, it can be concluded that dietary supplementation of grape pomace (*Vitis vinifera*) at a level of 5% improved semen quality in crossbred boars, with crossbred Yorkshire boars exhibiting the best overall performance.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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