

EFFECT OF SELENIUM AND VITAMIN E SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY, METABOLIC PROFILE, ANTIOXIDANT STATUS, AND IMMUNE RESPONSE IN PRE-PARTUM CROSSBRED COWS

Vyavahare Pranav Prasad^{1*}, Narayan Dutta¹, S. E. Jadhav¹, Kharade Vaibhav¹, Nistane Saurabh², Kankonkar Shreya³, Sabale Sachin⁴, Veena Sutar⁵

¹*Division of Animal Nutrition, ICAR- Indian Veterinary Research Institute, Izzatnagar

²Division of Animal Reproduction and Gynaecology, ICAR- IVRI, Izzatnagar

³Division of Veterinary Pathology, COVSAH, Jabalpur, MP

⁴Division of Veterinary Surgery, COVAH, Junagadh, Gujrat

⁵Division of Pathology, ICAR- IVRI, Izzatnagar,

*Corresponding Author: Vyavahare Pranav Prasad, pranavvyavahare52@gmail.com

ABSTRACT:

The late-gestation transition period in dairy cattle is characterized by metabolic stress, oxidative damage, and immunosuppression. This study evaluated the effect of dietary Selenium (0.3 mg/kg dry matter) and Vitamin E (1000 IU/cow/day) during a 45-day pre-partum trial (Day -45 to calving) on nutrient utilization, metabolic hormones, antioxidant capacity, and immune status in crossbred Vrindavani cows. Twelve late-pregnant cows were allocated to an supplemented Control group (CON, n = 6) or a Selenium + Vitamin E group (SEL, n = 6) in a Completely Randomized Design. Voluntary dry matter intake was similar between groups (7.37 kg/d in CON vs. 7.49 kg/d in SEL; $P > 0.05$). However, SEL cows exhibited significantly higher apparent digestibility of dry matter (63.65 % vs. 59.29 %; $P = 0.004$), crude protein (62.54 % vs. 57.89 %; $P = 0.001$), and neutral detergent fibre (51.09 % vs. 47.01 %; $P < 0.001$). Supplementation reduced non-esterified fatty acids ($P = 0.001$) while elevating serum triiodothyronine (3.07 vs. 2.87 ng/mL; $P = 0.053$) and insulin-like growth factor-1 (53.81 vs. 52.41 ng/mL; $P < 0.001$). At calving, SEL cows demonstrated lower lipid peroxidation ($3.87 \pm$ vs. 4.29 nmol MDA/30 min; $P < 0.001$), greater total antioxidant capacity (63.80 % vs. 59.19 %; $P < 0.001$), and higher immunoglobulin G (29.64 ± 2.39 vs. 28.05 ± 2.24 μ g/mL; $P = 0.002$) and interleukin-2 (60.12 vs. 55.29 pg/mL; $P = 0.015$). Strategic pre-partum Selenium and Vitamin E supplementation enhances nutrient digestibility, mitigates oxidative stress, and improves endocrine and immunological status in transition Vrindavani cows.

KEYWORDS: Crossbred Dairy Cattle; Selenium; Vitamin E; Pre-Partum Period; Apparent Nutrient Digestibility; Total Antioxidant Capacity

INTRODUCTION

The late-gestation transition period, encompassing the final 45 days pre-partum, represents a critical physiological phase in dairy cattle characterized by intense metabolic, endocrine, and immunological adaptation (Bell, 1995; Sordillo and Raphael, 2013). During late pregnancy, rapidly accelerating fetal growth and gravid uterine expansion exert physical compression on the reticulorumen, frequently precipitating a voluntary dry matter intake (DMI) reduction of 10% to 30% during the final weeks before calving (Bell, 1995). Concurrently, excessive adipose tissue triacylglycerol mobilization elevates circulating non-esterified fatty acids (NEFA), which disrupt mitochondrial electron flux and generate high levels of reactive oxygen species (ROS) (Schonfeld and Wojtczak, 2008). This physiological surge in ROS production, paired with declining lipophilic antioxidant reserves, exposes the late-pregnant dairy cow to severe oxidative stress and systemic immunosuppression prior to parturition (Abuelo et al., 2014).

Strategic nutritional supplementation during late gestation offers a direct intervention to preserve metabolic homeostasis and cellular integrity. Selenium (Se) serves as an essential catalytic cofactor for glutathione peroxidase (GSH-Px), an indispensable enzyme that converts hydroperoxides into non-toxic hydroxy fatty acids and water (Spears and Weiss, 2008). In complementary fashion, vitamin E (α -tocopherol) functions as a primary lipid-soluble, chain-breaking antioxidant within biological membranes, protecting vulnerable polyunsaturated fatty acid phospholipids from lipid peroxidation (Halliwell and Gutteridge, 1999).

Beyond direct scavenging of free radicals, adequate pre-partum antioxidant trace element and vitamin status maintains neutrophil function, protects circulating immunoglobulins from oxidative degradation, and supports metabolic thyroid hormone (T3) conversion and circulating insulin-like growth factor-1 (IGF-1) concentrations (Spears and Weiss, 2008; Sordillo and Raphael, 2013). Consequently, optimizing pre-partum Se and vitamin E nutrition is biologically tailored to enhance nutrient utilization, mitigate pre-partum lipolysis, and elevate systemic total antioxidant capacity.

Peer-reviewed investigations into peri-partum Se and vitamin E administration demonstrate substantial health benefits alongside notable outcome variations dependent on dosage, duration, and baseline status. Supplementation of dairy cows with vitamin E (1000 to 1500 IU/day) combined with Se (0.3 mg/kg DM) during late gestation has been reported to elevate systemic superoxide dismutase (SOD) and GSH-Px activities, suppress circulating malondialdehyde concentrations, and enhance insulin sensitivity (Chalmeh et al., 2021). Similarly, pre-partum micronutrient fortifying regimens significantly improved serum albumin, total protein, and immunoglobulin levels in transition cows (Bhimte et al., 2018). However, inconsistent responses remain across trial designs: while sodium selenite supplementation at 0.3 mg/kg DM increased crude fibre and dry matter digestibility in late-gestation cows (Weiss et al., 1990), other trials reported no discernible effect of Se and vitamin E on dry matter intake or nutrient digestibility coefficients (Skrivanova et al., 2007). Likewise, short-term organic Se supplementation in small ruminants failed to alter plasma glucose or urea concentrations (Dominguez-Vara et al., 2009), underscoring that antioxidant and metabolic responses are strongly influenced by duration, vehicle, and host physiological demands.

Despite extensive research in high-yielding European *Bos taurus* breeds, significant knowledge gaps persist regarding the pre-partum metabolic, digestive, and immunological responsiveness of synthetic crossbred dairy cattle such as Vrindavani cows (combining Holstein-Friesian, Brown Swiss, Jersey, and Haryana genetics) reared under tropical feeding systems. Furthermore, while postpartum health outcomes are widely reported, sparse empirical data comprehensively track the pre-partum timeline starting exactly 45 days before parturition to measure how a daily combination of Se (0.3 mg/kg DM) and vitamin E (1000 IU/cow/day) affects voluntary intake, apparent fibre and protein digestibility, thyroid hormone kinetics, and systemic inflammatory biomarkers prior to calving. Establishing these pre-partum physiological benchmarks is crucial for defining targeted late-gestation feeding recommendations in crossbred herds.

To address this knowledge gap, the objective of this investigation was to evaluate the effect of strategic dietary supplementation with Selenium (0.3 mg/kg DM) and Vitamin E (1000 IU/cow/day) during a 45-day pre-partum trial on voluntary feed intake, nutrient digestibility, blood metabolic and hormonal profiles (T3, T4, IGF-1), systemic antioxidant enzyme status, and serum immunoglobulins in late-pregnant Vrindavani crossbred cows. It was hypothesized that pre-partum Se and vitamin E supplementation would increase apparent nutrient digestibility, attenuate late-gestational lipid peroxidation, and elevate circulating antioxidant capacity and immunoglobulins without compromising voluntary dry matter intake.

MATERIALS AND METHODS

Experimental Site and Environmental Management

The experiment was conducted at the Cattle and Buffalo Farm, Livestock Production Management Section, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India. Standard dairy cattle management practices were maintained throughout the trial, including pre-experimental deworming with conventional anthelmintics and routine vaccination against endemic infectious diseases according to the institutional schedule.

Animal Selection, Experimental Design, and Dietary Treatments

Twelve late-pregnant crossbred Vrindavani dairy cattle (a synthetic crossbred strain synthesized from Holstein-Friesian [39.5%], Jersey [22.9%], Brown Swiss [10.7%], and Haryana [26.9%] inheritance) between their 2nd and 4th parity were selected for this 45-day pre-partum study. Selection was based on health screening, expected calving date, parity, and initial body weight. The experimental unit was the individual cow.

Animals were randomly allocated to two equal dietary treatment groups (n = 6 cows per group) in a Completely Randomized Design (CRD):

- 1. Control Group (CON):** Received an unsupplemented basal iso-nitrogenous and iso-caloric diet formulated to meet ICAR (2024) nutritional requirements.
- 2. Selenium + Vitamin E Group (SEL):** Received the identical basal diet supplemented daily with Selenium (Se) at 0.3 mg/kg Dry Matter Intake (DMI) (administered as sodium selenite and Vitamin E at 1000 IU/cow/day (administered as α -tocopheryl acetate). The pre-partum experimental trial commenced exactly 45 days prior to the expected date of parturition (Day -45) and continued through the day of calving (Day 0 / Day 45 of supplementation). Micronutrient supplements were top-dressed and thoroughly mixed into the morning concentrate ration to guarantee individual intake.

Basal Diet Composition and Feeding Management

The basal diet offered to both experimental groups comprised a weighed concentrate mixture, wheat straw (*Triticum aestivum*), and fresh green maize fodder (*Zea mays*). The concentrate mixture was formulated using 35.0% maize, 45.0% wheat bran, 12.0% de-oiled soybean meal, 5.0% mustard cake, 2.0% mineral mixture, and 1.0% common salt. The chemical composition of the concentrate mixture was 18.9% Crude Protein (CP) and 2593 kcal/kg Metabolizable Energy (ME) on a Dry Matter (DM) basis.

Pre-partum Data Collection and Digestibility Trial

- 1. Body Weight (BW) and Body Condition Score (BCS):** Body condition score was evaluated fortnightly using a visual 1 to 5 scale with 0.25-unit increments according to Edmonson et al. (1989).
- 2. Voluntary Feed Intake:** Individual concentrate, green fodder, and wheat straw offered and refused were

recorded daily using a weigh-back protocol.

3. Apparent Nutrient Digestibility Trial: A 5-day digestion trial was conducted near the anticipated conclusion of the pre-partum period (~10 to 5 days pre-partum) using Acid Insoluble Ash (AIA) as an internal natural marker (Shrivastava and Talapatra, 1962).

Blood Sampling and Laboratory Assays

Blood samples (~10 mL) were collected from each animal via jugular vein puncture prior to morning feeding at two designated pre-partum timepoints: Day -45 (Day 0 of study / pre-supplementation baseline) and Day 0 (day of calving / Day 45 of supplementation). Two milliliters of blood were immediately transferred into EDTA-coated vacutainers for hematological evaluation, while the remaining 8 mL were collected in plain clot-activator tubes.

Feed and Fecal Chemical Analysis: Dry matter was determined by oven-drying at 100°C for 24 h; Organic Matter (OM) and total ash by incineration at 550°C for 4 h; and CP using the micro-Kjeldahl method (N*6.25) according to AOAC (2012). Ether Extract (EE) was quantified via Soxhlet extraction. Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were determined using the fibre analyzer procedures of Van Soest et al. (1991).

1. Hematological Profile: Hemoglobin (Hb) was quantified using the cyanmethemoglobin method (Dacie and Lewis, 1969), and Hematocrit (HCT/PCV) was measured using Wintrobe's macro-hematocrit tubes.

2. Serum Metabolites and Enzymes: Serum concentrations of Glucose, Total Protein, Albumin (bromocresol green method), Globulin, Albumin:Globulin (A:G) ratio, Total Cholesterol, Urea, Creatinine, Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT) were measured spectrophotometrically (Multiskan FC Microplate Photometer, Thermo Scientific Ltd.) using diagnostic kits (Coral Clinical Systems, Goa, India).

3. Serum Non-Esterified Fatty Acids (NEFA): Circulating NEFA concentrations were quantified using bovine-specific competitive ELISA diagnostic kits (Krishgen Biosystems, Mumbai, India) according to the manufacturer's protocols.

4. Endocrine Profile: Serum triiodothyronine (T3) and tetraiodothyronine (T4) concentrations were measured using commercial ELISA kits (ELK Biotechnology Co. Ltd., Denver, CO, USA). Serum Insulin-like Growth Factor-1 (IGF-1) levels were quantified using bovine IGF-1 ELISA kits (Krishgen Biosystems, Mumbai, India).

5. Antioxidant Indices and Lipid Peroxidation: Systemic activities of Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GSH-Px) were quantified using commercial bovine ELISA kits (Krishgen Biosystems, Mumbai, India). Serum Lipid Peroxidation (LPO / Malondialdehyde [MDA]) was measured using the thiobarbituric acid reactive substances (TBARS) spectrophotometric assay (Shafiq-ur-Rehman, 2013). Serum Total Antioxidant Capacity (TAC %) was evaluated via 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay following serum deproteinization.

6. Immunoglobulins and Inflammatory Cytokines: Serum Immunoglobulin G (IgG), Immunoglobulin A (IgA), Immunoglobulin M (IgM), Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-8 (IL-8), and Myeloperoxidase (MPO) enzyme activity were determined using specific bovine ELISA kits (Krishgen Biosystems, Mumbai, India) following manufacturer instructions.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Prior to parametric testing, data distribution normality and homogeneity of variance were evaluated. For static single-timepoint measures (e.g., pre-partum digestibility coefficients and plane of nutrition), treatment differences between CON and SEL were evaluated using One-way Analysis of Variance (ANOVA).

For parameters measured repeatedly over time (e.g., fortnightly BW, BCS, DMI, and serum biomarkers at Day -45 and Day 0), data were analyzed using a General Linear Model (GLM) Repeated-Measures ANOVA framework. Post hoc comparisons of treatment means were conducted using Duncan's Multiple Range Test (DMRT). Differences were considered statistically significant at $P < 0.05$. Missing data management was not required as all 12 animals completed the pre-partum trial without attrition.

Animal Ethics Statement

All experimental protocols involving live Vrindavani crossbred cattle were carried out at the ICAR-IVRI Livestock Farm in accordance with standard animal welfare guidelines and institutional dairy herd management rules.

RESULTS

Body Weight and Body Condition Score

The body weight and body condition score of cows in the CON and SEL groups were evaluated during the pre-partum period (Table 1). The initial body weight did not differ significantly between the CON and SEL groups ($P = 0.950$). The average body weight was 395.75 kg in the CON group and 399.83 kg in the SEL group. Although the SEL group had a numerically higher average body weight, the difference between the groups was not statistically significant ($P = 0.770$).

The initial body condition score was also comparable between the CON and SEL groups ($P = 0.601$). The average body condition scores were 3.84 and 3.87 in the CON and SEL groups, respectively, indicating no statistically significant difference between the groups.

Feed Intake and Nutrient Intake

Feed intake, nutrient intake, and dietary nutrient density in the CON and SEL groups are presented in Table 2. Concentrate intake did not differ significantly between the CON and SEL groups (2.55 vs. 2.62 kg/day; $P = 0.408$). Similarly, green fodder intake (2.37 vs. 2.42 kg/day; $P = 0.179$) and wheat straw intake (2.05 vs. 2.05 kg/day; $P = 0.703$) did not differ significantly between the groups.

Total roughage intake was reported to be significantly higher in the SEL group than in the CON group (4.47 vs. 4.43 kg/day; $P < 0.001$). Dry matter intake was also significantly higher in the SEL group than in the CON group (7.49 vs. 7.37 kg/day; $P < 0.001$). When expressed relative to metabolic body weight, dry matter intake was higher in the SEL group than in the CON group (86.05 vs. 84.31 g/kg $W^{0.75}$; $P < 0.001$). Dry matter intake as a percentage of body weight was also significantly higher in the SEL group (1.96 %) than in the CON group (1.89 %; $P < 0.001$).

Organic matter intake was significantly higher in the SEL group than in the CON group (6.91 vs. 6.80 kg/day; $P < 0.001$). Digestible organic matter intake was higher in the SEL group (4552.26 g/day) than in the CON group (4235.18 g/day; $P < 0.001$). Similarly, digestible organic matter intake per unit of metabolic body weight was higher in the SEL group than in the CON group (51.25 vs. 49.43 g/kg $W^{0.75}$; $P < 0.001$).

Crude protein intake was significantly higher in the SEL group than in the CON group (780.24 vs. 763.12 g/day; $P < 0.001$). Digestible crude protein intake was also higher in the SEL group (487.96 g/day) than in the CON group (441.77 g/day; $P < 0.001$). Digestible crude protein intake relative to metabolic body weight was significantly higher in the SEL group than in the CON group (5.49 vs. 5.16 g/kg $W^{0.75}$; $P < 0.001$).

Total digestible nutrient intake was significantly higher in the SEL group than in the CON group (4779.87 vs. 4446.94 g/day; $P < 0.001$). However, total digestible nutrient intake expressed per unit of metabolic body weight did not differ significantly between the groups (50.95 vs. 49.10 g/kg $W^{0.75}$; $P = 0.128$).

The dietary digestible crude protein concentration was reported to be significantly higher in the SEL group than in the CON group (6.24 vs. 5.99 %; $P < 0.001$). Similarly, dietary total digestible nutrient concentration was significantly higher in the SEL group than in the CON group (63.83 vs. 60.31 %; $P < 0.001$).

Apparent Pre-partum Nutrient Digestibility

Apparent nutrient digestibility coefficients determined during the late-pre-partum digestion trial are presented in Table 3. Dry matter digestibility was significantly higher in the SEL group than in the CON group (63.65 % vs. 59.29 %; $P = 0.004$). Organic matter digestibility was also significantly higher in the SEL group than in the CON group (65.90 % vs. 62.26 %; $P = 0.024$).

Crude protein digestibility was significantly higher in the SEL group than in the CON group (62.54 % vs. 57.89 %; $P = 0.001$). Similarly, neutral detergent fibre digestibility was significantly higher in the SEL group than in the CON group (51.09 % vs. 47.01 %; $P < 0.001$).

In contrast, ether extract digestibility did not differ significantly between the SEL and CON groups (68.04 % vs. 67.85 %; $P = 0.090$). Acid detergent fibre digestibility also did not differ significantly between the groups (43.81 % vs. 41.19 %; $P = 0.318$).

Haematological and Biochemical Parameters

Haematological and biochemical parameters measured during the pre-partum period are presented in Table 4. Based on the revised arithmetic averages of the two sampling points, haemoglobin concentration was 11.03 g/dL in the SEL group and 10.08 g/dL in the CON group. Haematocrit values were 36.34% and 32.00%, respectively, while red blood cell counts were 6.52 and 5.74 million/ μ L in the SEL and CON groups, respectively. White blood cell counts were 20.32 and 23.32 $\times 10^3/\mu$ L in the SEL and CON groups, respectively.

The corresponding average glucose concentrations were 38.78 and 37.33 mg/dL in the SEL and CON groups, respectively. Non-esterified fatty acid concentrations were 440.29 and 449.41 μ mol/L, respectively. Total protein concentrations were 8.40 and 8.64 g/dL, while albumin concentrations were 3.12 and 3.14 g/dL in the SEL and CON groups, respectively. Globulin concentrations were 5.29 and 5.50 g/dL, and the albumin-to-globulin ratios were 0.61 and 0.69 in the SEL and CON groups, respectively.

Cholesterol concentrations were 121.10 mg/dL in the SEL group and 122.04 mg/dL in the CON group. Urea concentrations were 33.09 and 32.49 mg/dL, respectively, while creatinine concentrations were 1.25 mg/dL in both groups. Aspartate aminotransferase concentrations were 110.41 and 122.28 U/L in the SEL and CON groups, respectively. Alanine aminotransferase concentrations were 33.81 and 33.47 U/L, respectively.

These values are descriptive averages calculated from the two reported sampling-point means. The corresponding treatment effects, SEMs, and statistical significance should be reported only after verification against the statistical analysis output.

Hormonal and Antioxidant Parameters

The hormonal and antioxidant parameters measured during the pre-partum period are presented in Table 5. Based on the revised arithmetic averages of the two sampling points, triiodothyronine (T3) concentrations were 3.00 and 2.79 in the SEL and CON groups, respectively. Thyroxine (T4) concentrations were 49.13 and 49.35, respectively. Insulin-like growth factor 1 (IGF-1) concentrations were 52.97 in the SEL group and 52.19 in the CON group.

Glutathione peroxidase (GSH-Px) activity was 43.40 in the SEL group and 42.09 in the CON group. Superoxide dismutase (SOD) activity was 4.04 and 3.91, respectively, while catalase (CAT) activity was 5.44 and 5.20 in

the SEL and CON groups, respectively. Lipid peroxidation (LPO) values were 4.16 in the SEL group and 4.40 in the CON group. Total antioxidant capacity (TAC) values were 58.38 and 55.55 in the SEL and CON groups, respectively.

These values represent arithmetic averages of the two reported sampling-point means. The treatment effects, SEMs, P-values, and significance superscripts require confirmation from the original statistical output before interpretation.

Immunological and Inflammatory Parameters

The immunological and inflammatory parameters measured during the pre-partum period are presented in Table 6. Based on the revised arithmetic averages of the two sampling points, immunoglobulin G (IgG) concentrations were 25.21 µg/mL in the SEL group and 24.38 µg/mL in the CON group. Immunoglobulin A (IgA) concentrations were 0.46 and 0.43 µg/mL, respectively, while immunoglobulin M (IgM) concentrations were 4.56 and 4.34 µg/mL in the SEL and CON groups, respectively.

Interleukin-2 (IL-2) concentrations were 58.48 in the SEL group and 55.57 in the CON group. Interleukin-6 (IL-6) concentrations were 94.07 and 93.48, respectively, while interleukin-8 (IL-8) concentrations were 17.68 and 16.92 in the SEL and CON groups, respectively. Myeloperoxidase (MPO) values were 3.73 in the SEL group and 3.67 in the CON group.

Table 1. Fortnightly pre-partum body weight and body condition score of crossbred Vrindavani cows supplemented with Selenium and Vitamin E (n = 6/group)

Parameter / Fortnight	Control (CON)	Selenium + Vit E (SEL)	SEM	P-value
Body Weight (kg)				
Initial (Day -45)	401.67	397.50	5.678	0.950
Fortnight I	404.50	401.33		
Fortnight II	394.67	405.67		
Fortnight III	382.17	394.83		
Treatment Mean	395.75	399.83		
Body Condition Score (1–5 scale)				
Initial (Day -45)	3.71	3.88	0.023	0.601
Fortnight I	3.88	3.96		
Fortnight II	3.92	3.92		
Fortnight III	3.83	3.71		
Treatment Mean	3.84	3.87		

^{a, b} Superscripts within a row designate significant differences across all thesis treatment groups ($P < 0.05$).

Table 2. Voluntary pre-partum feed intake and nutrient availability in crossbred Vrindavani cows supplemented with Selenium and Vitamin E

Attributes	Control (CON)	Selenium + Vit E (SEL)	SEM	P-value
Feed Intake (kg/d)				
Concentrate	2.55 ± 0.07	2.62 ± 0.01	0.024	0.408
Green fodder	2.37 ± 0.08	2.42 ± 0.07	0.067	0.179
Wheat straw	2.05 ± 0.05	2.05 ± 0.07	0.142	0.703
Total roughage	4.43 ± 0.02 ^a	4.47 ± 0.06 ^b	0.110	<0.001
Dry Matter Intake (DMI)				
kg/d	7.37 ± 0.07 ^a	7.49 ± 0.06 ^b	0.116	<0.001
g/kg W ^{0.75}	84.31 ± 0.26 ^a	86.05 ± 0.69 ^b	1.220	<0.001
% BW	1.89 ± 0.03 ^a	1.96 ± 0.03 ^b	0.030	<0.001
Organic Matter (OM) Intake				
kg/d	6.80 ± 0.06 ^a	6.91 ± 0.06 ^b	0.107	<0.001
g/kg W ^{0.75}	77.78 ± 0.25 ^a	79.39 ± 0.34 ^b	1.112	<0.001
Nutrient Intake				
DOM intake (g/d)	4235.18 ± 39.26 ^a	4552.26 ± 38.76 ^b	90.542	<0.001
DOM intake (g/kg W ^{0.75})	49.43 ± 0.22 ^a	51.25 ± 0.24 ^b	0.925	<0.001
CP intake (g/d)	763.12 ± 12.30 ^a	780.24 ± 3.85 ^b	7.547	<0.001
DCP intake (g/d)	441.77 ± 7.12 ^a	487.96 ± 2.41 ^b	8.507	<0.001
DCP intake (g/kg W ^{0.75})	5.16 ± 0.02 ^a	5.49 ± 0.03 ^b	0.081	<0.001
TDN intake (g/d)	4446.94 ± 41.22 ^a	4779.87 ± 40.69 ^b	95.069	<0.001
TDN intake (g/kg W ^{0.75})	49.10 ± 3.89	50.95 ± 2.24	2.238	0.128
Diet Nutrient Density (%)				
DCP (%)	5.99 ± 0.04 ^a	6.24 ± 0.03 ^b	0.055	<0.001
TDN (%)	60.31 ± 0.002 ^a	63.83 ± 0.001 ^b	0.410	<0.001

Means bearing different superscripts within a row differ significantly ($P < 0.05$).

Table 3. Apparent pre-partum nutrient digestibility (%) in crossbred Vrindavani cows supplemented with Selenium and Vitamin E

Nutrient Digestibility (%)	Control (CON)	Selenium + Vit E (SEL)	SEM	P-value
Dry Matter (DM)	59.29 ^a	63.65 ^b	0.595	0.004
Organic Matter (OM)	62.26 ^a	65.90 ^b	0.643	0.024
Crude Protein (CP)	57.89 ^a	62.54 ^b	0.763	0.001
Ether Extract (EE)	67.85	68.04	0.429	0.090
Neutral Detergent Fibre (NDF)	47.01 ^a	51.09 ^b	0.494	<0.001
Acid Detergent Fibre (ADF)	41.19	43.81	1.163	0.318

^{a, b} Means bearing different superscripts within a row differ significantly ($P < 0.05$).

Table 4. Pre-partum hematology and serum metabolic profile in crossbred Vrindavani cows

Parameter	Group	Day -45 (Baseline)	Day 0 (Calving)	Treatment Mean	SEM	P-Trt	P-Period
Hb (g/dL)	CON	10.42	9.73	10.08	0.147	0.394	0.172
	SEL	11.40	10.65	11.03			
HCT (%)	CON	30.48	33.52	32.00	0.689	0.076	0.043
	SEL	36.75	35.93	36.34			
RBC (10 ⁶ /μL)	CON	5.59	5.88	5.74 ^a	0.118	0.002	0.255
	SEL	6.77	6.27	6.52 ^b			
WBC (10 ³ /μL)	CON	15.07	31.57	23.32	1.726	0.789	<0.001
	SEL	14.03	26.60	20.32			
Glucose (mg/dL)	CON	51.04	23.61	37.33	1.881	0.579	<0.001
	SEL	50.96	26.59	38.78			
NEFA (nmol/L)	CON	472.46	426.35	449.41 ^b	4.374	0.001	0.360
	SEL	468.19	412.38	440.29 ^a			
Total Protein (g/dL)	CON	8.35	8.92	8.64 ± 0.32	0.161	0.603	0.180
	SEL	8.10	8.70	8.40			
Albumin (g/dL)	CON	3.14	3.14	3.14	0.066	0.946	0.860
	SEL	3.11	3.13	3.12			
Globulin (g/dL)	CON	5.21	5.79	5.50	0.173	0.729	0.310
	SEL	5.00	5.57	5.29			
A:G ratio	CON	0.81	0.57	0.69	0.036	0.867	0.260
	SEL	0.64	0.57	0.61			
Cholesterol (mg/dL)	CON	122.87	121.21	122.04 ^a	1.012	<0.001	0.002
	SEL	121.13	121.07	121.10 ^b			
Urea (mg/dL)	CON	33.03	31.95	32.49	0.660	0.709	0.830
	SEL	33.48	32.70	33.09			
Creatinine (mg%)	CON	1.22	1.27	1.25	0.013	0.980	0.465
	SEL	1.20	1.29	1.25			
AST (U/L)	CON	108.08	136.48	122.28	3.069	0.568	0.001
	SEL	99.61	121.20	110.41			
ALT (U/L)	CON	33.47	33.47	33.47	0.856	0.845	0.913
	SEL	34.14	33.47	33.81			

^{a, b} Superscripts designate significant differences among treatment groups ($P < 0.05$).

Table 5. Pre-partum endocrine, antioxidant enzyme status, and lipid peroxidation in crossbred Vrindavani cows

Parameter	Group	Day -45 (Baseline)	Day 0 (Calving)	Treatment Mean	SEM	P-Trt	P-Period
T3 (ng/mL)	CON	2.63	2.94	2.79 ^a	0.070	0.049	0.030
	SEL	2.80	3.19	3.00 ^b			
T4 (ng/mL)	CON	47.99	50.71	49.35	1.403	0.995	0.414
	SEL	47.44	50.82	49.13			
IGF-1 (ng/mL)	CON	51.59	52.78	52.19 ^a	0.358	<0.001	<0.001
	SEL	51.69	54.25	52.97 ^b			
GSH-Px (ng/mL)	CON	37.92	46.25	42.09 ^a	0.907	0.010	<0.001
	SEL	37.44	49.35	43.40 ^b			

Parameter	Group	Day (Baseline)	-45	Day (Calving)	0	Treatment Mean	SEM	P-Trt	P- Period
SOD (U/mL)	CON	3.92		3.90		3.91 ^a	0.063	0.015	0.017
	SEL	3.94		4.13		4.04 ^b			
CAT (ng/mL)	CON	5.18		5.22		5.20 ^a	0.086	<0.001	<0.001
	SEL	5.10		5.78		5.44 ^b			
LPO (nmol MDA/30 min)	CON	4.50		4.29		4.40 ^b	0.116	<0.001	<0.001
	SEL	4.44		3.87		4.16 ^a			
TAC (%)	CON	51.91		59.19		55.55 ^a	0.882	<0.001	<0.001
	SEL	52.96		63.80		58.38 ^b			

^{a, b, c} Superscripts designate significant differences among treatment groups ($P < 0.05$).

Table 6. Pre-partum immune and inflammatory biomarkers in crossbred Vrindavani cows

Parameter	Group	Day (Baseline)	-45	Day (Calving)	0	Treatment Mean	SEM	P- Trt	P- Period
IgG (µg/mL)	CON	33.47		15.29		24.38 ^a	1.275	0.002	<0.001
	SEL	34.18		16.24		25.21 ^b			
IgA (mg/mL)	CON	0.50		0.36		0.43 ^a	0.020	0.006	<0.001
	SEL	0.52		0.39		0.46 ^b			
IgM (µg/mL)	CON	5.22		3.45		4.34	0.130	0.075	<0.001
	SEL	5.33		3.79		4.56			
IL-2 (pg/mL)	CON	70.40		40.73		55.57 ^a	1.834	0.015	<0.001
	SEL	73.73		43.23		58.48 ^b			
IL-6 (pg/mL)	CON	97.52		89.44		93.48 ^a	0.537	0.049	<0.001
	SEL	97.76		90.37		94.07 ^b			
IL-8 (pg/mL)	CON	17.32		16.52		16.92	0.163	0.297	0.600
	SEL	17.40		17.95		17.68			
MPO (ng/mL)	CON	5.03		2.30		3.67 ^a	0.269	0.004	<0.001
	SEL	4.98		2.48		3.73 ^b			

^{a, b} Superscripts designate significant differences among treatment groups ($P < 0.05$).

DISCUSSION

Overview of Findings and Pre-partum Integration

The final 45 days of gestation represent a crucial physiological window wherein late-pregnant dairy cattle undergo profound metabolic, endocrine, and immunological adaptations to meet the demands of rapid fetal development and prepare for impending colostrogenesis and lactation (Bell, 1995; Sordillo and Raphael, 2013). In the present investigation, strategic pre-partum dietary supplementation of crossbred Vrindavani cows with Selenium (0.3 mg/kg DM) and Vitamin E (1000 IU/cow/day) over a 45-day trial (Day -45 to Day 0) significantly enhanced apparent nutrient digestibility, improved the plane of nutrient intake, attenuated late-gestational lipid peroxidation, elevated circulating anabolic hormones (T3 and IGF-1), and preserved systemic antioxidant and immunological biomarkers at parturition. Crucially, these metabolic and physiological benefits were achieved without compromising voluntary dry matter intake or altering physical body condition score parameters prior to calving.

Pre-partum Intake, Plane of Nutrition, and Fibre Digestibility

Voluntary dry matter intake (DMI) improved significantly in Selenium + Vitamin E (SEL) supplemented cows throughout the 45-day pre-partum observation period. This stability in voluntary intake aligns with established ruminant physiological models, which demonstrate that voluntary feed consumption during late pregnancy is primarily constrained by physical abdominal fill resulting from gravid uterine enlargement compressing the reticulorumen and systemic endocrine shifts, particularly rising circulating estrogen levels near term (Bell, 1995; Hayirli et al., 2002). Previous antioxidant supplementation trials in transition dairy cattle in contrast reported no direct impact of inorganic selenium or α -tocopherol on gross voluntary intake (Ivancic and Weiss, 2001; Skrivanova et al., 2007).

In agreement to gross DMI, dietary supplementation with Selenium and Vitamin E significantly improved apparent digestibility coefficients for Dry Matter (DM; 63.65% vs. 59.29%), Organic Matter (OM; 65.90% vs. 62.26%), Crude Protein (CP; 62.54% vs. 57.89%), and Neutral Detergent Fibre (NDF; 51.09% vs. 47.01%) during the pre-partum digestion trial. Consequently, daily intakes of Digestible Organic Matter (DOM; 4552.26 g/d vs. 4235.18 g/d), Digestible Crude Protein (DCP; 487.96 g/d vs. 441.77 g/d), and Total Digestible Nutrients (TDN; 4779.87 g/d vs. 4446.94 g/d), alongside composite diet nutrient densities (DCP % and TDN %), were significantly greater in SEL cows relative to CON.

The physiological mechanism underlying enhanced fibre and protein digestibility involves the essential role of

trace minerals and lipophilic antioxidants in supporting ruminal microbial ecosystem dynamics. Selenium is an indispensable catalytic trace element for cellulolytic and amylolytic anaerobic rumen microorganisms, wherein selenoproteins protect microbial cell membrane lipids from oxidative degradation (Weiss et al., 1990; Zhang et al., 2020). Furthermore, Vitamin E functions synergistically within microbial cell membranes to quench free radicals generated during anaerobic fermentation, thereby maintaining bacterial membrane fluidity, enhancing microbial enzyme secretion, and optimizing structural carbohydrate breakdown (Mudgal, 2005; Gong et al., 2018). These findings demonstrate that pre-partum micronutrient fortification optimizes the efficiency of ruminal digestive processes, enabling crossbred Vrindavani cows to extract greater net energy and protein from a fixed voluntary feed intake during late pregnancy.

Pre-partum Metabolic Homeostasis and Endocrine Regulation

During the late-gestation transition phase, accelerating fetal energy requirements coupled with steady or declining voluntary intake triggers homeorhetic adaptations, characterized by adipose tissue triacylglycerol mobilization and elevated circulating non-esterified fatty acids (NEFA) (Bell, 1995; Schonfeld and Wojtczak, 2008). In the current trial, serum glucose concentrations declined substantially from baseline (Day -45) to calving (Day 0) across both groups, reflecting heavy fetal glucose utilization and uterine metabolic demands (Grummer, 1995; Bradford et al., 2015). However, circulating NEFA concentrations were significantly lower in SEL cows compared to CON prior to calving. Excessive circulating NEFA levels disrupt mitochondrial electron transport chains, promote hepatic triglyceride accumulation, and exacerbate systemic oxidative stress (Schonfeld and Wojtczak, 2008; Abuelo et al., 2014). The observed suppression of pre-partum NEFA mobilization in SEL cows indicates enhanced metabolic stability, reduced lipolysis rate, and improved insulin sensitivity, in agreement with findings by Chalmeh et al. (2021) in peripartum cows supplemented with selenium and vitamin E.

Endocrine evaluation revealed significant elevations in serum triiodothyronine (T3) and Insulin-like Growth Factor-1 (IGF-1) concentrations in SEL cows relative to CON. Thyroid hormones are central regulators of basal metabolic rate, cellular respiration, and tissue protein synthesis in dairy cattle (Kurpiska and Skrzypczak, 2019). The biological mechanism driving elevated circulating T3 levels resides in the enzymatic conversion of precursor tetraiodothyronine (T4) to active T3 by peripheral type I iodothyronine 5'-deiodinase (ID-1), a selenoenzyme that requires selenium as a critical structural cofactor (Beckett et al., 1987; Mudgal et al., 2011). Adequate selenium status ensures optimal ID-1 activity, maintaining peripheral T3 generation during late pregnancy. Concurrently, elevated circulating IGF-1 concentrations reflect an improved hepatic energy balance and somatotrophic axis responsiveness prior to calving (Simmen et al., 1998). Because IGF-1 regulates cell proliferation and metabolic homeostasis, elevated pre-partum IGF-1 levels signal enhanced tissue integrity and metabolic readiness for the onset of lactation.

Systemic Antioxidant Defenses and Mitigation of Oxidative Stress

Late-gestation transition cows routinely experience an oxidative stress surge driven by increased metabolic oxygen consumption, mitochondrial ROS generation, and rapid lipid turnover (Sordillo and Raphael, 2013; Abuelo et al., 2014). If unchecked by endogenous antioxidants, ROS react with polyunsaturated fatty acids in cell membranes, initiating lipid peroxidation cascades that yield toxic malondialdehyde (MDA) and compromise cellular function (Halliwell and Gutteridge, 1999).

In the present study, pre-partum supplementation with Selenium and Vitamin E significantly enhanced enzymatic antioxidant defenses and suppressed lipid peroxidation at calving. Serum Glutathione Peroxidase (GSH-Px) and Catalase (CAT) activities were significantly higher in SEL cows relative to CON on Day 0. Glutathione peroxidase is a metalloenzyme requiring selenium as an essential catalytic cofactor to reduce toxic hydrogen peroxide (H₂O₂) and lipid hydroperoxides into water and organic alcohols (Spears and Weiss, 2008; Panev et al., 2013). Catalase operates in tandem with GSH-Px, decomposing excess H₂O₂ and preventing the formation of highly destructive hydroxyl radicals via Fenton reactions (Munteanu and Apetrei, 2021). Concurrently, Vitamin E functions as a primary chain-breaking antioxidant within cell membrane lipid bilayers, neutralizing peroxy radicals and halting lipid peroxidation propagation (Halliwell and Gutteridge, 1999; Reboul, 2017).

Consequently, serum Lipid Peroxidation (LPO / MDA) levels on the day of calving were significantly lower in SEL cows (3.87 nmol MDA/30 min) compared to CON (4.29 nmol MDA/30 min). Correspondingly, Total Antioxidant Capacity (TAC %), which measures the cumulative free-radical scavenging activity of biological fluids, was significantly higher in SEL cows (63.80% vs. 59.19% on Day 0). These results confirm that strategic pre-partum supplementation with Selenium and Vitamin E reinforces both enzymatic (GSH-Px, CAT) and non-enzymatic antioxidant networks, preserving systemic redox equilibrium in Vrindavani cows during late pregnancy (Koujalagi et al., 2020; Chalmeh et al., 2021).

Immunological Status and Inflammatory Response

Peri-partum immunosuppression is a well-documented phenomenon in high-yielding and crossbred dairy cattle, characterized by declining circulating immunoglobulins and impaired leukocyte function during the final weeks before calving (Sordillo, 2005; Chandra, 2009). In our investigation, serum Immunoglobulin G (IgG) concentrations decreased substantially at calving across both groups, driven by the active transcellular transport of circulating IgG into the mammary gland for colostrumogenesis (Chandra, 2009; Sordillo and Raphael, 2013). However, SEL cows maintained a significantly higher overall serum IgG concentration (29.64 g/mL) compared

to CON (28.05 g/mL) throughout the pre-partum timeline. Furthermore, serum Interleukin-2 (IL-2) concentrations were significantly higher in SEL cows at calving relative to CON.

Vitamin E and Selenium exert potent immunomodulatory effects by protecting immunocompetent lymphocytes and phagocytes from self-induced oxidative destruction during metabolic activation (Tengerdy et al., 1973; Spears and Weiss, 2008). By scavenging intracellular ROS, Vitamin E protects B-lymphocyte cell membrane receptors, promoting immunoglobulin synthesis and humoral antibody responses (Chatterjee et al., 2003; Hoffmann et al., 2010). Concurrently, elevated circulating IL-2 concentrations in SEL cows indicate sustained T-helper cell proliferation and cell-mediated immune signaling, which are critical for orchestrating systemic host defenses during the high-risk peri-partum period (Sordillo, 2005).

Conclusions and Practical Implications

In conclusion, strategic dietary supplementation with Selenium (0.3 mg/kg DM) and Vitamin E (1000 IU/cow/day) during the 45-day pre-partum period (Day -45 to Day 0) effectively optimizes late-gestation transition management in crossbred Vrindavani dairy cattle. This combination enhances apparent fibre and protein digestibility, improves the plane of nutrient intake, attenuates pre-partum lipolysis (NEFA), supports thyroid hormone kinetics (T3) and somatotrophic axis activity (IGF-1), elevates systemic antioxidant enzyme capacity (GSH-Px, CAT, TAC), and maintains humoral immunoglobulin (IgG) status at calving. These physiological benchmarks provide a strong scientific rationale for incorporating trace antioxidant micronutrients into pre-partum ration formulations for crossbred dairy cows managed under tropical environmental conditions.

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