

Effects of supranutritional organic selenium with vitamin E on antioxidant status and reproductive traits in Wagyu heifers

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ABSTRACT. Oxidative stress during the periparturient period can impair immune and reproductive functions in cattle. Thus, antioxidant supplements such as selenium (Se) and vitamin E (VE) are increasingly used to support redox balance. However, evidence regarding the effects of combined Se–VE supplementation on gestation length, particularly in beef breeds such as Wagyu, remains limited. This study evaluated supranutritional supplementation with organic Se yeast (Sel-Plex 2000) and VE in pregnant Wagyu heifers ($n = 24$) fed from approx. 100 days of prepartum to 7 days of postpartum. Treatments included a basal diet (CON), T1 (0.5 mg Se/kg dry matter (DM) + 800 IU VE/kg DM), and T2 (1.0 mg Se/kg DM + 1 600 IU VE/kg DM). Whole-blood Se, serum α -tocopherol, whole-blood glutathione peroxidase (GPx), and erythrocyte superoxide dismutase (SOD) were measured at day 7 and 60 after starting supplementation, and at day 7 of postpartum. Gestation length was the primary reproductive endpoint, while age at first calving, inter-calving period, and service period were also recorded. Compared with CON, both supplemented diets increased whole-blood Se and serum α -tocopherol levels (diet $P \leq 0.014$), and elevated GPx activity throughout the trial (diet $P \leq 0.019$). A significant diet \times day interaction was observed for SOD activity ($P < 0.001$), indicating a time-dependent response to supplementation. Gestation length decreased in a dose-dependent manner (least square means 284.25, 276.63, and 270.38 days for CON, T1, and T2, respectively; $P < 0.001$), whereas other reproductive intervals were not affected ($P > 0.05$). In conclusion, organic Se–VE supplementation during late-gestation improved systemic antioxidant status and moderately shortened gestation length in Wagyu heifers, supporting its use to improve periparturient redox balance and optimise calving management.

Introduction

Oxidative stress is a major physiological challenge during the periparturient period, because rapid changes in metabolism, immune function, and endocrine status increase the generation of reactive oxygen species (ROS). When antioxidant defences are insufficient, ROS can damage lipids, proteins, and DNA, contributing to immune dysfunction, periparturient disorders, and reduced reproductive

performance (Spears and Weiss, 2008; Sordillo, 2016; Xiao et al., 2021). Consequently, antioxidant supplementation, such as selenium (Se), vitamin E (VE), vitamin C, and other bioactive compounds, has become a common strategy to support ruminant health under metabolic or environmental stress. Recent studies in heat-stressed ruminants have demonstrated that commercial antioxidant formulations, such as Se–VE combinations and trace mineral blends, as well as, vitamin C-containing

phytobiotics, and plant-derived extracts, can reduce oxidative damage, improve immune and reproductive function, and support productive performance (Ullah et al., 2020; Salles et al., 2022; Martínez et al., 2022; Chen et al., 2023; Guo et al., 2023; Purba et al., 2025).

Nevertheless, most available evidence relates to dairy cows, and information regarding the effects of targeted antioxidant strategies on reproductive traits in beef breeds such as Wagyu remains limited. Given the distinct growth patterns and metabolic characteristics of Wagyu cattle, it is important to determine whether combined Se–VE supplementation can improve antioxidant status and reproductive performance in Wagyu, with potential implications for both herd health and productivity.

Selenium and VE are complementary components of the antioxidant defence system. Selenium is an essential constituent of selenoproteins, including glutathione peroxidases (GPx), which detoxify hydrogen peroxide and lipid hydroperoxides in aqueous compartments, whereas VE acts as a lipid-soluble, chain-breaking antioxidant in biological membranes. Numerous studies have shown that improving Se–VE status reduces the incidence of retained foetal membranes (RFM), improves udder health, and subsequent fertility (Weiss et al., 1997; Smith et al., 1997; Bourne et al., 2007; Pontes et al., 2015). Low periparturient serum α -tocopherol levels have been associated with an increased disease risk (LeBlanc et al., 2004), and meta-analytic research confirms the benefits of VE supplementation for udder health and milk performance in transition cows (Moghimi-Kandelousi et al., 2020). Selenium and VE also interact synergistically: VE scavenges lipid radicals in membranes, while Se-dependent GPx reduces the resulting lipid hydroperoxides, thereby preserving membrane integrity and limiting excessive VE oxidation (Xiao et al., 2021). This biochemical interaction provides a rationale for combined Se–VE supplementation rather than increasing the dietary supply of Se or VE individually.

The biological effects of Se depend on its chemical form and rumen metabolism. Inorganic Se sources, such as selenite and selenate, are readily reduced by rumen microorganisms, whereas organic Se supplied as selenised yeast is mainly incorporated into body proteins as selenomethionine, improving Se retention and transfer to the offspring (Abdelrahman and Kincaid, 1995; Xiao et al., 2021). Experimental work in ruminants and other livestock indicates that Se–yeast supplementation generally results in higher blood and tissue Se

concentrations and a more favourable antioxidant status than inorganic Se supplied at comparable doses (Bourne et al., 2007; Smith et al., 1997; Weiss et al., 1997). Similarly, the physiological activity and stability of VE depend on its chemical form. All-rac- α -tocopheryl acetate is commonly used in ruminant nutrition because it is substantially more stable in premixes and total mixed rations than free α -tocopherol, while still effectively increasing circulating α -tocopherol levels (Brzezinska-Slebodzinska et al., 1994; Xiao et al., 2021). Recent studies in lambs fed lipid-rich diets have further indicated that antioxidant efficacy and tissue tocopherol profiles depend not only on dosage but also on the specific form and combinations of Se and VE administered (Białek et al., 2021; 2022).

In line with this evidence, the current study applied supranutritional organic Se as Se-enriched *Saccharomyces cerevisiae* (Sel-Plex 2000) and VE in the form of all-rac- α -tocopheryl acetate. Although controlled supplementation trials in Wagyu are limited, a case report from a stud Wagyu herd in New South Wales associated severe Se deficiency with poor performance and an outbreak of bovine respiratory disease, with clinical improvement following combined Se and vitamin A–D–E treatment. This observation highlights the importance of adequate Se supply in this breed (Davis and Gardiner, 2011; Li et al., 2014).

The target Se concentrations (0.5 and 1.0 mg Se/kg dry matter (DM)) were intentionally set above nutritional requirements, but remained within established safety and regulatory limits, in order to test whether supranutritional Se–VE supply can further strengthen antioxidant protection in late-gestation heifers. This combination was selected to provide a chemically stable dietary source of VE together with a highly bioavailable organic Se source, with the expectation of synergistic effects through increased Se incorporation into GPx and improved protection of membrane lipids by VE. Although Se–VE supplementation during the periparturient period has been shown to reduce the incidence of RFM and support postpartum reproductive performance in dairy cows (Julien et al., 1976; Bourne et al., 2007; Pontes et al., 2015), relatively few trials have evaluated its effect on gestation length itself. Moreover, at least one study using prepartum Se–VE injections reported no effect on gestation length despite favourable impact on udder health (Moeini et al., 2009). Furthermore, data for Wagyu heifers, particularly under Central European management conditions, are almost entirely lacking.

Therefore, it was hypothesised that supranutritional organic Se yeast (0.5–1.0 mg Se/kg DM), combined with high-dose vitamin E from late gestation to early postpartum, would increase blood Se and serum α -tocopherol concentrations, GPx and superoxide dismutase (SOD) activities, as well as reproductive performance, with particular emphasis on a reduction in gestation length in Wagyu heifers.

Material and methods

Animals, diets, and experimental design

The experiment was conducted at the experimental facility of the Research Institution Agrovyz-kum Rapotin in the Czech Republic. All animal procedures were reviewed and approved by the Ministry of Agriculture of the Czech Republic (protocol No. 14608/2021-MZE-18134).

Animals were housed in an insulated free-stall barn with separate sections for each experimental group, and each group had access only to its assigned diet. Water was provided *ad libitum*, and diets were formulated to meet energy and nutrient requirements according to NRC (2016) recommendations.

Before the start of the experiment, 36 Wagyu heifers (17.9 ± 0.8 months of age; 471.2 ± 28.3 body weight) were inseminated using a synchronisation protocol presented in Table 1.

Table 1. Synchronization protocol used for artificial insemination of Wagyu heifers

Day 0	Hormone application (Oestrophan 0.25 mg/ml)
Day 11	Hormone application (Oestrophan 0.25 mg/ml)
Day 14	Insemination
Day 17	Re-insemination

The artificial insemination (AI) procedure, preceded by a routine veterinary examination, was performed using semen from two state-registered Japanese Black Wagyu bulls (Itofuji Wag-12 and Hisamoto Wag-25), selected according to the established mating plan. Pregnancy was confirmed by transrectal ultrasonography 40 days after AI. Based on these results, 24 of the 36 heifers were diagnosed as pregnant, corresponding to an insemination success rate of 66.7%.

Successfully inseminated and fertilised heifers ($n = 24$) were randomly assigned to one of three dietary treatment groups ($n = 8$), differing in selenium and vitamin E supplementation levels. The control group (CON) was fed the basal diet (BD) as a total mixed ration (TMR) without additional Se–VE

premix. Selenium in the supplemented diets was provided as Sel-Plex 2000, an organic Se source derived from Se-enriched, inactivated *S. cerevisiae* CNCM I-3060, and VE was supplied as all-rac- α -tocopheryl acetate. The first group (Diet 1; T1) received the BD supplemented with 150 g Sel-VE premix/animal per day, formulated to achieve total dietary concentrations of 0.5 mg Se/kg DM and 800 IU VE/kg DM in the complete TMR. The second treatment group (Diet 2; T2) received the BD supplemented with 300 g Sel-VE premix/animal per day, formulated to obtain a total concentration of 1.0 mg Se/kg DM and 1 600 IU VE/kg DM in the complete TMR.

Selenium and VE supplementation was administered for 100 days before the expected calving date and continued until 7 day postpartum. Both Se levels used in the present study (0.5 and 1.00 mg Se/kg DM) exceeded the nutritional (supranutritional) nutritional requirement for cattle (≈ 0.10 mg/kg DM) (NRC, 2016) and the currently authorised maximum contents in the European Union of 0.5 mg total Se/kg complete feed and 0.2 mg supplemented organic Se/kg complete feed (EFSA, 2016; 2024; EU Regulation 2022/1459). Nevertheless, the higher experimental dose applied in the present study (1.0 mg/kg DM) was still below the maximum tolerable level for ruminants, estimated at approximately 5 mg Se/kg DM (EFSA FEEDAP Panel, 2016; Salles, 2017). Additionally, in Wagyu cows, lower serum Se concentrations have been associated with an increased risk of stillbirth (Uematsu et al., 2016), suggesting that this breed may have distinct patterns of Se deposition and sensitivity to Se supply in relation to reproductive outcomes. In this context, evaluation of supranutritional Se–VE supplementation in Wagyu heifers, including a high experimental dose (1.0 mg Se/kg DM), is scientifically justified to better characterise the dose–response relationship in this genetic type.

All three diets were offered as total mixed rations in controlled daily amounts. The daily ration was divided into two equal portions and delivered at 07:00 and 17:00. The Se–VE premix (Sel-VE) was weighed separately for each treatment and manually added to the morning portion immediately before feeding to ensure complete consumption of the supplement. The daily amounts of TMR and Sel-VE premix provided in each treatment are specified in Table 2. The ingredient composition, and calculated nutrient profile of the experimental diets, together with the chemical characteristics of the feed ingredients and the total mixed ration, are presented in Tables 2, 3, and 4, respectively.

Table 2. Ingredient composition of the experimental diets

Ingredients	CON		T1		T2	
	% of DM	kg DM, as-fed	% of DM	kg DM, as-fed	% of DM	kg DM, as-fed
Meadow hay	20.51	8.00	20.43	8.00	20.85	8.00
Alfalfa silage	20.51	8.00	20.43	8.00	20.26	8.00
Corn silage	38.46	15.00	38.31	15.00	37.90	15.00
GFM	20.51	8.00	20.43	8.00	20.23	8.00
Premix Sel-VE ¹	0.00	0.00	0.38	0.15	0.76	0.30
Total	100	39.00	100	39.15	100	39.30

CON – basal diet without selenium–vitamin E (Se–VE) premix, T1 – basal diet formulated to 0.5 mg Se/kg dry matter (DM) and 800 IU VE/kg DM, T2 – basal diet formulated to 1.0 mg Se/kg DM and 1 600 IU VE/kg DM; GFM – granulated feed mixture BIOSTAN (Biokron, s.r.o., Czech Republic), quantity per kg of product: %: barley 13.3, oat mill feed 57.5, wheat 13.3, malt flour 8.8, sunflower expellers 4.4, soybean meal 2.7. ¹ premix Sel-VE (MIKROP CEBIN, a.s., Czech Republic) contained organic selenium from selenium-enriched, inactivated *Saccharomyces cerevisiae* CNCM I-3060 (Sel-Plex) and vitamin E (all-rac- α -tocopheryl acetate) on a wheat carrier

Analyses

Samples of the TMR and feed ingredients (Table 2) were collected to analyse DM content and nutrient composition. Chemical analyses were performed at the Department of Animal Nutrition and Forage Production, Mendel University, Brno, Czech Republic. The chemical composition of all diets (Table 3) was determined for dry matter, crude protein, crude fat, crude fibre, and ash contents, following the guidelines of the EC Commission Regulation (2009). The concentrations of acid detergent fibre and neutral detergent fibre were determined sequentially using an ANKOM A200 Fibre Analyzer (ANKOM Technology, Macedon, NY, USA) according to the manufacturer's instructions and methods described by Van Soest et al. (1991). Starch concentration was analysed using the Ewers polarimetric method, based on partial acid hydrolysis of starch followed by measurement of the optical rotation of the hydrolysate, following STN EN ISO 10520 (1997).

Table 3. Chemical composition of diets

Nutrient composition, %	CON	T1	T2
Dry matter	92.96	92.95	92.96
Crude protein	9.54	9.55	9.55
Ash	5.47	5.48	5.49
Crude fibre	17.51	17.46	17.49
Fat	2.28	2.28	2.27
Starch	15.73	15.71	15.60
Cellulose	21.49	21.48	21.53
NDF	35.36	35.30	35.39
ADF	24.60	24.58	24.62

CON – basal diet without selenium–vitamin E (Se–VE) premix, T1 – basal diet formulated to 0.5 mg Se/kg dry matter (DM) and 800 IU VE/kg DM, T2 – basal diet formulated to 1.0 mg Se/kg DM and 1 600 IU VE/kg DM; NDF – neutral detergent fibre, ADF – acid detergent fibre

To estimate baseline Se and VE concentrations in the feed, determine the required dietary inclusion levels, and monitor Se and VE supply throughout the experiment, Se and VE contents were determined in the TMR and each feed ingredient before the start of the experiment and at 2-week intervals thereafter. Selenium was measured after microwave-assisted nitric acid digestion, in accordance with EN 13805:2014 (2014) and quantified by inductively coupled plasma–mass spectrometry (ICP-MS) according to ISO 17294-2:2016 (2016). Reproductive performance was assessed using five key indicators: (1) age at first calving (AFC, year), calculated from birth to first calving; (2) gestation length (day), defined as the interval from successful AI to calving; (3) service period (day), calculated as the number of days from calving to the subsequent successful insemination; (4) and inter-calving period (ICP, day), defined as the interval between two consecutive calvings. Reproductive records, including dates of oestrus detection, AI, pregnancy diagnosis, and calving, were obtained from herd management software. All intervals were calculated on a calendar-day basis.

To assess the effects of organic Se and VE supplementation on antioxidant status in Wagyu heifers before and after parturition, blood samples ($n = 72$) were collected during supplementation with Se and VE (days 7 and 60), and 7 days after calving. Blood was sampled from the tail vein using a disposable vacuum blood collection tube. Samples for whole blood Se, GPx, and SOD analyses were collected into heparinised tubes (HEMOS H-02, GAMA Group, Czech Republic). For serum VE analysis, blood was collected into tubes without anticoagulant (HEMOS H-02; GAMA Group, Czech Republic).

For serum preparation, blood samples were allowed to clot and were incubated at room temperature until serum separation, after which they were centrifuged at 3 000 rpm for 10 min.

Table 4. Chemical composition of feed ingredients

Nutrient composition	Meadow hay	Alfalfa silage	Corn silage	GFM ¹	Premix Sel-VE ²	TMR
Dry matter (DM), % of as-fed	94.37	91.67	93.67	91.56	92.00	92.96
Crude protein, % of DM	6.84	16.27	6.95	10.36	13.40	9.54
Ash, % of DM	5.87	9.70	3.74	4.10	7.70	5.47
Crude fibre, % of DM	29.47	21.80	15.51	5.00	5.61	17.51
Fat, % of DM	1.22	2.06	3.07	2.06	2.55	2.28
Starch, % of DM	4.80	0.25	38.15	0.11	8.80	15.73
Cellulose, % of DM	31.73	23.56	17.80	16.10	17.89	21.49
NDF, % of DM	59.55	32.89	31.44	21.00	19.60	35.36
ADF, % of DM	36.11	28.58	19.86	18.01	17.51	24.60
Initial Se, mg/kg DM ³	0.05	0.07	0.01	0.02	-	0.08
Se, mg/kg DM ⁴	0.04 ± 0.001	0.06 ± 0.005	0.02 ± 0.001	0.03 ± 0.001	-	0.09 ± 0.01

¹ GFM – granulated feed mixture (Biostan, s.r.o., Czech Republic). Example batch composition (% of product): barley 13.3, oat mill feed 57.5, wheat 13.3, malt flour 8.8, sunflower expellers 4.4, soybean meal 2.7 (sums to 100%); ² premix Sel-VE (MIKROP CEBIN, a.s., Czech Republic) contained organic selenium from selenium-enriched, inactivated *Saccharomyces cerevisiae* CNCM I-3060 (Sel-Plex) and vitamin E (all-rac- α -tocopheryl acetate) on a wheat carrier; ³ selenium concentration in total mixed ration (TMR) and feed ingredients before the start of the experiment; ⁴ selenium concentration in TMR and feed ingredients throughout the experimental period. Results are presented as mean ± standard error of the mean (SEM); NDF – acid detergent fibre; ADF – neutral detergent fibre

Serum samples were either analysed immediately or stored at -70°C . For plasma preparation, whole blood was centrifuged at 3 000 rpm for 10 min, and plasma samples were stored at -70°C until analysis. Concentrations of selected markers of antioxidant status including Se, VE, GPx, and SOD were determined in a total of 72 blood samples. The activities of the antioxidant enzymes GPx and SOD were directly affected by dietary Se supplementation.

GPx activity in whole blood was determined using a RANSEL kit (Randox Laboratories Ltd., Crumlin, UK) and a UV method based on the procedure of Paglia and Valentine (1967). The assay measures the decrease in absorbance at 340 nm resulting from NADPH oxidation catalysed by glutathione reductase (GR). GPx activity was analysed using a Konelab 20XT automatic biochemical analyser (Thermo Fisher Scientific, Vantaa, Finland). Whole-blood Se concentration was determined by hydride generation atomic absorption spectrometry (HG AAS) (SOLAAR, Thermo Fisher Scientific, Waltham, MA, USA). Samples were prepared by mineralisation with HNO_3 and H_2O_2 using an ETHOS TOUCH CONTROL microwave digestion system (Milestone, Sorisole, Italy), followed by evaporation. The activity of SOD in erythrocyte lysates was measured using a commercially available kit (Randox Laboratories Ltd., UK) and analysed with a Cobas Mira Plus automatic biochemical analyser (Roche, Switzerland).

Serum VE (alpha-tocopherol) concentration was determined using the method described by McMurray and Blanchflower (1979) with minor modifications. Briefly, serum samples were deproteinised with ethanol and extracted with hexane.

The resulting organic phase was evaporated, and the dried residue was reconstituted in methanol before analysis. High-performance liquid chromatography (HPLC) was performed using an Ultimate 3000 system (Thermo Fisher Scientific, Sunnyvale, CA, USA). Chromatographic separation was carried out on an ACCLAIM 120 C18 column (3 μm , 120 \AA , 4.6 \times 100 mm) with methanol as the mobile phase at a flow rate of 1.2 ml/min. A 20 μl sample was injected, the column was maintained at 30°C , and fluorescence detection was used with excitation and emission wavelengths of 292 and 325 nm, respectively.

Chemicals and standards

All reagents were of analytical grade. Calibration of hydride-generation AAS for selenium determination was performed using certified aqueous selenium standards. Quantification of serum α -tocopherol by HPLC was based on external calibration with certified α -tocopherol standards prepared in methanol. Commercial kits for GPx (RANSEL) and SOD were supplied by Randox Laboratories Ltd. (Crumlin, UK). Nitric acid and hydrogen peroxide used for mineralisation prior to Se analysis were of suprapure grade.

Statistical analysis

Statistical analyses were performed using STATISTICA software (TIBCO Software Inc., Palo Alto, CA, USA). Antioxidant biomarkers SOD, GPx, whole-blood Se, and serum α -tocopherol were analysed using linear mixed models with diet and time relative to supplementation (7 and 60 days after supplementation and 7 day postpartum) as

fixed effects, including their interaction, and animal as a random effect. No additional covariates were included, as heifers did not differ between treatments in age or body weight at the beginning of the trial. Continuous reproductive traits, i.e., gestation length, service period, ICP, and AFC, were analysed using one-way ANOVA, with diet as the main factor. Results are presented as least squares means \pm standard error of the mean (SEM). Differences were considered statistically significant at $P \leq 0.05$, and trends were noted when $0.05 < P \leq 0.10$.

Results

Reproductive performance

Table 5. Effects of Se and vitamin E supplementation on reproductive indices of Wagyu heifers. Least squares means (LSMs) and standard error of the mean (SEM), $n = 24$

Items	Diets			SEM	P-value
	CON	T1	T2		
Gestation length, days	284.25 ^a	276.63 ^b	270.38 ^c	1.52	<0.001
AFC, years	2.27	2.21	2.17	0.05	0.38
ICP, days	363.5	362.00	358.88	3.25	0.59
Service period, days	50.25	51.63	53.00	1.11	0.23

CON – control diet without added selenium and vitamin E premix, T1 – diet contains 0.5 mg/kg dry matter (DM) of selenium and 800 IU of vitamin E, T2 – diet contains 1.0 mg/kg DM of selenium and 1 600 IU of vitamin E., D – diet effect; AFC – age at first calving, ICP – inter-calving period; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Dietary supplementation with Se and VE significantly affected gestation length ($P < 0.001$; Table 5). Heifers in the supplemented groups had shorter gestation periods than those in the control group. The average gestation length was 284.25, 276.63, and 270.38 days for the CON, Diet 1 (0.5 mg Se/kg DM + 800 IU vitamin E), and Diet 2 (1.0 mg Se/kg DM + 1 600 IU VE) treatments, respectively. Post hoc Tukey HSD tests confirmed that all pairwise differences were significant (CON > Diet 1 > Diet 2; $P < 0.05$). These findings indicate a dose-dependent reduction in gestation length with increasing Se and VE supplementation, suggesting that improved antioxidant status may influence parturition timing in Wagyu heifers. In contrast, AFC, ICP, and service period were not affected by the diet ($P > 0.05$), indicating that supranational Se and VE supplementation shortened gestation length without altering other reproductive interval traits in Wagyu heifers.

Effects of selenium–vitamin E on antioxidant biomarkers

Dietary Se–VE supplementation increased systemic selenium and α -tocopherol levels and modified antioxidant enzyme activities (Table 6). Seven days after the start of supplementation, Se concentrations were higher in T1 than in CON, with T2 showing intermediate values ($P = 0.014$). By day 60, both supplemented groups exceeded CON ($P = 0.001$), and at day 7 postpartum, a clear dose-related pattern was observed ($P < 0.001$). VE showed a similar pat-

Table 6. The effect of selenium (Se) and VE supplementation on the antioxidant status of Wagyu heifers. Least squares means (LSMs) and standard errors of the mean (SEM), $n = 72$

Items	Diets			SEM	P-value		
	CON	T1	T2		D	SD ²	D x SD ²
7 days after supplementation							
Se, $\mu\text{g/l}$	166.29 ^b	249.13 ^a	232.44 ^{ab}	19.01	0.014	0.50	0.20
VE, $\mu\text{mol/l}$	2.45 ^b	3.66 ^{ab}	3.97 ^a	0.34	0.012	<0.001	0.16
SOD, U/ml	180.86 ^b	284.65 ^a	296.83 ^a	7.44	<0.001	<0.001	<0.001
GPx, $\mu\text{kat/l}$	690.16 ^b	817.90 ^{ab}	916.99 ^a	51.94	0.019	0.007	0.52
60 days after supplementation							
Se, $\mu\text{g/l}$	154.34 ^b	212.10 ^a	237.91 ^a	13.32	0.001		
VE, $\mu\text{mol/l}$	4.57	4.77	4.95	0.25	0.57		
SOD, U/ml	211.65	199.81	202.87	7.95	0.028		
GPx, $\mu\text{kat/l}$	765.19 ^b	946.89 ^a	1031.13 ^a	38.76	<0.001		
7 days postpartum							
Se, $\mu\text{g/l}$	154.01 ^c	212.10 ^b	270.38 ^a	14.14	<0.001		
VE, $\mu\text{mol/l}$	3.77 ^b	4.77 ^a	5.44 ^a	0.26	0.001		
SOD, U/ml	204.97	199.81	204.38	8.00	0.88		
GPx, $\mu\text{kat/l}$	741.66 ^b	946.89 ^a	1018.96 ^a	37.94	<0.001		

CON – control diet without added Se and vitamin E premix; T1 – diet contains 0.5 mg/kg DM of Selenium and 800 IU of vitamin E; T2 – diet contains 1.0 mg/kg DM of Selenium and 1600 IU of vitamin E; ¹ For each biomarker, sampling day (SD) and the diet \times sampling day interaction (D \times SD) P-values are identical across the three day-specific rows. They are therefore displayed only on the first row (7 days after supplementation); D – diet effect; SD – sampling day effect; S \times D – diet and sampling day effect; VE – vitamin E; SOD – superoxide dismutase; GPx – glutathione peroxidase. ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

tern, with higher concentrations in T2 compared to CON at 7 day after supplementation ($P = 0.012$) and greater values in both supplemented groups postpartum ($P = 0.001$). Superoxide dismutase (SOD) activity was significantly higher in supplemented heifers 7 days after supplementation ($P < 0.001$), but differences between groups declined by day 60 (diet $P = 0.028$) and were no longer evident postpartum (diet $P = 0.88$). Correspondingly, both sampling day and the diet \times sampling day interaction were significant for SOD ($P < 0.001$). GPx activity remained higher in supplemented groups at all time points (e.g., 690.16, 817.90, 916.99 $\mu\text{kat/l}$ at 7 days after supplementation; diet $P = 0.019$), with a significant effect of sampling day ($P = 0.007$) but no diet \times day interaction ($P = 0.52$). Overall, Se–VE supplementation increased Se and α -tocopherol levels and sustained elevated GPx activity, while SOD response was transient and varied over time.

Discussion

This study investigated whether feeding supra-nutritional levels of organic Se and VE from 100 days before calving to 7 days postpartum would improve systemic antioxidant status and reproductive performance in Wagyu heifers. We hypothesised that combined Se–VE supplementation would increase Se, α -tocopherol concentrations as well as antioxidant enzyme activities, and positively affect key reproductive traits through an improved redox balance.

The present results partially confirmed this hypothesis as both supplemented diets increased blood Se and α -tocopherol levels and caused a sustained increase in GPx activity. On the other hand, SOD showed a transient, time-dependent response. Gestation length was markedly shortened in a clear dose-dependent manner.

From a reproductive standpoint, the shorter gestation observed in both Se–VE treatments indicates that improved antioxidant status during late gestation can influence the timing of parturition. Selenium and VE act in complementary compartments: Se through selenoproteins such as GPx in aqueous phases, and VE as a lipid-phase chain-breaking antioxidant in cell membranes. Together, they reduce oxidative damage in reproductive tissues and support immune and uterine function (Xiao et al., 2021).

Large field and herd-level studies in dairy cows have shown that targeted Se/VE supplementation around calving reduces the incidence of RFM and improves subsequent fertility, indicating

better uterine health and more efficient postpartum recovery (Bourne et al., 2007; Pontes et al., 2015; Chen et al., 2023). Observational studies have also associated lower periparturient α -tocopherol levels with increased disease risk and impaired reproductive performance (LeBlanc et al., 2004; Moghimi-Kandelousi et al., 2020). The present results extend this evidence by indicating that an elevated Se–VE status may also modulate gestation length in beef-type animals, although no effects were observed for other reproductive intervals.

In contrast, several studies have reported no effect of antioxidant supplementation on gestation length. For example, prepartum Se/VE injections in heifers improved periparturient health but did not alter gestation length (Kim et al., 1997; Moeini et al., 2009). Such inconsistencies may be explained by differences in Se source (organic yeast versus injectable or inorganic forms), dosage, duration of administration, breed, and baseline Se/VE status.

Organic Se supplied as selenised yeast is incorporated into body proteins as selenomethionine and generally results in higher maternal and foetal Se levels than some inorganic forms provided at comparable dietary concentrations (Abdelrahman and Kincaid, 1995; Xiao et al., 2021).

These carry-over effects correspond to the gradual increase in blood Se concentration and GPx activity observed across sampling days in the current trial. Such changes may contribute to improved placental function and foetal development.

Mechanistically, improved antioxidant capacity may affect multiple pathways involved in the initiation of parturition. Oxidative stress has been linked to changes in placental steroid production, prostaglandin synthesis, and foetal–maternal cortisol signalling, all of which play a role in the regulation of labour timing (Spears and Weiss, 2008; Sordillo, 2016). By limiting oxidative damage in placental and uterine tissues, Se–VE supplementation could normalise endocrine signalling and uterine contractions, thereby influencing the timing of calving in heifers, particularly under conditions predisposing to prolonged gestation. In parallel, an increased maternal Se supply facilitates foetal Se accumulation and colostrum transfer (Abdelrahman and Kincaid, 1995), potentially helping the foetus prepare for birth. However, as endocrine or placental biomarkers were not assessed in this study, these mechanisms remain hypothetical and require further targeted research.

Regarding antioxidant biomarkers, dietary Se and VE consistently increased blood Se and α -tocopherol levels and elevated GPx activity

across all sampling days, indicating a sustained enhancement of systemic antioxidant capacity.

This pattern is biologically plausible given the key roles of Se-dependent GPx and VE in detoxifying hydrogen peroxide and lipid hydroperoxides. It is consistent with classic studies showing improved antioxidant status and reduced markers of periparturient disorders in transition cows receiving combined Se–VE supplementation (Brzezinska-Slebodzinska et al., 1994; Smith et al., 1997; Weiss et al., 1997). Conversely, the SOD response was strongly time-dependent, with treatment differences evident during supplementation but diminishing by 60 days and absent postpartum. This pattern indicates dynamic redox adjustments during late gestation and early lactation, rather than a uniform upregulation of all antioxidant pathways, likely reflecting temporal changes in oxidative and inflammatory load, reactive oxygen species production, and immune-metabolic interactions during the transition period (Spears and Weiss, 2008; Sordillo, 2016).

Importantly, Se, VE, and GPx showed consistent dietary effects across sampling days, indicating that these components of the Se–VE–dependent antioxidant network responded in a coordinated manner to the supplemented doses under the present physiological conditions. This coordinated response aligns with the functional interactions described by Xiao et al. (2021), whereby VE scavenges lipid radicals within membranes, while Se-dependent GPx reduces the resulting lipid hydroperoxides, thereby restoring membrane integrity and preventing VE from excessive oxidation. The increases in α -tocopherol and GPx observed here are comparable to those reported in lambs receiving Se and VE, where changes in tocopherol distribution and improved antioxidant status were associated with reduced lipid oxidation in tissues (Białek et al., 2021; 2022).

A key limitation of the present study is the absence of direct markers of lipid peroxidation, such as malondialdehyde (MDA) or related thiobarbituric-acid-reactive substances, which are widely used to quantify oxidative damage and assess the effectiveness of antioxidant interventions (Białek et al., 2021; 2022). Consequently, it is not possible to directly relate the observed improvements in Se–VE status and GPx activity to reductions in lipid peroxidation. Future studies should include plasma or tissue MDA measurements to better characterise changes in oxidative stress. Additional limitations include

the relatively modest number of heifers, single-herd design, lack of detailed neonatal outcomes, and absence of endocrine or placental measurements. Together, these factors may reduce the external validity of the findings and emphasise the need for multi-herd trials that would include a wider range of physiological and production-related endpoints.

The supranutritional Se levels applied (0.5 and 1.0 mg/kg DM) were intentionally selected to examine responses within the upper safe range of organic Se supplementation. No clinical signs of Se toxicity were observed, and whole-blood Se concentrations remained within levels considered safe for cattle. Nevertheless, these findings should be interpreted in light of regulatory limits, and any practical use of higher dietary Se doses must comply with local legislation and account for long-term Se accumulation in animals and the environment.

Taken together, these findings indicate that supranutritional organic Se–VE supplementation during late gestation improves systemic antioxidant status. It is also associated with a low but biologically significant reduction of gestation length in Wagyu heifers, without adverse effects on other reproductive intervals. The results support the concept that strengthening Se- and VE-dependent antioxidant systems can positively influence periparturient physiology. Larger, multi-farm studies incorporating lipid peroxidation markers, endocrine and placental assessments, and detailed calf outcomes are necessary to confirm these observations and to determine optimal Se–VE supplementation strategies within safe and legally permitted limits.

Conclusions

Supplementing Wagyu heifers with organic selenium (Se) (0.5–1.0 mg/kg dry matter (DM) and vitamin E (VE) (800–1 600 IU/kg DM) from approximately 100 days prepartum to 7 days postpartum consistently increased circulating Se and α -tocopherol levels, as well as induced a sustained increase in glutathione peroxidase activity. Gestation length was reduced in a dose-dependent manner by approximately 7 days in T1 (0.5 mg Se/kg DM + 800 IU VE/kg DM), and 14 days in T2 (1.0 mg Se/kg DM + 1 600 IU VE/kg DM) compared with the control, whereas age at first calving, intercalving period, and service period remained unaffected. From a practical perspective, a 1–2-week shortening of gestation may facilitate more compact calving, improve the organisation of calving facili-

ties and labour, and lower the risk of periparturient complications associated with prolonged gestation, provided calf viability is not compromised. Within the conditions of this herd, supplementation with organic Se yeast combined with VE during the last trimester appears to be a justified management practice to enhance periparturient redox balance and optimise calving timing. Nonetheless, confirmation in different herds, production systems, and regulatory environment is required, with particular emphasis on neonatal outcomes and long-term Se safety.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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