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*Research article*

# EFFECTS OF β-CAROTENE AND SELENOPYRAN SUPPLEMENTATION ON NEONATAL PERFORMANCE AND COLOSTRUM COMPOSITION IN GILTS

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Colostrum quality is a key factor influencing neonatal survival, passive transfer of immunity, and early postnatal development in pigs. This study evaluated the effects of β-carotene (Carofertin®) and selenopyran supplementation on neonatal performance, colostral protein profile, and oxidative stress parameters in gilts. Thirty gilts were allocated to three groups (n=10 each): control, β-carotene-treated (CF), and selenopyran-treated (SE). Colostrum samples collected immediately after farrowing were analyzed using digital Brix refractometry, agarose protein electrophoresis (APE), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and oxidative stress assays. Total protein concentration, γ-globulin concentration, and proteins corresponding to immunoglobulin G (IgG) were determined. Redox status was assessed by measuring total oxidant status (TOS), total antioxidant capacity (TAC), and oxidative stress index (OSI). Piglets from the CF group had significantly higher birth weight (1.29 ± 0.19 kg) compared with both the control ($p < 0.001$) and SE groups ($p < 0.01$). However, no significant differences were observed among groups in total colostral protein concentration, γ-globulin concentration determined by APE, or IgG concentration determined by SDS-PAGE. Strong positive correlations were found between Brix values and total protein, γ-globulin, and IgG concentrations in the control and SE groups ($\rho$=0.657–0.936; $p<0.05$). The CF group showed significantly higher TOS and OSI values and lower TAC values than the control and SE groups ($p<0.01$), indicating increased oxidative stress. In contrast, selenopyran supplementation maintained oxidative homeostasis without affecting colostral protein composition. These findings

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suggest that β-carotene and selenopyran exert distinct effects on neonatal performance and oxidative status in gilts.

**Keywords:** gilts; β-carotene; selenopyran; colostral whey proteins; immunoglobulin G; oxidative stress.

## INTRODUCTION

An increased mortality rate of piglets represents a serious problem in pig production [1]. Piglets are born with low body fat reserves and an underdeveloped immune system [2]. Since the placenta in pigs is impermeable to immunoglobulins (epitheliochorial placenta), piglets are born without maternal antibodies. They acquire passive immunity until their own immune system matures only through colostrum and milk [3]. The piglet's survival in the first hours and days of life depends on the availability of adequate nutrients to meet their energy requirements as their glycogen reserves are sufficient to sustain bodily functions for no longer than 16 hours [4]. Therefore, it is crucial that piglets consume colostrum immediately after birth. Colostrum is the first mammary gland secretion synthesized immediately preparturition (farrowing) [5,6]. During the first 24 hours colostrum contains, besides the basic components such as proteins, fats, and lactose, high concentrations of immunoglobulins, certain minerals and fat-soluble vitamins, hormones [7], essential growth factors and antioxidants crucial for neonatal survival and development of pigs [6]. Immunoglobulin G (IgG) of colostral origin predominates in piglets' peripheral blood during the first weeks of life. This colostral IgG is 100% derived from sow/gilt serum [8]. Since the epitheliochorial placenta of swine prevents prenatal transfer of immunoglobulins, newborn piglets are highly dependent on adequate colostrum intake immediately after birth to acquire passive immunity and protection against infectious diseases [9,10]. In addition to immunoglobulins, numerous bioactive proteins and peptides in the colostrum contribute to intestinal maturation, metabolic adaptation and regulation of immune responses in neonatal animals [11].

The quality of sow colostrum may be influenced by several factors including parity, nutrition, environmental conditions, oxidative stress and maternal metabolic status during late gestation [12]. Modern intensive production systems expose gilts and sows to considerable physiological and metabolic challenges during pregnancy and farrowing, often resulting in increased production of reactive oxygen species and dysregulation of redox homeostasis [13]. Oxidative stress occurring during late gestation may negatively affect reproductive performance, mammary gland function and colostrum composition [14]. Therefore, maintaining adequate antioxidant capacity in gilts during the periparturient period is considered important for both maternal health and neonatal viability.

Dietary supplementation with antioxidants, trace elements and functional feed additives has attracted increasing attention as a strategy for improving reproductive performance and immune status in swine production [15]. Selenium, as an essential component of

glutathione peroxidase and other selenoproteins, plays an important role in antioxidant defense mechanisms, regulation of immune responses and maintenance of cellular integrity. Selenopyran is an organoselenium compound with pronounced antioxidant and immunomodulatory properties. Previous experimental studies demonstrated its ability to improve antioxidant defense mechanisms and protect biological systems against oxidative damage [16]. Previous studies demonstrated that selenium supplementation may improve antioxidant status, immunoglobulin concentrations and productive performance in sows and piglets [17]. β-Carotene is a naturally occurring carotenoid and a precursor of vitamin A that plays an important role in antioxidant protection, immune regulation and reproductive function. In addition to its provitamin A activity, β-carotene can scavenge reactive oxygen species and contribute to maintenance of cellular redox balance during periods of increased metabolic demand [15,16]. Similarly, supplementation with biologically active compounds and nutritional additives during gestation has been associated with improved colostrum composition, enhanced antioxidant capacity and better neonatal outcomes [18,19].

Protein profiling techniques, including agarose gel electrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), enable detailed characterization of colostrum protein fractions and may provide valuable insight into immunological and metabolic changes associated with nutritional interventions [20]. Moreover, biomarkers such as total antioxidant capacity (TAC), total oxidant status (TOS), advanced oxidation protein products (AOPP), advanced glycation end products (AGEs) and oxidative stress index (OSI) are widely used for evaluation of redox balance in veterinary studies [21].

Data regarding the relationship between supplementation, colostrum protein profile and oxidative stress biomarkers in gilts are still limited. Therefore, the aim of the present study was to evaluate whether pre-breeding intramuscular administration of the organic selenium compound selenopyran or β-carotene was associated with subsequent neonatal performance, colostrum protein fractions, and colostral oxidative stress markers at farrowing.

## MATERIALS AND METHODS

### Animals and experimental design

The study was conducted on the experimental pig farm of the Institute for Animal Husbandry, Belgrade, Serbia. A total of 30 commercial gilts were included in the experiment at the age of six months. At the start of the study, body weight, backfat thickness at two anatomical locations and depth of the musculus longissimus dorsi (MLD) were measured in all animals.

Following baseline measurements, the gilts were randomly allocated to three experimental groups, each consisting of 10 animals. Body weight, backfat thickness,

and musculus longissimus dorsi depth were recorded before treatment to assess baseline comparability among groups. During the experimental period, environmental conditions in the housing facility were continuously monitored using a Testo 174H data logger (Testo SE & Co. KGaA, Germany), which recorded ambient temperature and relative humidity at one-hour intervals.

All animals were managed under the same feeding programme and received a standard farm diet based on cereals, feed flour, a protein concentrate, and a vitamin-mineral premix. The complete diet contained 0.1 mg Se/kg feed, and water was provided ad libitum throughout the experiment. One experimental group received intramuscular selenopyran (9-phenyl-symmetrical octahydroselenoxanthene, C19H22Se; SE), dissolved in sterile olive oil, at 0.1 mg/kg body weight. The second experimental group received intramuscular Carofertin® (β-carotene, 10 mg/mL; CF) 7 mL per animal. Both treatments were administered twice during the pre-breeding period: immediately after detection of the first oestrus and after confirmation of the second oestrus, respectively. Animals in the control group received sterile olive oil intramuscularly at the corresponding time points to control for the injection procedure. Following confirmation of the third oestrus, at 200–215 days of age, the gilts were introduced into reproduction. After farrowing, the numbers of live-born and stillborn piglets were recorded, and piglets were weighed immediately after birth. Colostrum samples were collected immediately after farrowing; approximately 2–7 mL was obtained from each gilt and stored at −20 °C until analysis.

The interventions were therefore completed during the pre-breeding period, rather than during late gestation or the periparturient period. This protocol was selected to explore whether antioxidant administration before conception could have carry-over associations with subsequent reproductive and neonatal outcomes. A persistent biological effect was considered plausible because lipophilic β-carotene may be stored in maternal tissues and selenium may contribute to longer-lived tissue selenium and selenoprotein pools. However, circulating or tissue concentrations of β-carotene, vitamin A, selenium, and selenoproteins were not monitored between treatment and farrowing; thus, persistence of either compound until farrowing was not demonstrated and should be regarded as a limitation of this exploratory study.

## Ethics

All procedures involving animals were performed in accordance with national regulations on animal welfare and were approved by the Veterinary Directorate of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (Approval No. 002066765 2024 14841 002 000 323 022).

### Sampling

Colostrum samples were collected from each gilt immediately after farrowing (2–3 h). The sample volume ranged from 2 to 7 mL. The samples were then stored at −20 °C until further analysis.

### Brix refractometry

The Brix refractometer was calibrated with distilled water before analysis of every sample. Then, colostrum was homogenized by inverting 10 to 15 times, approximately 500 μL of the sample was placed, using a sterile pipette, on the prism of the Brix refractometer (Digital Refractometer MA871, Milwaukee, Romania), and %Brix value was read. The digital Brix refractometer has a range from 0 to 85% Brix [23].

### Preparation of colostral whey and serum samples

Frozen colostrum samples were thawed at 37°C, homogenized and defatted by removal of the lipid layer using a vacuum pump. The resulting colostral whey fraction was collected and used for electrophoretic and oxidative stress analyses. Blood serum obtained from a healthy gilt was prepared by centrifugation of whole blood (800 × g, 15 min) and used as a reference control for electrophoretic analyses.

### Agarose gel protein electrophoresis (APE)

Agarose protein electrophoresis (APE) was performed using 1% agarose gel prepared in Tris–Tricine buffer (8 g Tris and 3.75 g Tricine/L, pH 8.6) [23]. Colostral whey and serum samples (3 μL) were applied onto the gel and electrophoretically separated into a horizontal electrophoresis system at a constant voltage (up to 200 V) for approximately 30 min. Following separation, gels were fixed in 45% methanol and 10% acetic acid, stained with 0.12% Coomassie Brilliant Blue R, destained, and air-dried. The relative content of γ globulins (percentage) was quantified by densitometry using Image Master Total- Lab v1.11 software (Amersham Pharmacia Biotech, Uppsala Sweden). The concentration of γ globulins was calculated based on total protein concentration determined by the commercial BCA assay (Pierce, Rockford IL, USA).

### Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein composition of colostral whey was additionally evaluated by non-reducing SDS-PAGE [24]. After electrophoretic separation, gels were stained with Coomassie Brilliant Blue and protein bands were visualized using Coomassie brilliant blue R-250, C.I. 42660 (Sigma) and imaged with ChemiDoc 2.0 (Bio-Rad, Hercules, CA). Densitometry was performed using the ImageMaster Total Lab TL 120 software (GE HealthCare LifeScience, NJ, USA). The relative abundance and concentration of IgG fractions were calculated based on total protein concentrations determined by the BCA assay (Pierce, Thermo Fisher, USA).

## Oxidative stress analysis

The oxidative stress status of colostral whey was evaluated by determination of total oxidant status (TOS) and total antioxidant capacity (TAC). The oxidative stress index (OSI) was calculated as the ratio of TOS to TAC and expressed in arbitrary unit. All results were normalized to total protein concentration and reported per milligram of protein.

### TAC Determination

Total antioxidant capacity was determined according to Erel's method [25]. The assay used hydrogen peroxide in an acidic medium to oxidise reduced 2,2-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), resulting in discoloration of the reagent. The intensity of the discoloration was proportional to the concentration of antioxidants in the sample that neutralized $H_2O_2$. The results were measured at 660 nm using an UltroSpect 3300 Pro UV/Visible spectrophotometer (Amersham Biosciences Corp., Piscataway, NJ, USA).

### TOS Determination

Total oxidative status was determined with a method described by Erel [26]. Namely, the oxidation of the ferrous ion-o-dianisidine complex to ferric ion is carried out by oxidants from colostral whey samples, where the intensity of colour produced is proportional to the total amount of oxidant molecules present. Absorbance was measured spectrophotometrically at 560 nm using an UltroSpect 3300 Pro UV/Visible spectrophotometer (Amersham Biosciences Corp., Piscataway, NJ, USA).

## Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and OriginPro 8.0 (OriginLab Corporation, Northampton, MA, USA). Data are presented as mean ± standard deviation (SD) and standard error of the mean (SE). The normality of data distribution was assessed with the Shapiro–Wilk test. Differences among the three experimental groups (control, CF and SE) were evaluated by one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) post hoc test for multiple comparisons. Associations between Brix values and colostrum quality parameters, including total protein concentration, γ-globulin concentration determined by agarose protein electrophoresis (APE), and IgG concentration determined by SDS-PAGE, were assessed using Spearman's rank correlation coefficient. For colostrum variables and within-group correlations, the gilt was the independent experimental unit ($n = 10$ per group). Piglet body weights were analysed using the available individual piglet records; the potential non-independence of piglets within the same litter is acknowledged as a limitation. Differences and correlations were considered statistically significant at $p < 0.05$.

## RESULTS

Reproductive and neonatal parameters are presented in Table 1. Piglets from the CF-treated group had significantly higher body weight at birth (1.29 ± 0.19 kg) compared with both the control (1.19 ± 0.22 kg; $p < 0.001$) and SE-treated groups (1.20 ± 0.24 kg; $p < 0.01$). No significant difference was observed between the control and SE groups. Similarly, body weight on the second day after birth was significantly higher in piglets from the CF group (1.50 ± 0.17 kg) than in the control group (1.40 ± 0.19 kg; $p < 0.001$), whereas the difference between the CF and SE groups (1.44 ± 0.21 kg) was not statistically significant. Body weight of gilts progressively increased throughout the experimental period in all experimental groups.

**Table 1**. Reproductive performance, piglet viability and body weight changes in gilts during the experimental period.

| Parameter | Control | CF group | SE group |
|---|---|---|---|
| Total piglets born | 114 | 119 | 101 |
| Piglet mortality at first day (%) | 6.14 | 5.04 | 8.91 |
| Piglet birth weight (kg) | $1.19 \pm 0.22^{a}$ | $1.29 \pm 0.19^{b}$ | $1.20 \pm 0.24^{a}$ |
| Piglet body weight, day 2 (kg) | $1.40 \pm 0.19^{a}$ | $1.50 \pm 0.17^{b}$ | $1.44 \pm 0.21^{a,b}$ |
| Gilts BW at first estrus (kg) | 106.5 ± 10.5 | 101.4 ± 15.7 | 102.6 ± 11.3 |
| Gilts BW at second estrus (kg) | 118.0 ± 9.6 | 112.3 ± 11.5 | 112.6 ± 7.5 |
| Gilts BW at third estrus (kg) | 129.7 ± 10.5 | 127.9 ± 15.4 | 126.2 ± 11.5 |

Within a row, means with different superscript letters differ significantly ($p < 0.05$).
Piglet mortality data are presented descriptively and were not subjected to inferential statistical analysis. Data are presented as mean ± SD, except total piglet born and piglet mortality, which are shown as counts and percentages, respectively. BW—body weight.

Colostrum quality parameters, including total protein concentration and Brix values, are presented in Figures 1A and 1B. The highest average concentration of total proteins was recorded in the SE-treated group (104.2 ± 18.0 mg/mL), followed by the control group (102.8 ± 24.8 mg/mL), while the lowest values were observed in the CF-treated animals (94.9 ± 16.0 mg/mL). Similarly, the highest average Brix values were observed in the SE-treated group (22.1 ± 2.3%), whereas slightly lower values were recorded in the control (21.4 ± 3.4%) and CF-treated gilts (20.6 ± 1.8%). Despite the observed variations, the differences between groups were not statistically significant.

The concentrations of colostral γ-globulin fractions determined by APE and the concentrations of proteins corresponding by molecular weight to IgG (hereafter referred to as total IgG) are presented in Figure 1C–F; representative APE and SDS-PAGE profiles are shown in Figures 2 and 3, respectively. No statistically significant differences were observed among the experimental groups regarding γ-globulin percentage, γ-globulin concentration, total IgG percentage or total IgG concentration. The highest average γ-globulin concentration determined by APE was observed in the

control group (74.0 ± 16.7 g/L), while lower values were recorded in the CF-treated (62.5 ± 14.6 g/L) and SE-treated animals (69.1 ± 15.1 g/L). Similarly, the highest total IgG concentration determined by SDS-PAGE was observed in the control group (77.8 ± 20.4 g/L), followed by the SE-treated (75.0 ± 19.0 g/L) and CF-treated group (65.4 ± 14.8 g/L). Despite the absence of statistically significant differences between experimental groups, comparable distribution patterns have been obtained by agarose electrophoresis and SDS-PAGE analysis.

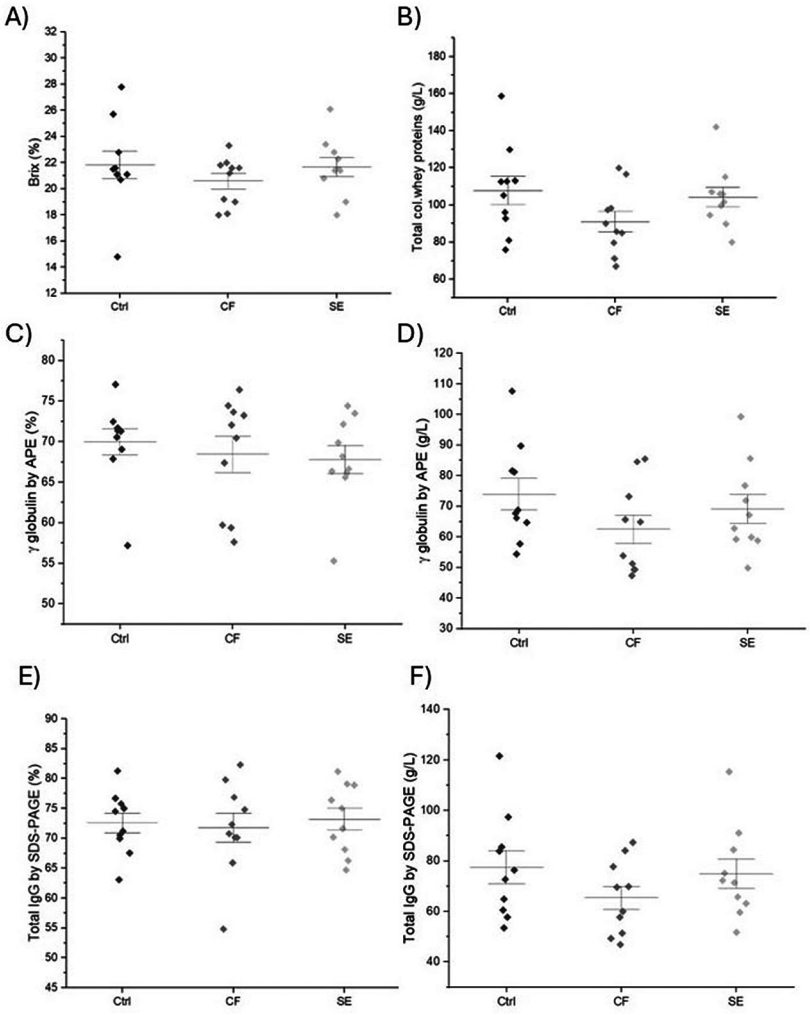


**Figure 1.** Colostrum quality parameters at farrowing following pre-breeding β-carotene or selenopyran administration in gilts. Individual values and mean ± SEM are shown for (A) Brix value (%), (B) total colostral whey proteins (g/L), (C) γ-globulin fraction determined by agarose protein electrophoresis (APE, %), (D) γ-globulin concentration determined by APE (g/L), (E) total IgG determined by SDS-PAGE (%), and (F) total IgG concentration determined by SDS-PAGE (g/L). Ctrl—control group; CF—Carofertin-treated group; SE—selenopyran-treated group.

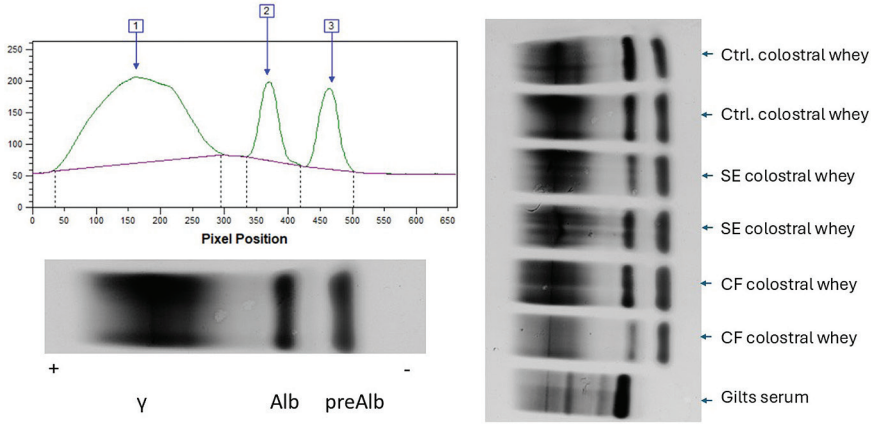


**Figure 2. APE** colostral whey proteins. (A) – a representative sample - control group; γ – γ globulin; Alb - albumin; preAlb - prealbumin; green line: densitometrically recorded color intensity. (B) - Ctrl - control group; CF - carofetrin-treated group; SE - selenopyran-treated group.

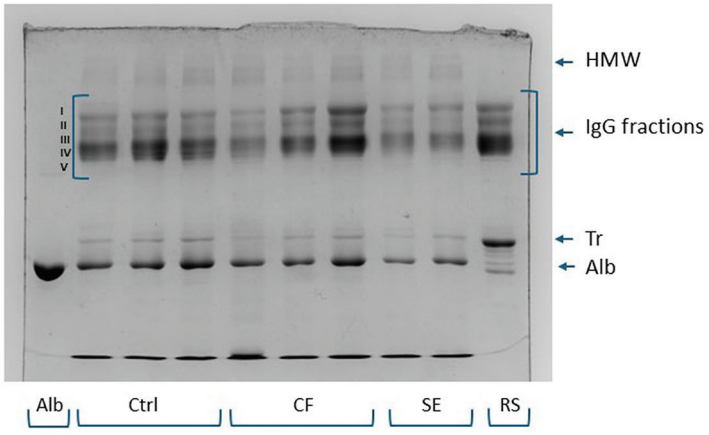


**Figure 3.** Non-reducing SDS-PAGE of colostral whey proteins. Alb- albumin; Ctrl - control group; CF - carofertin-treated group; SE - selenopyran-treated group; RS- serum proteins remaining soluble after precipitation with rivanol; Tr- transferrin; HMW- high molecular weight proteins.

Correlation analysis revealed significant positive associations between Brix values and colostrum quality parameters within the experimental groups (Figure 4). In the control group, a strong positive correlation was observed between Brix values and total colostrum protein concentration ($\varrho = 0.857$, $p = 0.001$), as well as between Brix values and γ-globulin concentration determined by APE ($\varrho = 0.936$, $p < 0.001$). A similarly strong positive correlation was found between Brix values and total IgG concentration determined by SDS-PAGE analysis ($\varrho = 0.908$, $p < 0.001$). In the CF-treated group,

no statistically significant correlations were observed between Brix values and total proteins, γ-globulin concentration, or total IgG concentration (n = 10; ϱ = 0.154, p > 0.05). Given the small group size, the absence of statistical significance should not be interpreted as evidence that no biological association exists. In the SE-treated group, Brix values showed a strong positive correlation with total colostrum protein concentration (ϱ = 0.894, p < 0.001), γ-globulin concentration determined by APE (ϱ = 0.657, p = 0.039) and total IgG concentration determined by SDS-PAGE (ϱ = 0.766, p = 0.010). These findings indicate that Brix refractometry represents a useful indirect indicator of colostrum protein and immunoglobulin content, only in control and SE-treated animals.

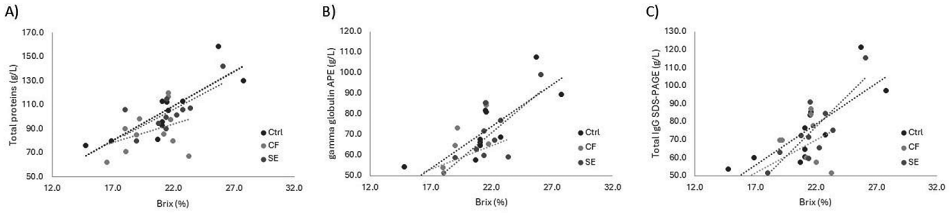


**Figure 4.** Correlation between Brix scores (%) and total colostral proteins (A), APE determined concentration of γ globulins (B) and SDS-PAGE determined concentration of colostral IgG (C). (●) Experimental data; Ctrl- control group; CF- carofertin-treated group; SE- selenopyran-treated group.

Oxidative stress parameters in the colostrum samples are presented in Figure 5. The CF-treated group showed significantly higher TOS and OSI values compared to the control and SE-treated animals (p < 0.01), while TAC values were significantly lower (p < 0.001). No significant differences were observed between the control and SE-treated groups regarding TOS, TAC and OSI values. These findings indicate increased oxidative status and reduced antioxidant capacity in CF-treated animals.

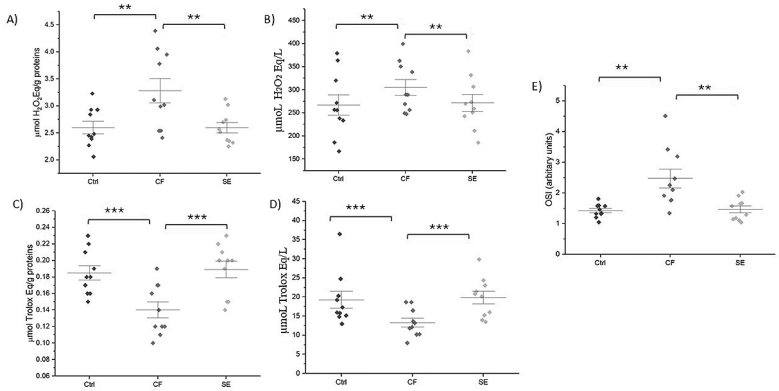


**Figure 5.** Oxidative stress parameters in colostral whey samples of the experimental groups: (A) total oxidant status normalized to total protein; (B) total oxidant status (TOS); (C) total antioxidant capacity normalized to total protein; (D) total antioxidant capacity (TAC); and (E) oxidative stress index (OSI). Individual values and mean ± SEM are shown. **p < 0.01; ***p < 0.001. Ctrl—control group; CF—Carofertin-treated group; SE—selenopyran-treated group.

## DISCUSSION

The present study evaluated the associations of pre-breeding selenopyran and β-carotene administration with reproductive performance, colostrum composition, immunoglobulin content, and colostral oxidative stress parameters at farrowing. The obtained results demonstrated that the treatments significantly influenced neonatal body weight and colostral oxidative status, while only minor variations were observed in total colostrum protein concentration and immunoglobulin fractions.

Piglets originating from the CF-treated gilts showed significantly higher body weight at birth and on the second day after birth compared with the control animals. Although β-carotene was administered before breeding in the present study, several biologically plausible pathways may contribute to a later association with fetal growth. β-Carotene serves as a precursor of vitamin A, which is involved in cell differentiation, placental development, and embryonic growth, and it may also modulate immune and antioxidant responses [27,28]. In addition, the lipophilic nature of β-carotene permits its storage in maternal tissues and subsequent mobilization. However, maternal β-carotene and vitamin A concentrations, placental function, and metabolic or hormonal mediators were not measured; therefore, these mechanisms remain hypothetical and the higher neonatal body weight cannot be attributed directly to a specific β-carotene-mediated pathway.

Despite the absence of significant differences in total colostrum protein concentration among experimental groups, electrophoretic analyses revealed variability in γ-globulin fraction and IgG subfraction. Agarose protein electrophoresis and SDS-PAGE analysis showed highly comparable protein distribution patterns, indicating consistency between the applied analytical methods. The predominance of γ-globulin fractions observed in the present study is consistent with the known immunological role of porcine colostrum as the principal source of passive immunity for neonatal piglets [9,10]. Similar protein distribution patterns in sow colostrum have previously been reported using electrophoretic techniques [29].

An important finding of the present study is the strong positive correlation between Brix values and concentrations of total proteins, γ-globulins, and IgG respectively. Particularly high correlations are observed in the control and SE-treated groups. As stated by Souza et al., [30] and others [31-34] the predominant protein of γ-globulin fraction present in colostrum is IgG, which significantly contributes to the total solids content, and Brix refractometer can be used to indicate their concentration, as IgG represents main colostral protein fraction. Our findings are supported by numerous reports, suggesting Brix refractometry, in both pigs and ruminants, as rapid, reliable and inexpensive for indirect evaluation of colostrum quality [33,34]. Because Brix method is reproducible and neither temperature nor storage duration affects IgG assessment, it is convenient for on-farm use [32].

The oxidative stress analysis revealed significantly increased TOS and OSI values together with reduced TAC values in the CF-treated group. These findings indicate increased oxidative stress and reduced antioxidant capacity in these animals. Although β-carotene is generally considered as an antioxidant compound, an increase, probably short-term, in oxidative parameters observed in the present study may reflect enhanced metabolic activity associated with intensified fetal growth and increased neonatal body weight. Similar findings have been previously reported in animals exposed to increased metabolic demands during late gestation and lactation [13,35]. Increased production of reactive oxygen species during the periods of intensified metabolism may temporarily exceed the body's natural antioxidant defense system (TAC) and contribute to elevated oxidative stress markers [36]. The increased oxidative stress parameters observed in the CF group may reflect elevated metabolic activity associated with enhanced fetal growth and increased birth weight rather than a direct pro-oxidant effect of β-carotene supplementation [37].

In contrast, SE-treated gilts showed colostral whey TOS, TAC, and OSI values comparable to those of the control group. Selenium is an essential component of several antioxidant enzymes, particularly glutathione peroxidase, and plays a key role in cellular antioxidant defence [16]. Selenium supplementation may affect the health of pregnant sows and neonatal pigs through antioxidant or immunomodulatory pathways [17,22,38]. Nevertheless, systemic selenium status, selenoprotein activity, and blood oxidative stress markers were not measured in the present study; consequently, these results should not be interpreted as direct evidence that selenopyran maintained systemic redox homeostasis.

Several limitations should be considered when interpreting these findings. The number of gilts per group was limited, and the concentrations of the administered compounds and their metabolites were not measured during gestation or at farrowing. Systemic oxidative stress and metabolic parameters in gilt blood were not assessed; therefore, colostral whey biomarkers cannot be extrapolated to whole-animal redox status. Piglet body-weight analyses used individual piglet records; consequently, possible within-litter dependence may have influenced the precision of the estimated treatment effects, and these neonatal findings require confirmation in a larger study designed for litter-level or mixed-effects analysis. In addition, the pre-breeding administration schedule and the absence of direct measurements of placental function preclude causal conclusions regarding the mechanisms underlying neonatal body weight. The correlations obtained in the relatively small groups should also be interpreted cautiously and confirmed in larger studies.

## CONCLUSIONS

In conclusion, pre-breeding β-carotene administration was associated with higher neonatal body weight, whereas selenopyran-treated gilts showed colostral whey oxidative stress parameters comparable to those of the control group. Electrophoretic

analyses did not demonstrate significant treatment-related differences in the γ-globulin/IgG fraction of colostrum.

Further studies involving a larger number of animals, a clearly defined interval between supplementation and farrowing, measurement of maternal β-carotene, vitamin A, and selenium status, and additional methods for confirming immunoglobulin fractions are needed to verify these findings and clarify their biological basis.

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**Authors' contributions**

AVP and BMS conceived and designed the study. AVP performed animal experiments and sample collection. MVK carried out Brix refractometry, electrophoretic analyses, data interpretation and preparation of the manuscript. JDGM performed oxidative stress analyses and participated in data interpretation. VLjI and ISM contributed to laboratory analyses and manuscript revision. NPF provided veterinary supervision and participated in evaluation of animal health and reproductive parameters. BMS supervised the study and contributed to manuscript revision. All authors read and approved of the final manuscript.

**Declaration of conflicting interests**

The author(s) reported no potential conflicts of interest concerning the research, authorship, or publication of this article.

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# EFEKTI SUPLEMENTACIJE B-KAROTENOM I SELENOPIRANOM NA NEONATALNE POKAZATELJE I SASTAV KOLOSTRUMA KOD NAZIMICA

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Kvalitet kolostruma predstavlja važan faktor koji utiče na preživljavanje novorođene prasadi, prenošenjem prirodnog pasivnog imuniteta, i rani postnatalni razvoj. Cilj ovog istraživanja bio je da se ispita uticaj suplementacije β-karotenom (Carofertin®) i selenopiranom na neonatalni razvoj, proteinski profil kolostruma i parametre oksidativnog stresa kod nazimica. U istraživanje je uključeno 30 nazimica raspoređenih u tri grupe (n=10): kontrolnu (Ctrl), grupu suplementiranu β-karotenom (CF) i grupu suplementiranu selenopiranom (SE). Uzorci kolostruma prikupljeni neposredno nakon prašenja analizirani su primenom digitalne Brix refraktometrije, agarozne elektroforeze proteina (APE), SDS-PAGE elektroforeze i testova za procenu oksidativnog stresa. Određivane su koncentracije ukupnih proteina, γ-globulina i imunoglobulina G (IgG), kao i ukupni oksidativni status (TOS), ukupni antioksidativni kapacitet (TAC) i indeks oksidativnog stresa (OSI). Prasad poreklom od nazimica iz CF grupe imala su značajno veću telesnu masu na rođenju (1,29 ± 0,19 kg) u poređenju sa kontrolnom ($p < 0,001$) i SE grupom ($p < 0,01$). Između grupa nisu utvrđene značajne razlike u koncentracijama ukupnih proteina, γ-globulina i IgG u kolostrumu. U kontrolnoj i SE grupi ustanovljene su visoko pozitivne korelacije između Brix vrednosti i koncentracija ukupnih proteina, γ-globulina i IgG ($\rho = 0,657–0,936$; $p < 0,05$). CF grupa pokazala je više vrednosti TOS i OSI, kao i niže vrednosti TAC ($p < 0,01$), što ukazuje na izraženiji oksidativni stres. Nasuprot tome, suplementacija selenopiranom održala je redoks ravnotežu i nije pokazala značajan uticaj na proteinski sastav kolostruma. Rezultati ukazuju na različite efekte β-karotena i selenopirana na neonatalne performanse i oksidativni status kod nazimica.