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ORIGINAL ARTICLE

Wine Grape Pomace as a Dietary Supplement to Improve Semen Quality in Boars

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ABSTRACT

Boar spermatozoa are highly susceptible to oxidative damage due to their high content of unsaturated fatty acids, which are prone to disruption by reactive oxygen species (ROS). Excessive ROS can induce lipid peroxidation, DNA fragmentation and impaired enzyme activity, ultimately reducing sperm quality and reproductive performance. Wine grape pomace (WGP), a by-product of the winemaking process, is rich in polyphenols, including flavonoids (anthocyanins and quercetin), stilbenes (resveratrol) and tannins, which possess strong antioxidant properties. This study aimed to evaluate the effects of dietary supplementation with 4% WGP on boar ejaculate output and sperm quality during storage. Twenty boars were divided into two groups: a control group fed a standard diet and a WGP group supplemented with 4% WGP for 4 months. Semen samples were collected and analysed for ejaculate number of sperm per ml, total antioxidant capacity (in seminal plasma) and quality parameters (motility, kinematic parameters, mitochondrial activity, acrosome integrity, viability) after 1, 3 and 5 days of refrigerated storage. Results showed that WGP supplementation increased the number of sperm per ml compared to the control group ($p < 0.05$), resulting in approximately two additional seminal doses per ejaculate, without negatively affecting other seminal parameters ($p > 0.05$) and refrigeration storage ($p > 0.05$). This improvement in sperm concentration could enhance the profitability of swine semen production by increasing the number of doses produced per boar annually. Given the low cost of WGP (10.03 €/boar/year), this strategy could offer a cost-effective approach to improving reproductive performance in boars. These findings support further research into optimising WGP inclusion levels and exploring its broader impacts on boar fertility and reproductive efficiency.

1 | Introduction

The membrane of boar spermatozoa contains a large amount of unsaturated fatty acids, making these cells highly susceptible to the harmful effects of reactive oxygen species (ROS). These ROS can induce lipid peroxidation, disrupt various cellular structures and impair functions such as plasma membrane integrity and enzyme activity (White 1993). Additionally, ROS can cause DNA fragmentation (Baumber et al. 2003), reduce cell mass, trigger apoptosis and consequently decrease sperm quality (Sanocka-Maciejewska et al. 2005). A positive and significant correlation has been shown between ROS concentrations and the percentage of sperm exhibiting morphological abnormalities in the head and midpiece, acrosome disruption, tail defects

and increased cytoplasmic droplets (Kobayashi and Suda 2012). In human reproduction, it has been established that men with fertility problems associated with teratozoospermia have ejaculates with relatively higher concentrations of ROS compared to those without fertility problems (Agarwal and Said 2005; Rato et al. 2012).

Physiologically, ROS concentrations are maintained at optimal levels due to the presence of antioxidants in seminal plasma, such as β-mercaptoethanol, vitamins E and C, cysteamine, cysteine, taurine and hypotaurine (Holmes et al. 1992; Chen et al. 1993; Rolf et al. 1999; Kitagawa et al. 2004; Bucak et al. 2007). Based on this knowledge, experiments have been conducted proposing dietary antioxidant supplementation as a potential strategy to improve reproductive outcomes in subfertile males (Wong et al. 2000; Eskenazi et al. 2005; Michael et al. 2007) or to increase seminal quality in animals (Marin-Guzman et al. 1997; Castellini et al. 2002; Deichsel et al. 2008; Contri et al. 2011). Another proposed approach for improving semen quality involves adding antioxidants to diluents during the processing of chilled or frozen semen to promote optimal ROS concentrations and maintain high sperm quality during storage. Experimental results have varied, ranging from satisfactory outcomes to no noticeable antioxidant effects. These studies have included the supplementation of hypotaurine in human semen (Brugnon et al. 2013), crocin in bull sperm (Sapanidou et al. 2015), and vitamin C, taurine, catalase, vitamin E and vitamin B16 in cryopreserved dog semen (Michael et al. 2007). Other investigations have examined the use of astaxanthin to assess the effect of refrigerated boar semen storage (Basioura et al. 2018), and alpha-tocopherol, cysteine and rosemary for boar semen cryopreservation (Jeong et al. 2009; Malo et al. 2010).

Wine grape pomace (WGP) is the residue left after grape pressing during wine production or following fermentation in the case of red wine. It primarily consists of grape skins, residual pulp, seeds and stems. This by-product is rich in polyphenols (such as flavonoids, including anthocyanins and quercetin), stilbenes (e.g. resveratrol) and tannins (Beres et al. 2017), retaining approximately 30%–40% of these compounds after processing (Ky et al. 2014). These components exhibit various bioactive properties, including antioxidant (Auger et al. 2004), anti-inflammatory (Gessner et al. 2013; Fiesel et al. 2014; Tian et al. 2023), anti-aging and antimicrobial properties (Xia et al. 2010; Tian et al. 2023; Kohut et al. 2024). In the pig industry, WGP has been predominantly used as a dietary supplement for piglets during the weaning and transition phases (7–30 kg/live weight) to evaluate its role as a growth promoter. Studies have shown that WGP supplementation enhances productive performance (e.g. feed intake, average daily gain and feed conversion), improves blood profiles and boosts immunity through the antimicrobial modulation of the gastrointestinal tract. Additionally, it enhances antioxidant capacity in the intestine, liver, spleen and kidneys (Brenes et al. 2016; Chedea et al. 2019; Costa et al. 2022; Tian et al. 2023; Ospina-Romero et al. 2024; Proca et al. 2024). Moreover, the effects of dietary WGP supplementation on male reproductive performance have also been studied in various species, including rabbits (Eid 2008), rams (Zhao et al. 2017) and boars (Gloria et al. 2019). In the latter, a 10-month study demonstrated that supplementing boar diets with 2% and 4% WGP improved seminal parameters compared to baseline semen quality before the dietary intervention (Gloria et al. 2019). However, since boar seminal parameters can fluctuate significantly across the seasons (Ciereszko et al. 2000), further research is needed to compare supplemented and non-supplemented groups simultaneously.

Based on this, we hypothesise that dietary supplementation with WGP, a low-cost by-product of the wine industry, could enhance boar semen production and quality by protecting sperm cells from oxidative damage during storage. Therefore, this study aimed to assess the effect of dietary supplementation with 4% WGP on ejaculate output in boars and sperm quality in diluted semen stored under refrigeration for 5 days.

2 | Material and Methods

2.1 | Ethics Statement

The experiments presented in this study were carried out in accordance with the Spanish Policy for Animal Protection RD 53/2013, which aligns with the European Union Directive 2010/63/EU on animal protection. This project was approved by the Research Ethics Committee of Miguel Hernández University with the ethical approval code UMH.DTA.PLL.050423, according to the Spanish Royal Decree RD 53/2013 and the EU Directive 2010/63/EU for the protection of animals used for experimental research and other scientific purposes.

2.2 | Wine Grape Pomace

The red grape pomace came from the vinification process carried out in a winery associated with the BOCOPA Cooperative (Petrer, Alicante, Spain). It consisted of a semi-solid heterogeneous mixture of skins, remains of pulp and seeds left after the vinification process and separation of the liquid phase. This pomace was transferred to the Pilot Plant for the Conservation of Agro-industrial By-products at the Escuela Politécnica Superior de Orihuela of the Miguel Hernández University of Elche (Spain), where it was subjected to a drying process in a 3-phase rotary trommel using indirectly heated air in a biomass boiler (Rial, Sigüeiro-Oroso, A Coruña, Spain). For the test, 5000 kg of fresh pomace with 56% dry matter (DM) was used, which was dehydrated to 92% DM. After dehydration, four random samples were taken and analysed for bromatological composition. The mean values found are shown in Table 1. The composition was determined using AOAC (Association of Analytical Communities) procedures: Ether extract (EE, g/kg MS, 920.39), crude protein (CP, g/kg MS, 988.05), crude fibre (CF, g/kg MS, 978.10), ash (g/kg MS, 934.01) and total sugars (TS, g/kg MS, 974.06). Neutral detergent fibre (NDF, g/kg DM), acid detergent fibre (ADF, g/kg DM) and acid detergent lignin (ADL, g/kg DM) contents were analysed according to Van Soest et al. (1991). Starch determination was carried out by the Ewers polarimetric method (ISO 10520:1997).

TABLE 1 | Chemical composition of dehydrated red grape pomace.

DM	OM	EE	CP	CF	NDF	ADF	ADL	ST	ASH	TS	TP
922.0	888.5	95.8	111.8	384.0	552.5	467.5	332.2	25.8	43.5	5.5	45.5

Abbreviations: ADF, acid detergent fibre (g/kg DM); ADL, acid detergent lignin (g/kg DM); ASH, ash (g/kg DM); CF, crude fibre (g/kg DM); CP, crude protein (g/kg DM); DM, dry matter (g/kg DM); EE, ether extract (g/kg DM); NDF, neutral detergent fibre (g/kg DM); OM, organic matter (g/kg DM); ST, starch (g/kg DM); TP, total phenol (mg gallic acid equivalent/g DM); TS, total sugars (g/kg DM).

2.3 | Animals

Boars from a commercial Artificial Insemination (AI) centre (Spermatica Reproduccion, Lorca, Murcia, Spain) were used in this study. Twenty boars (age: 16.25 ± 0.12 months) of the Duroc breed (Danish genetics), of proven fertility, were individually housed in pens with sawdust under the same conditions.

2.4 | Extraction and Processing of Ejaculate

The rich fraction of ejaculates was collected using the gloved-hand method, with a collection frequency of approximately 7-day intervals between collections. Sperm concentration was determined using Metrosperm C (Import-Vet, Barcelona, Spain). For semen collection, 100 mL of commercial extender (Androstar Plus, Minitube International, Tiefenbach, Germany) at 32°C was placed in a plastic container. After collection, the container was filled with the same extender until reaching a final volume of 455 mL. The semen samples were then diluted with the commercial extender at 32°C to adjust the final concentration to 33×10^6 sperm cells/ml. Finally, seminal doses were packaged in plastic bags containing 2000×10^6 sperm/60 mL and incubated at room temperature (18°C–22°C) for 2 h. Finally, seminal doses were refrigerated at 16°C.

The seminal plasma was obtained from raw samples (before dilution) after double centrifugation at 1500 g for 10 min (centrifuge Model 5418 R, Eppendorf, Hamburg, Germany) of 10 mL semen samples. Then, the supernatant was examined using microscopy to ensure a sperm-free sample. The seminal plasma samples were frozen to −80°C and stored until use. On the day of the analysis, the seminal plasma samples were thawed at room temperature.

2.5 | Determination of Total Antioxidant Capacity (TAC) in Seminal Plasma Samples

The total antioxidant capacity (TAC) of seminal plasma was determined using an automated analyser (AU 400, Olympus, Minneapolis, USA). The method was based on the ability of antioxidants to decolorize 2,2′-azinobis-(3-ethylbenzothiazolin e-6-sulfonate), measured according to their concentration and antioxidant capacity. This change was quantified by the variation in light absorbance at 660 nm. The assay calibration was performed using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carb oxylic acid (Trolox), and the TAC values of the seminal plasma samples were expressed as equivalents of the mM concentration of the Trolox solution.

2.6 | Evaluation of Spermatozoa Motility and Kinetic Parameters

Sperm motility and kinetic parameters were assessed by a computer-assisted semen analysis (CASA; ISAS software, PROiSER R + D S.L., Valencia, Spain) coupled to phase-contrast microscopy (negative-pH 10x objective; Leica DMR, Wetzlar, Germany) and a digital camera (Basler Vision, Ahrensburg, Germany). Before evaluation, sperm samples were warmed at 38°C for 10 min (heat block CH100, Biosan Laboratories Inc., MI, USA). Then, a 4 μL drop of the sample was placed in a pre-warmed (38°C) chamber (20-μm Spermtrack chamber, Proiser R + D, SL; Paterna, Spain) and evaluated using phase-contrast microscopy, and at least three different fields per sample were analysed. The parameters evaluated were the following: Total motility (%), progressive motility (%), curvilinear line velocity (VCL, μm/s), average path velocity (VAP, μm/s), straight line velocity (VSL, μm/s), amplitude of lateral head displacement (ALH, μm), percentage linearity (LIN, ratio of VSL/VCL, %), percentage straightness (STR, ratio of VSL/VAP, %), percentage oscillation (WOB, %) and beat-cross frequency (BCF, Hz). Sperm cells were motile when there was a VAP > 10 μm/s, and progressive motile when there was a straightness (STR) > 45%. Additionally, 25 frames per second and particle size area between 10 and 80 μm^2 were used as CASA setting parameters.

2.7 | Evaluation of Spermatozoa Mitochondrial Activity

Sperm mitochondrial activity was assessed by using JC-1 (5,5′, 6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; Thermo Fisher Scientific Inc., MA, USA). The staining solution, composed of 10 μL of JC-1 (0.017 μg/mL) in 10 mL of PBS without Ca^{2+} and Mg^{2+}, was prepared. Then, sperm samples underwent a 30-min incubation with the JC-1 solution in darkness. Evaluation of the samples took place under fluorescence microscopy (40 × objective; Leica DM4000 Led, Wetzlar, Germany, 495/520 nm), with a minimum of 200 cells counted per sample. Sperm were categorised based on mitochondrial membrane potential: (1) low potential, indicated by green fluorescence in the midpiece, and (2) high potential, characterised by orange fluorescence in the midpiece.

2.8 | Evaluation of Spermatozoa Morphology

For sperm morphology, a buffered 0.3% formal saline solution was used to fix sperm cells and store them at 4°C until the evaluation. Regarding sperm morphology, wet amounts of fixed samples were prepared, evaluated using a phase-contrast

TABLE 2 | Characteristics of ejaculates (sperm concentration, spz/ml), sperm quality parameters (normal and abnormal spermatozoa, cytoplasmatic droplet, abnormal tail) and seminal plasma (total antioxidant capacity) from both experimental groups (CG and WGP). Data are expressed as the mean, standard deviation (SD) and coefficient of variation (CV, %).

	CG ($n = 30$)			WGP ($n = 30$)		
Parameters	**Mean**	**SD**	**CV (%)**	**Mean**	**SD**	**CV (%)**
Concentration (10^6 spz/mL)	182.9	72.2	39.7	191.6	50.0	26.1
Total abnormal spermatozoa (%)	39.6	20.9	52.8	31.1	19.7	63.3
Total normal spermatozoa (%)	60.4	24.5	40.6	68.9	23.2	33.7
Cytoplasmic droplet (%)	28.7	20.5	71.4	23.6	18.3	77.5
Abnormal tail (%)	10.7	14.6	136.4	7.4	9.9	133.8
Total antioxidant capacity (mmol/L)	0.40	0.15	37.5	0.35	0.14	40.0

microscope (100x objective, Nikon, Eclipse E2000) and classified into the following categories: normal morphology, cytoplasmic droplet, abnormal tail (folded or coiled tail) and others.

2.9 | Experimental Design

Twenty boars were randomly distributed as follows: (1) control group (CG; $n = 10$): animals (aged 16.46 ± 0.16 months) were fed 2.5 kg per day of a commercial diet; (2) WGP group ($n = 10$): animals were fed the commercial pelleted diet used in CG supplemented with 100 g of WGP per day for four consecutive months (July to October 2023) (aged 16.01 ± 0.17 months). After 3 months of treatment, three ejaculates were collected from each boar (one per week) to obtain a total of three ejaculates per male and treatment. For each ejaculate, sperm concentration/ml and TAC (in seminal plasma) were assessed. Moreover, seminal doses were refrigerated, and sperm quality (motility and kinetic parameters, mitochondrial activity and morphology) was evaluated on days 1, 3 and 5 of storage.

TABLE 3 | Differences in ejaculate characteristics (sperm concentration, spz/ml), sperm quality parameters (normal spermatozoa, cytoplasmic droplet, abnormal tail) and seminal plasma (total antioxidant capacity) between CG and WGP groups.

Parameters	**D**	**$HPD_{95\%}$**	***p* (%)**
Concentration (10^6 spz/mL)	−19.5	−41.3; 3.1	96
Total abnormal spermatozoa (%)	8.5	−14.6; 31.7	77
Total normal spermatozoa (%)	−8.5	−32.7; 13.7	77
Cytoplasmic droplet (%)	5.1	−12.9; 23.2	71
Abnormal tail (%)	3.3	−8.4; 15.3	72
Total antioxidant capacity (mmol/L)	0.05	−0.10; 0.19	79

Abbreviations: $HPD_{95\%}$, highest posterior density at 95% of probability; *p* (%), probability of the difference being > 0 when D > 0 or being < 0 when D < 0.

2.10 | Statistical Analysis

The model used to analyse sperm concentration (sperm/ml), sperm morphology (normal spermatozoa, cytoplasmic droplets, abnormal tail) and TAC included the effects of treatment (CG and WGP groups), week (three levels), male as a random effect and the error.

For kinetic parameters and mitochondrial activity, the model included the fixed effect of treatment (CG and WGP groups), days of storage (1, 3, and 5), treatment × days of storage interaction, male as a random effect and the error.

Bayesian methodology was applied for all statistical analyses. Uniform bounded priors were used for all systematic effects, except for the male effect, which was normally distributed with a mean 0 and a variance $I\sigma^2_m$. The male and residual effects were considered independent. Residuals were normally distributed with a mean 0 and a variance $I\sigma^2_e$. The a priori variances were defined as flat-bounded. The marginal posterior distributions of the parameters of interest were estimated using Gibbs sampling. All analyses were performed using the Rabbit software developed by the Institute of Animal Science and Technology (Valencia, Spain). After some exploratory analyses, a chain of 60,000 iterations was generated, with a burn-in period of 10,000 iterations, and only one sample out of 10 was considered. Convergence was tested using Geweke's Z criterion, and Monte Carlo errors were obtained using the time series procedure.

3 | Results

Table 2 presents the descriptive analysis of ejaculate characteristics, sperm quality parameters and seminal plasma from both the CG and WGP groups. Sperm concentration (sperm/ml) was statistically greater in the WGP group compared to the CG ($p > 95\%$; Table 3). However, no significant differences were observed between groups for total abnormal spermatozoa, the presence of cytoplasmic droplets, abnormal tails or TAC ($p < 95\%$; Table 3).

Table 4 shows the descriptive statistics for sperm motility, kinematic parameters and mitochondrial activity in both groups (CG and WGP). All parameters showed a higher coefficient of variation (CV) in CG compared to the WGP group. Total motility,

TABLE 4 | Values of sperm motility (total and progressive motility), kinematic parameters and mitochondrial activity from both experimental groups (CG and WGP). Data are expressed as the mean, standard deviation (SD) and coefficient of variation (CV, %).

	CG ($n = 30$)			WGP ($n = 30$)		
Sperm descriptors	**Media**	**SD**	**CV (%)**	**Media**	**SD**	**CV (%)**
Total motility (%)	69.0	24.5	37.4	68.5	21.5	31.4
Progressive motility (%)	40.4	18.6	46.0	39.9	17.5	43.9
VCL (μm/s)	69.6	17.1	24.6	65.7	12.5	19.0
VSL (μm/s)	28.8	7.2	25.0	28.3	6.6	23.3
VAP (μm/s)	41.8	9.1	21.8	40.6	8.3	20.4
LIN (%)	42.4	11.6	27.4	44.1	11.0	24.9
STR (%)	67.8	9.9	14.6	68.8	9.1	13.2
WOB (%)	60.6	8.9	14.7	61.8	8.5	13.8
ALH (μm)	2.5	0.6	24	2.3	0.5	21.7
BCF (Hz)	6.8	1.2	17.6	6.8	0.9	13.2
Mitochondrial activity (%)	89.2	7.2	8.1	87.2	5.7	6.5

TABLE 5 | Differences in sperm motility (total and progressive motility), kinematic parameters, and mitochondrial activity from both experimental groups (CG and WGP).

Sperm descriptors	**D**	**$HPD_{95\%}$**	***p* (%)**
Total motility (%)	0.5	−12.2; 12.9	54
Progressive motility (%)	0.5	−8.9; 9.7	55
VCL (μm/s)	3.9	−6.2; 13.4	80
VSL (μm/s)	0.5	−3.7; 4.1	59
VAP (μm/s)	1.2	−3.3; 5.8	72
LIN (%)	−1.7	−9.2; 5.0	70
STR (%)	−1.0	−6.6; 4.6	65
WOB (%)	−1.2	−5.8; 3.5	71
ALH (μm)	0.2	−0.2; 0.5	81
BCF (Hz)	0.0	−0.5; 0.4	58
Mitochondrial activity (%)	2.0	−0.6; 4.9	93

Abbreviations: $HPD_{95\%}$, highest posterior density at 95% of probability; *p* (%), probability of the difference being > 0 when D > 0 or being < 0 when D < 0.

progressive motility, VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF and mitochondrial activity were similar between the CG and WGP groups ($p < 93\%$, Table 5).

Figures 1 and 2 show the interaction between the two groups (CG and WGP) at 1, 3, or 5 days of storage for sperm motility, kinematic parameters and mitochondrial activity. Total motility, progressive motility, VSL, VAP and WOB decreased over the days of storage in both groups. While storage time did not affect CG, day 5 of storage resulted in a lower VCL compared to days 1 and 3 for the WGP group. ALH was unaffected by either feeding treatment or storage time. LIN and STR showed different patterns based on feeding, decreasing from day 1 in the CG and from day 3 in the WGP group. BCF was lower on day 3 than on day 1 in the CG, with similar values between days 3 and 5. However, in the WGP group, BCF continued to decrease between days 3 and 5 of storage. Mitochondrial activity decreased from day 3 of storage, regardless of feeding treatment. Both CG and WGP groups displayed similar values on the same storage day, except for VSL and VAP, which were lower in the WGP group compared to the CG on day 5 of storage.

4 | Discussion

Although WGP supplementation had no effect on most sperm quality parameters, the results of the present study indicate that dietary supplementation with 100 g of WGP (4% of dietary feed) improves sperm concentration. These findings are consistent with previous studies conducted in other species such as rabbits (Eid 2008; Derbali et al. 2024) and humans (Silberstein et al. 2016). In rabbits, however, the effectiveness of GP supplementation varies between studies. While Eid (2008) reported improvements in several parameters (ejaculate volume, sperm concentration, plasma membrane integrity and sperm motility), Derbali et al. (2024) observed enhancements specifically in sperm concentration and motility. These discrepancies may be attributed to the different GP inclusion levels in the diets, which ranged from 1% to 2% (Derbali et al. 2024) to 10%–20% (Eid 2008). In our study, the WGP supplementation represented 4% of the diet (100 g of GP per 2.5 kg of feed), yielding results similar to those obtained in studies with lower GP percentages in rabbits. This suggests that increasing the GP content in the diet could further enhance reproductive parameters. It is important to highlight that increasing the percentage of this by-product in the diet had no significant effect on body weight gain, at least in rabbits (Eid 2008), which is relevant for boars, as excessive weight gain can reduce libido and lead to lameness issues during semen collection (Wang et al. 2016). In contrast to

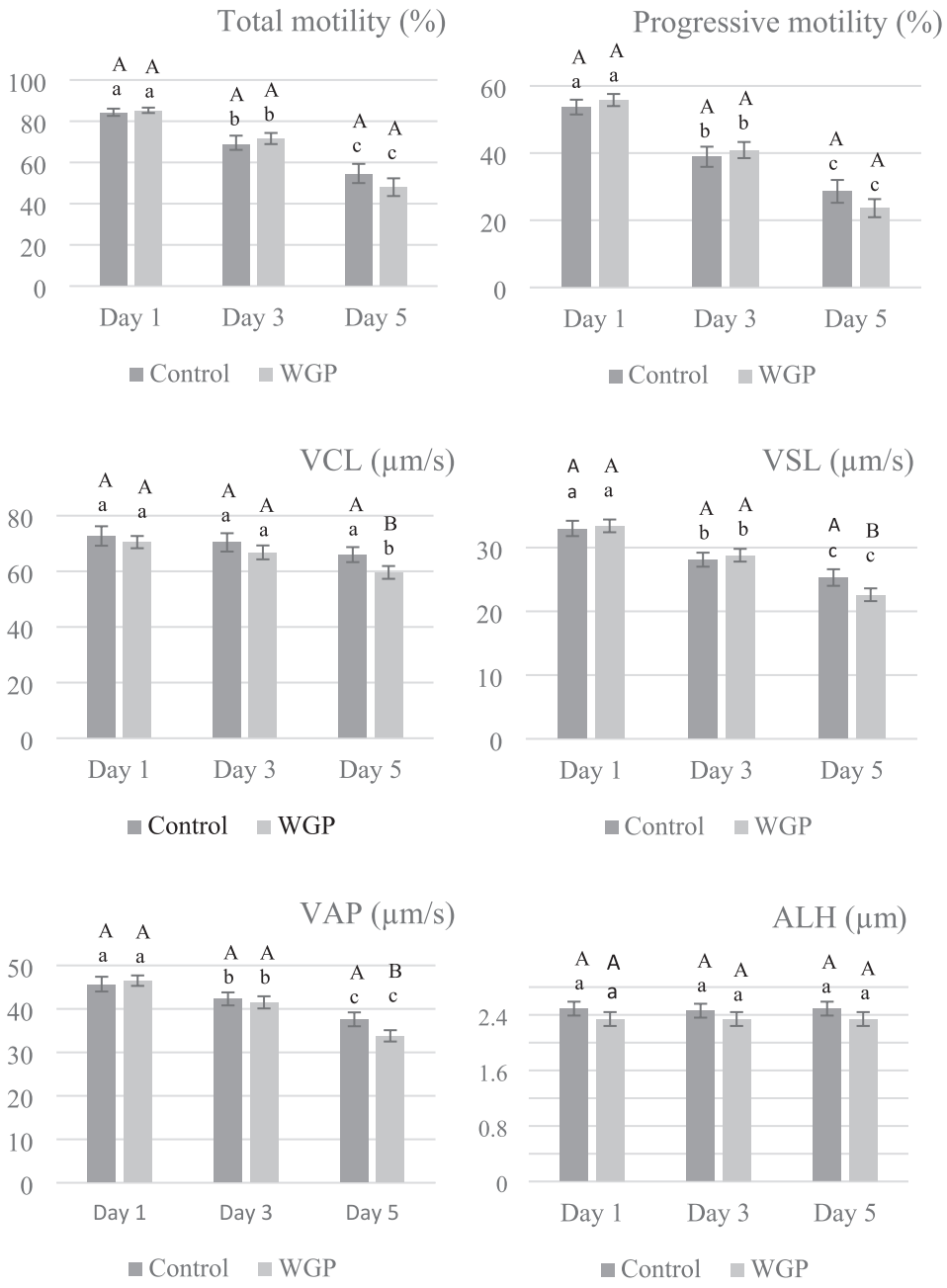

FIGURE 1 | Total motility, progressive motility, VCL, VSL, VAP and ALH values (mean ± standard error of the posterior marginal distribution) for treatment and storage day. Different capital letters (A, B) between treatments on the same day indicate that the probability of the difference between treatments being greater than 0 (if the difference is > 0) or less than 0 (if the difference is < 0) is higher than 90%. Different lowercase letters (a, b, c) between days for the same treatment indicate that the probability of the difference between treatments being greater than 0 (if the difference is > 0) or less than 0 (if the difference is < 0) is higher than 90%.

our study, the only previous report on WGP supplementation in boars did not observe any increase in sperm concentration with a similar supplementation regimen (Gloria et al. 2019). These mismatches could be attributed to differences in experimental design between studies or to variations in porcine breeds, as breed-specific factors have been shown to influence sperm quality when different dietary additives are used (Yeste et al. 2010, 2011). In our study, we used two separate, homogeneous groups of animals simultaneously (control and WGP-supplemented), whereas the previous study used the same animals throughout the entire experiment.

The increase in sperm concentration could be attributed to improved hormonal regulation of the hypothalamus-pituitary-testicular axis within the seminiferous tubule of the testicular parenchyma, resulting in a higher number of spermatozoa per millilitre of ejaculate. This hypothesis is based on the findings in female reproductive physiology, which state that grape seed extract and grape polyphenols positively influence oocyte quality and development, the regulation of reproductive hormones (GnRH, FSH, LH and steroid hormones), steroid hormone receptors, proliferation markers and apoptosis (Kohut et al. 2024). This finding is relevant, as an increase in sperm concentration

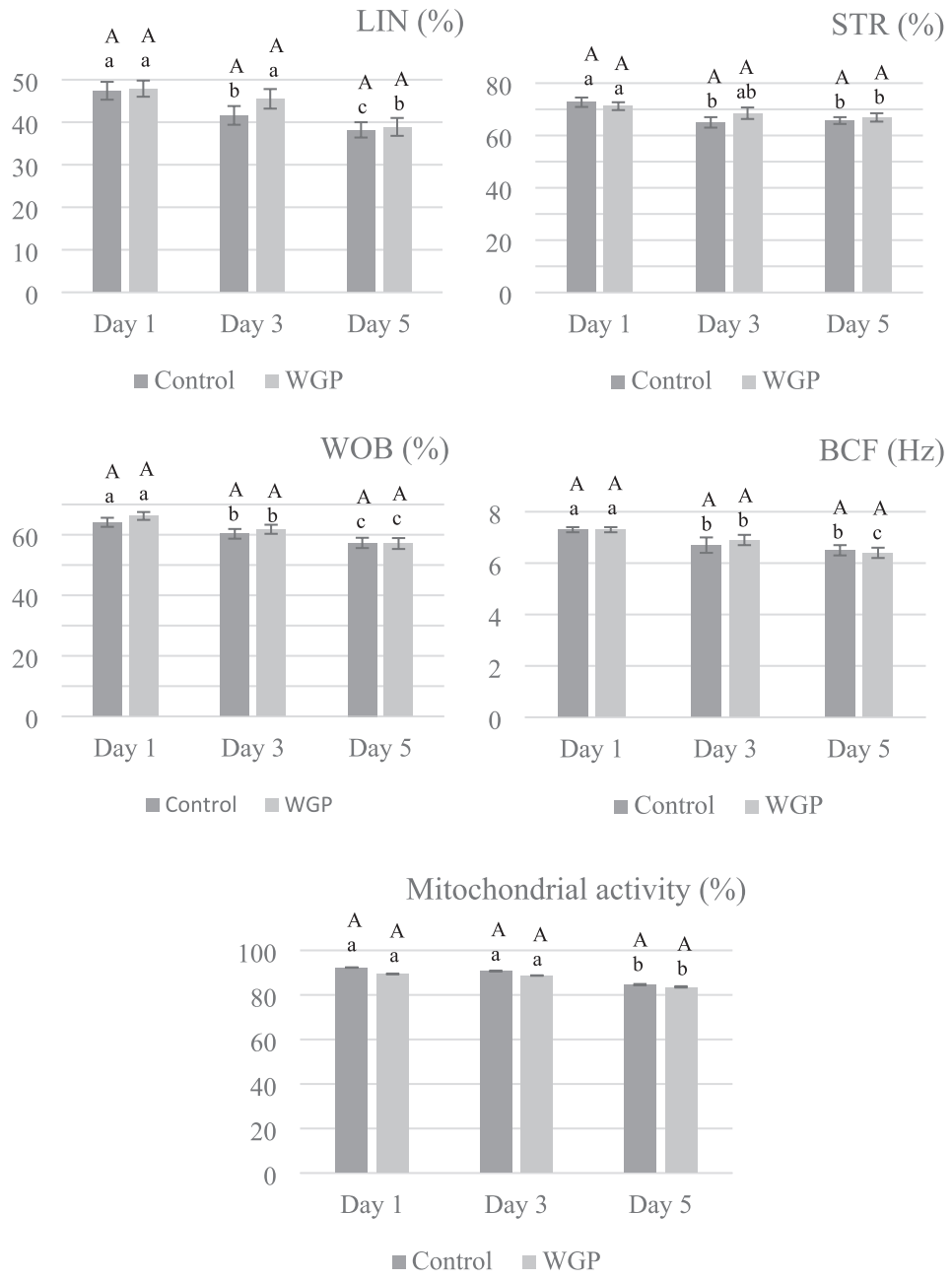

FIGURE 2 | LIN, STR, WOB, BCF and mitochondrial activity values (mean ± standard error of the posterior marginal distribution) for treatment and storage day. Different capital letters (A, B) between treatments on the same day indicate that the probability of the difference between treatments being greater than 0 (if the difference is > 0) or less than 0 (if the difference is < 0) is higher than 90%. Different lowercase letters (a, b, c) between days for the same treatment indicate that the probability of the difference between treatments is greater than 0 (if the difference is > 0) or less than 0 (if the difference is < 0) is higher than 90%.

leads to a higher number of seminal doses. In this experiment, an increase of ~4×10^9 sperm per ejaculate was observed in the WGP group, resulting in approximately two additional seminal doses per ejaculate (calculated as 2000×10^6 sperm per seminal dose). This results in the production of 156 additional doses per boar per year (calculated as 1.5 ejaculate per week × 52 weeks per year × 2 additional seminal doses). Considering the cost per seminal dose (3.9 €, Luongo et al. 2022), this could yield an additional profit of 608.4 € per boar annually from the sale of these extra doses. It should be noted that this improvement was not consistent across all boars, indicating individual variations that may be attributed to physiological and testicular status. Furthermore, it has been reported that there is a considerable variability in response among boars due to differences in sensitivity to dietary polyphenol supplementation (Gloria et al. 2019). The cost estimation of WGP after solid-state fermentation is around 275 € per tonne (Cebrián et al. 2024). If a boar is fed 100 g of WGP daily, the annual consumption would be 36.5 kg (100 g/day × 365 days). Given the price of 275 € per tonne, the cost per kilogramme is 0.275€. Therefore, feeding a boar for a year would incur a total cost of 10.04 € boar/year (36.5 kg × 0.275€/kg). Thus, this strategy could be considered cost-effective, as the benefits of increasing the number of seminal doses produced outweigh the cost of dietary supplementation.

Moreover, although not evaluated in the present study, WGP has shown nutritional benefits in the pig industry, particularly for improving gut microbiota health in piglets (Kafantaris et al. 2018). WGP's ability to modulate gut health and systemic immunity could benefit overall reproductive efficiency by enhancing the health and productivity of both sows and boars throughout the reproductive cycle. These combined effects highlight the potential of WGP as a cost-effective dietary supplement for optimising reproductive outcomes in the pig industry.

5 | Conclusions

The results of this study indicate that supplementation with WGP in boar diets improves sperm concentration without negatively affecting other seminal parameters. This finding is particularly significant given the effect of sperm concentration on the profitability of swine semen production centres and the swine industry, combined with the low cost of WGP. These results encourage further research to evaluate different WGP concentrations and supplementation protocols to optimise its use and maximise reproductive performance.

Author Contributions

A.Q.M., C.M.-L., C.L., F.A.G.V., P.J.L.L., J.R.D.: methodology. M.L.G., M.J.A., G.R.: investigation and statistical analysis. C.P.P.: investigation. J.R.D.: conceptualization. A.Q.M., F.A.G.V.: wrote the initial draft. F.A.G.V., P.J.L.L.: Supervision. P.J.L.L.: Funding acquisition. All authors have approved the final draft of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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