



Organic Selenium and Zinc: Their Effects in Feed on Blood Profiles and Antioxidant Capacity in Early-Lactating Dairy Cows

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Abstract | During early lactation, dairy cows often experience reduced immune cell function, including lymphocytes, and changes in blood metabolites, which can impact their health and production. Essential trace minerals (TM), such as selenium and zinc. They support enzyme function, immune response, and antioxidant defences. Moreover, this current research assessed the impact of supplementing organic selenium and zinc on dairy cows' blood biochemical, haematology, and total antioxidant capacity (T-AOC) during early lactation. Sixteen crossbred Friesian Holstein cows were divided into two distinct groups. The first group (control: CON) was provided with a basal diet. In contrast, the treatment group (SUP) received the same basal diet but with added selenium and zinc supplements at dosages of 0.45 mg/kg and 60 mg/kg of dry matter (DM), respectively, based on our previous studies. The selenium and zinc were provided in organic chelated-methionine forms. The experimental feeding lasted for 49 days. Blood samples were drawn from each cow at the trial's conclusion and placed in tubes containing anticoagulants. Whole blood was used for haematological analysis with a haematology analyzer. Additional samples were collected in sterile tubes without anticoagulants and centrifuged to obtain serum. The serum was then used to assess blood biochemistry parameters and T-AOC with an automatic biochemical analyzer and a commercial kit. The findings showed no significant changes in the levels of AST, ALT, total protein, glucose, BUN, triglycerides, cholesterol, HDL, LDL, or creatinine ($P > 0.05$) due to supplementation. However, the SUP group showed a significant increase in WBC (41.50%), lymphocyte count (74.70%), and MCHC (2.20%) ($P < 0.05$), with no influence on other haematological parameters ($P > 0.05$). Additionally, the SUP group exhibited significantly higher T-AOC levels (63.28%) ($P < 0.05$). In conclusion, supplementing with combined organic selenium and zinc (0.45 and 60 mg/kg DM) can improve the antioxidant status of early-lactating dairy cows without negatively affecting their blood metabolite profiles. These results suggest potential implications for using essential organic TM during early lactation. However, further research with a longer duration related to dairy cow productivity is needed to confirm these findings.

Keywords | Antioxidant capacity, Blood biochemistry, Dairy cows, Haematology

Received | July 22, 2024; **Accepted** | August 11, 2024; **Published** | November 01, 2024

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Citation | Anam MS, Agus A, Widyobroto BP, Gunawan, Astuti AA (2024). Organic selenium and zinc: Their effects in feed on blood profiles and antioxidant capacity in early-lactating dairy cows. Adv. Anim. Vet. Sci. 12(12): 2512-2522.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2024/12.12.1512.1522>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331



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The initial 60 to 90 days post-calving in dairy cows are critical, requiring significant physiological adjustments. During this period, cows face substantial metabolic stress due to increased energy demands for peak milk production. A negative energy balance (NEB) is expected, characterized by elevated blood non-esterified fatty acid levels, indicating body fat mobilization to meet energy (Brady *et al.*, 2021; Gross, 2023). These metabolic changes are accompanied by complex hormonal fluctuations, such as growth hormone and cortisol increases and insulin decreases (Barletta *et al.*, 2017; Qiao *et al.*, 2024). Cortisol, the stress hormone, can suppress immune function, increasing susceptibility to infections such as mastitis (Sordillo and Mavangira, 2014). During the initial phases of lactation, dairy cows experience a reduction in both the quantity and function of immune cells, such as lymphocytes, which are vital in protecting against infections (Lacetera *et al.*, 2005). The NEB and accompanying hormonal changes have been associated with this decline in immune function.

Another significant challenge dairy cows face during early lactation is increased oxidative stress. This condition is associated with elevated levels of reactive oxygen species (ROS) generated as byproducts of energy production in the mitochondria. Oxidative stress occurs when the body's ability to neutralize ROS is overwhelmed by its excessive production through its antioxidant defence mechanisms. Under normal physiological conditions, the production and elimination of ROS are balanced, keeping ROS levels very low, where they are both beneficial and non-harmful. However, during early lactation, ROS production tends to increase while antioxidant defence capacity decreases (Abuelo *et al.*, 2015). In this phase, cows experience a higher level of oxidative stress and a reduced antioxidant defence compared to the late pregnancy stage (Tufarelli *et al.*, 2023). Additionally, increased free radicals and oxidative stress during this period can damage cells and tissues, further reducing the total antioxidant capacity in cows (Lopreato *et al.*, 2020). This can cause cell and tissue damage and potentially affect the health and productivity of dairy cows. Oxidative stress heightens the vulnerability of dairy cows to various diseases (Lalot *et al.*, 2020).

Nutrition for ruminants is vital for the well-being of dairy cows' productivity, especially during the intensive lactation stage. Trace minerals (TM), such as selenium and zinc, are crucial for numerous physiological processes, including enzyme function, antioxidant defences, and the immune response. Selenium is a fundamental part of the glutathione peroxidase (GSH-Px) enzyme, which helps alleviate oxidative stress, while zinc is vital for protein synthesis, enzyme activity, and the maintenance of cell membrane integrity (Spears and Weiss, 2008; Yatoo *et al.*, 2013). Selenium is

an effective antioxidant in neutralizing free radicals and preventing the formation of ROS (Kang *et al.*, 2020). Zinc functions as an antioxidant by serving as a crucial cofactor for superoxide dismutase (SOD), which plays a crucial role in eliminating intracellular ROS that can damage biomolecules like DNA, proteins, and lipids. This enzyme protects cells from ROS-induced damage by converting superoxide anions into oxygen and hydrogen peroxide (Li *et al.*, 2015). However, the availability of selenium and zinc as TM in feed varies widely depending on soil conditions, feed type, and other factors (Overton and Yasui, 2014; Wang *et al.*, 2019). Since the ruminant's body cannot synthesize these trace minerals, they must be provided externally to meet their needs, especially during the early lactation phase in dairy cows, such as through supplementation (Ullah *et al.*, 2020). Research indicates that supplementing with selenium or zinc in cows can boost immune function and antioxidant capacity, particularly during the crucial early lactation period (Duffy *et al.*, 2023; Xiao *et al.*, 2021).

The focus on organic forms of selenium and zinc has grown due to their superior bioavailability to inorganic forms (Anam *et al.*, 2023a; Mion *et al.*, 2023). Additionally, organic forms offer increased tissue retention and less toxicity than inorganic forms (Barbé *et al.*, 2019; Cortinhas *et al.*, 2012). Many researches have shown the advantages of these organic minerals for animal health, rumen functionality, and overall productivity (Anam *et al.*, 2022; Anam *et al.*, 2023b; Chen *et al.*, 2020; Danesh Mesgaran *et al.*, 2022; Hachemi *et al.*, 2023; Salama *et al.*, 2003; Salles *et al.*, 2022). Research by Gong *et al.* (2014), indicated that organic selenium supplementation improves dairy cows' antioxidant status and immune function. Selenium enhances the antioxidant system in cows by boosting antioxidant defences and lowering oxidative stress levels (Surai *et al.*, 2019). Cows receiving organic selenium during the transition period demonstrated decreased lipid peroxidation, lower reactive oxygen species and plasma hydrogen peroxide levels, and improved GSH-Px activity concentrations (Gong and Xiao, 2018). Furthermore, cows supplemented with organic selenium showed greater total antioxidant capacity (T-AOC) during heat stress than those fed inorganic selenium (Sun *et al.*, 2017). Likewise, Oconitrillo *et al.* (2024) discovered that cows fed organic zinc as methionine at 100 mg/kg in their diet exhibited enhanced milk production and immune function. There were also improvements in blood haematology profiles in dairy cows supplemented with organic zinc (Dresler *et al.*, 2023). Through *in vitro* studies, our previous research recommended the use of 0.45 mg/kg dry matter (DM) of selenium and 60 mg/kg DM of zinc (Anam *et al.*, 2023a,b) as they positively impact rumen enzyme activity in ruminant animals.

Interest in evaluating the impact of combining antioxidants has recently increased. Since many antioxidants function

synergistically, using a combination of antioxidants in treatments appears more beneficial than relying on a single antioxidant alone. The combined supplementation of selenium and zinc was observed to enhance antioxidant levels in mature rams synergistically. This increase in antioxidant levels may improve reproductive performance (Ghorbani *et al.*, 2024). Positive effects of combined selenium and zinc treatment in reducing oxidative stress have also been documented in Nile tilapia (Ghazi *et al.*, 2022). However, those two studies were not conducted on dairy cows. There is limited evidence on the combined impacts of dietary selenium and zinc, and further research is needed to explore their potential impact on antioxidant status and biochemical-hematological blood parameters in early-lactating dairy cows. We hypothesized that supplementing organic selenium and zinc would enhance the T-AOC in early-lactating dairy cows, as well as improve several blood metabolites, which serve as critical indicators of overall health, metabolic status, and the animal's ability to cope with physiological challenges during lactation. Hence, this current research assessed the impact of supplementing organic selenium and zinc on dairy cows' blood biochemical, haematology, and T-AOC during early lactation.

MATERIALS AND METHODS

DESIGN OF THE TRIAL

This current trial was conducted at Sarono Makmur Farm in Yogyakarta, Indonesia (-7.65798° N, 110.44358° E) from November 2023 to February 2024. All experimental procedures were approved by the Institutional Ethics Committee and Animal Care, Universitas Gadjah Mada (Approval No. 025/2023). The experiment involved sixteen Friesian Holstein crossbred dairy cows, both multiparous (2.38 ± 0.52) and primiparous, with body weight (452.09 ± 35.45 kg) in early lactation (32.65 ± 5.22 days in milk) and good health. The cows were allocated into two equal groups, each consisting of eight individuals. The first group (CON) was provided with a basal diet. In contrast, the treatment group (SUP) received the same basal diet but with added selenium and zinc supplements at dosages of 0.45 mg/kg and 60 mg/kg DM, respectively. The addition used chelated methionine forms of selenium and zinc based on dosage recommendations from our previous studies (Anam *et al.*, 2024; Anam *et al.*, 2023a,b). Selenium and zinc supplementation was carried out for 49 days. This duration supplementation method was limited to a short period based on previous research (Kumar *et al.*, 2023). Selenium and zinc were administered by top-dressing and thoroughly mixed into the concentrate feed to ensure even distribution.

All groups received identical diets in composition and quantity, the only difference being the inclusion or exclusion of selenium and zinc. The basal diet consisted of 29.25% el-

ephant grass, 12.40% corn plants, 3.83% rice straw, 7.93% Bermuda grass, and 46.59% mixed concentrate, all proportions on a dry matter basis. The nutrient content of the basal diet included 52.20% DM, 89.31% organic matter, 15.76% crude protein, 4.79% ether extract, 31.33% acid detergent fiber, and 54.25% neutral detergent fiber. The animals were fed twice daily, with milking performed beforehand. All the cows were also given unlimited access to water. During the study, feed intake was calculated by measuring the amount of feed given and the amount remaining. The average feed intake for the CON and SUP groups was 17.27 and 17.08 kg DM, respectively. Since this study occurred in a tropical country, daily measurements of the temperature-humidity index (THI) were taken following the procedure detailed by Yan *et al.* (2021). The average THI recorded during the trial was 76.06.

BLOOD COLLECTION AND DETERMINATION

Blood samples were drawn from each cow at the end of the feeding period. Sampling was done four hours post-morning feeding via the jugular vein, using 3-ml EDTA K3 tubes (Vaculab®, Surabaya, Indonesia) for haematological analysis. Blood samples were analyzed for haematological parameters on the same day. Haematological parameters were measured using a 5-diff fully auto haematology analyzer. For biochemical analysis, fresh blood was collected into 10-ml sterile tubes without anticoagulants (Vaculab®, Surabaya, Indonesia). After clotting, the serum was isolated by centrifugation at 2,000 rpm for 15 minutes (Anam *et al.*, 2021). Serum biochemical parameters were evaluated using an automatic biochemical analyzer (DiaSys Diagnostic System, Holzheim, Germany). T-AOC of the serum was measured using a commercial kit by the manufacturer's guidelines (Elabscience Biotechnology Inc., Texas, HT, USA).

STATISTICAL ANALYSIS

Statistical analysis was performed with IBM SPSS® Statistics (V26) software. The Shapiro-Wilk test was employed to evaluate data normality, confirming that all data followed a normal distribution. The impact of the treatment was analyzed with an independent sample T-test (SUP vs CON). A *P-value* of less than 0.05 was deemed statistically significant. Additionally, Cohen's *d* was used to calculate the effect size, categorizing it as small (≥ 0.2), moderate (≥ 0.5), or large (≥ 0.8) (Cohen, 1988).

RESULTS AND DISCUSSION

BLOOD BIOCHEMISTRY

The biochemical blood profile of early-lactating dairy cows receiving organic selenium and zinc supplements is presented in Table 1. Blood metabolites are commonly regarded as rapid and reliable indicators for assessing the health status of livestock (Ellis *et al.*, 1997; Longnecker *et al.*, 1996). In this research, the supplementing selenium

Table 1: Blood biochemical profiles of early-lactating dairy cows receiving organic selenium and zinc supplementation.

Items	Treatment		SEM	P-value	95% Confidence Interval		Effect Size
	CON	SUP			Lower	Upper	
AST, U/l	70.96	67.03	2.01	0.345	-1.477	0.516	-0.489
ALT, U/l	26.15	22.43	1.11	0.093	-1.923	0.146	-0.903
Glucose, mg/dl	50.01	49.75	1.83	0.946	-1.014	0.946	-0.035
Total protein, g/dl	7.88	7.87	0.10	0.939	-1.018	0.942	-0.039
BUN, mg/dl	16.57	16.31	0.59	0.833	-1.086	0.875	-0.107
Triglycerides, mg/dl	5.33	6.35	0.54	0.357	-0.528	1.463	0.476
Cholesterol, mg/dl	142.84	155.18	7.08	0.402	-0.568	1.417	0.432
HDL, mg/dl	56.09	65.58	2.70	0.078	-0.105	1.976	0.950
LDL, mg/dl	39.26	41.15	2.15	0.676	-0.773	1.193	0.214
Creatinine, mg/dl	0.74	0.80	0.02	0.175	-0.312	1.716	0.714

AST: aspartate aminotransferase; **ALT:** alanine aminotransferase; **BUN:** blood urea nitrogen; **HDL:** high-density lipoprotein; **LDL:** low-density lipoprotein. **SEM:** standard error of the mean.

and zinc did not significantly influence the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum ($P>0.05$). Specifically, the AST level in the CON was 70.96 U/l, while the SUP recorded 67.03 U/l. Furthermore, ALT levels were 26.15 U/l in the CON group and 22.43 U/l in the SUP group. The measurement of AST and ALT activity is a good indicator for assessing liver metabolic function in livestock (AlSuwaiegh *et al.*, 2022). The lack of changes in AST and ALT levels indicates that supplementing with selenium and zinc at 0.45 mg/kg DM did not impact liver function. Similar findings were outlined by Żarczyńska *et al.* (2020), who found that the daily addition of 300 mg of selenium as selenitetriglycerides had no impact on dairy cows's liver function. Juniper *et al.* (2006) found no changes in AST and ALT blood in cows given selenium-formed organic or inorganic. Even when high doses of selenium (0.5 and 5 mg Se/kg) were used, there was no impact on AST levels in cows, suggesting that these doses are safe concerning liver function (Sun *et al.*, 2021). An elevation in AST levels is a reliable marker of selenium toxicity in ruminants (Żarczyńska *et al.*, 2020). Additionally, ALT and AST levels remained unaffected by various dietary zinc sources (Alijani *et al.*, 2020). Juniper *et al.* (2006) observed a comparable pattern with selenium supplementation. Normal ranges for AST are typically between 68-82 U/l (Zaitsev *et al.*, 2020), while ALT levels observed in this study fall within the normal range of 11-40 U/l (Kaneko *et al.*, 2008).

Serum glucose levels were not significantly altered by selenium and zinc supplementation ($P>0.05$). The serum glucose concentration in the CON group was 50.01 mg/dl, while the SUP group recorded 49.75 mg/dl. These values fall within the reference range for dairy cows, typically between 40 and 60 mg/dl (Mair *et al.*, 2016). The absence of an effect on serum glucose levels may be linked to feed metabolism within the rumen. Rumen serves as a sophisticated ecosystem where microbes ferment dietary components.

The efficiency of this fermentation process plays a crucial role in determining the availability of glucose for absorption. For instance, elevated levels of volatile fatty acids (VFA) produced in the rumen can enhance glucose uptake by the liver, thereby influencing blood glucose concentrations (Zheng *et al.*, 2024). Tian *et al.* (2022) demonstrated that administering 4.8 mg of selenium yeast did not change the total VFA levels in the rumen. Furthermore, after four weeks of supplementation, 0.40 mg/kg DM selenium yeast had no significant impact on serum glucose levels in dairy cows (Juniper *et al.*, 2006).

No significant differences were found in total protein levels and blood urea nitrogen (BUN) between the groups ($P>0.05$), indicating that supplementation did not influence the cows' protein status. The total protein levels observed are consistent with findings from Mohammed *et al.* (2021). According to Broderick (2003), maintaining good protein status is essential for animal health and productivity. The stable BUN levels indicate that supplementation did not negatively impact nitrogen metabolism. Juniper *et al.* (2006) observed that administering selenium yeast at doses up to 0.45 mg/kg did not affect dairy cows' total protein, albumin, or BUN levels. However, Bakhshizadeh *et al.* (2019) found that using various forms of zinc at 60 mg/kg DM reduced serum BUN levels in dairy cows. This discrepancy may be attributed to differences in TM form, dosage, duration, and other experimental protocols. According to Webb and de Bruyn (2021), in ruminant metabolism, BUN functions as a critical end product of the urea cycle and serves as an essential marker for assessing nitrogen metabolism. The breakdown and utilization of BUN involve the degradation of dietary proteins by rumen microbes, deamination to produce ammonia, conversion of ammonia to urea, recycling of urea back to the rumen, and utilization of nitrogen by microbes for protein synthesis. This complex process ensures efficient nitrogen utilization and maintains

the productivity and health of ruminants (Maxiselly *et al.*, 2022). However, some studies have not shown any changes in nitrogen fermentation in the rumen with the dietary of selenium or zinc (Liu *et al.*, 2020; Petrič *et al.*, 2021; Zhang *et al.*, 2020).

Regarding the blood lipid profiles, the supplementation of selenium and zinc did not significantly affect triglycerides, cholesterol, high-density lipoprotein (HDL) or low-density lipoprotein (LDL) ($P>0.05$). Overall, the blood lipid levels observed in the current trial were consistent with findings from Cozzi *et al.* (2011). The blood lipid profile in cows is affected by the formulation of their diet, rumen digestive fermentation, lipid mobilization from adipose tissue, lipoprotein metabolism, and hormonal regulation (Di Meo *et al.*, 2023; Imhasly *et al.*, 2015). Selenium and zinc supplements did not impact blood creatinine levels ($P>0.05$). The serum creatinine level in the CON was 0.74 mg/dl, while the SUP group recorded 0.80 mg/dl. Creatinine levels serve as indicators of kidney function, with reference ranges for dairy cattle (Cozzi *et al.*, 2011). Kumar *et al.* (2023) indicated that adding selenium combined with probiotics did not change tropical dairy cows' blood creatinine levels.

In this current investigation, combining organic selenium and zinc did not affect the blood biochemical parameters in early lactation cows. Several studies have reported that selenium addition in feed has minimal impact on blood biochemistry (Juniper *et al.*, 2006; Phipps *et al.*, 2008; Sun *et al.*, 2017). Various factors can influence the biochemical blood profile, including the form of the mineral used in the feed, the type and physiological condition of the livestock, and the composition and nutrient content of the ration (Arshad *et al.*, 2021; Cortinhas *et al.*, 2012; Ullah *et al.*, 2020). Furthermore, the differences observed could also be due to variations in the form of TM, dosage, duration, and other experimental protocols.

BLOOD HAEMATOLOGY

The blood haematology profile is a valuable indicator for assessing the physiological status of animals and is positively correlated with nutrient status (Dalia *et al.*, 2020). Table 2 shows the haematological profile, where selenium and zinc supplementation significantly increased white blood cell (WBC), lymphocyte count, and mean corpuscular haemoglobin concentration (MCHC) ($P<0.05$) but did not substantially affect neutrophil, neutrophil, monocyte, eosinophil counts, red blood cell (RBC), haemoglobin, and others ($P>0.05$).

The findings align with those of Shinde *et al.* (2009), who administered 0.3 mg/kg DM sodium selenite to young buffaloes and found no significant changes in RBC count,

hematocrit, or haemoglobin levels. Similarly, during the transition period, Khalili *et al.* (2020), observed no alterations in haematological parameters such as RBC, haemoglobin, and hematocrit in dairy cows feeding organic or inorganic selenium. Bakhshizadeh *et al.* (2019) also reported no changes in blood haematology parameters in Holstein dairy cows after supplementing their diet with 60 mg/kg DM of different zinc forms, including oxide, glycine, and nano zinc. In contrast, Hachemi *et al.* (2023) noted that incorporating lactating dairy cows with 0.1 mg/kg DM hydroxy-selenomethionine (OH-SeMet) increased RBC and haemoglobin but a higher dose of 0.3 mg/kg DM had no significant impact. Additionally, Ulutaş *et al.* (2020), also documented an increase in RBC and haemoglobin levels in goats given a high dose of 1,000 mg/kg zinc. Sobhanirad and Naserian (2012) similarly found that a 500 mg/kg zinc dose elevated RBC and haemoglobin levels in dairy cows.

In this current observation, adding selenium and zinc at 0.45 and 60 mg/kg DM resulted in a 74.70% increase in lymphocyte levels. The effect size of -1.797 suggests that the SUP treatment greatly influenced lymphocyte levels. Selenium functions as an antioxidant by mitigating oxidative stress in lymphocytes. It helps in scavenging active oxygen species, thereby protecting lymphocytes from damage caused by ROS. This is evident from studies showing that selenium supplementation decreases lipid peroxide levels in lymphocytes, which indicates oxidative stress (Huang *et al.*, 2012; Jin *et al.*, 2023). Zinc is crucial for lymphocyte activation. It promotes the upregulation of A20 mRNA, which inhibits NF- κ B activation, resulting in decreased inflammation and a strengthened immune response (Prasad, 2008). Lymphocytes are crucial for adaptive immunity and vital in controlling humoral and cellular immunities (Broome *et al.*, 2004; Hoffmann and Berry, 2008). However, other studies have not reported changes in lymphocyte levels with dietary selenium supplementation (Khalili *et al.*, 2020; Ulutaş *et al.*, 2020) or zinc (Bakhshizadeh *et al.*, 2019; Oconitillo *et al.*, 2024).

This study's increase in WBC with selenium and zinc supplementation was 41.50% compared to the un-supplemental group. The effect size of 2.370 suggests that selenium and zinc supplementation greatly impacted WBC levels. This phenomenon is due to increased individual cell counts, particularly lymphocytes, which showed significantly higher values than the control. Lymphocytes are generated in lymphoid tissue and comprise WBC (Roland *et al.*, 2014). Lymphocytes comprise more than 50% of the blood leukocyte population in cattle (Farschtschi *et al.*, 2022). An increase in WBC indicates enhanced immune function (Kvidera *et al.*, 2017). This study aligns with Hachemi *et al.* (2023), who reported a 9.41% increase in WBC with 0.3 mg/kg hydroxy-selenomethionine addition in dairy cows.

Table 2: Blood haematological profiles of early-lactating dairy cows receiving organic selenium and zinc supplementation.

Parameter	Treatment		SEM	P-value	95% Confidence Interval		Effect Size
	CON	SUP			Lower	Upper	
WBC, 10 ³ /μl	8.86 ^b	12.52 ^a	0.68	0.003	0.594	2.956	1.797
Neutrophils, 10 ³ /μl	4.01	4.73	0.23	0.114	-0.198	1.857	0.843
Lymphocytes, 10 ³ /μl	3.64 ^b	6.35 ^a	0.44	<0.001	1.037	3.657	2.370
Monocytes, 10 ³ /μl	0.20	0.32	0.04	0.184	-0.325	1.701	0.699
Eosinophils, 10 ³ /μl	1.01	1.13	0.24	0.803	-0.856	1.106	0.127
As % of the total							
Neutrophils, %	46.24	38.10	2.26	0.170	-2.010	0.078	-0.982
Lymphocytes, %	41.75	50.45	2.26	0.050	0.000	2.112	1.072
Monocytes, %	2.10	2.46	0.27	0.527	-0.669	1.306	0.324
Eosinophils, %	9.91	8.94	1.59	0.771	-1.127	0.836	-0.149
RBC, 10 ⁶ /μl	5.56	5.83	0.13	0.319	-0.490	1.506	0.517
Haemoglobin, g/dl	9.21	9.23	0.13	0.964	-0.958	1.003	0.023
PLT, 10 ³ /μl	474.00	466.25	38.23	0.923	-1.028	0.932	-0.049
Hematocrit, %	28.33	27.71	0.42	0.485	-1.341	0.637	-0.358
MCV, fL	51.13	47.80	0.97	0.086	-1.945	0.129	-0.922
MCH, pg	16.66	15.91	0.28	0.192	-1.687	0.337	-0.686
MCHC, g/dl	32.60 ^b	33.31 ^a	0.13	0.002	0.722	3.154	1.961
RDW-CV, %	20.16	21.41	0.42	0.144	-0.258	1.782	0.775
MPV, fL	6.21	6.70	0.14	0.089	-0.137	1.935	0.913
PDW	15.24	15.09	0.07	0.332	-1.491	0.503	-0.502
PCT, %	0.29	0.22	0.03	0.208	-1.658	0.361	-0.659

^{ab}Superscript letters on the same row denote statistically significant differences at a significance level of $P < 0.05$; **WBC**: white blood cell; **RBC**: red blood cell; **PLT**: platelets; **MCV**: mean corpuscular volume; **MCH**: mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration; **RDW-CV**: red cell distribution width-standard deviation; **MPV**: mean platelet volume; **PDW**: platelet distribution width; **PCT**: plateletcrit; **SEM**: standard error of the mean.

Similarly, [Dresler et al. \(2023\)](#) found a 33.61% increase in WBC with 30 mg zinc/kg DM in the chelated-methionine form in postpartum dairy cows at 60 days compared to the control. Nevertheless, some researchers have reported no changes in WBC levels with selenium or zinc supplementation ([Oconitrillo et al., 2024](#); [Żarczyńska et al., 2020](#)).

Furthermore, the combination of selenium and zinc increased MCHC by up to 2.20%. The effect size of 1.961 suggests that the SUP treatment significantly impacted MCHC levels relative to CON. This effect may be related to the ability of trace minerals to regulate the hematopoietic system. Selenium is crucial for the effective operation of the hematopoietic system. It helps synthesize haemoglobin, which can lead to an increase in MCHC. Research has demonstrated that elevated serum selenium levels are linked to higher serum iron levels and increased MCHC ([Zhou et al., 2021](#)). Moreover, high levels of zinc sulfate monohydrate and zinc-methionine (500 mg/kg) increased MCHC in early-lactating dairy cows ([Sobhanirad and Naserian, 2012](#)). In contrast, a combination of selenium, zinc, vitamin E, copper, and manganese in dairy cows did not affect MCHC before and after calving ([Chen et al.,](#)

[2023](#)). Nonetheless, the blood haematology profiles in this observation remained within normal ranges ([Wood and Quiroz-Rocha, 2010](#)).

TOTAL ANTIOXIDANT CAPACITY

T-AOC is an essential indicator of the body's ability to counter oxidative stress, which can lead to cellular and tissue damage ([Tufarelli et al., 2023](#)). Compared to CON, the SUP group exhibited significantly higher T-AOC levels ($P < 0.05$) ([Figure 1](#)). This study found that dietary selenium and zinc increased T-AOC levels by 63.28%. The effect size of 1.891 suggests that the supplementation treatment greatly impacted T-AOC levels. Selenium is a crucial TM in the GSH-Px, an enzyme detoxing hydrogen peroxide and lipid peroxides ([Khalili et al., 2020](#); [Xiao et al., 2021](#)). This activity helps neutralize oxidative stress, thereby enhancing the T-AOC of dairy cows. Zinc is a critical element in various antioxidant enzymes, such as SOD and GSH-Px. These enzymes help neutralize ROS, thereby reducing oxidative ([Chen et al., 2023](#)). The finding aligns with the reports of [Sun et al. \(2021\)](#), who found that selenium-yeast addition tends to increase T-AOC in dairy cows. Mid-lactation dairy cows fed hydroxy-analogue of

selenomethionine showed increased serum T-AOC in both inorganic selenium-supplemented and control groups (Sun *et al.*, 2017). Patel *et al.* (2021) found that under heat-stress challenges, feeding with 60 mg/kg DM of zinc increased T-AOC levels in Karan-Freis cows after 45 days postpartum. Additionally, Chen *et al.* (2020) noted that feeding 40 and 60 mg/kg DM organic zinc significantly increased T-AOC levels in multiparous dairy cows. These findings imply that dietary selenium and zinc in organic form could effectively enhance cows' antioxidant capacity, potentially reducing the negative impacts of oxidative stress. Although T-AOC in serum can be a valuable biomarker for assessing health conditions, it also has several limitations that should be considered, including diet, age, environmental conditions, and other factors (Kim *et al.*, 2016; Michałek *et al.*, 2020; Rubio *et al.*, 2016).

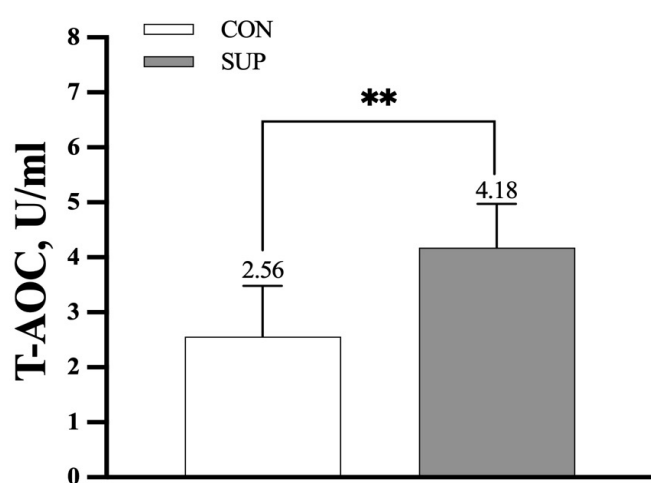


Figure 1: Effect of dietary selenium and zinc on dairy cows total antioxidant capacity (T-AOC). Data are presented as mean $\pm$ SE, n=8. CON: control group; SUP: selenium and zinc supplementation group. P-value: 0.002. 95% confidence interval, lower: 0.668, upper: 3.070. Effect size: 1.891.

The current study's limitation is that the supplementation of selenium and zinc was conducted over a relatively short period, which may explain why many blood biochemical parameters were not significantly affected. Additionally, the limited sample size may have constrained the robustness of the data. Future studies should investigate the impacts of selenium and zinc supplementation over a longer duration, using a larger sample size, and consider relevant production and economic parameters.

CONCLUSIONS AND RECOMMENDATIONS

Based on our findings, supplementation with organic selenium and zinc can enhance the antioxidant status in early-lactating dairy cows without negatively affecting their blood metabolite profiles. However, significant changes were observed only in some parameters, while

others showed non-significant changes. It is essential to acknowledge that this study has limitations, including a short observation period and a small sample size. Therefore, specific recommendations for dairy farmers should be considered carefully, and it is crucial to evaluate the potential side effects and economic considerations of this supplementation. Future research is recommended to explore the long-term impacts of organic selenium and zinc supplementation and to test it in different farming conditions to obtain a more comprehensive understanding. Scaling up this study and conducting trials in various farm conditions may provide additional insights into the benefits and challenges of this supplementation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support and assistance the BRIN dan LPDP for Research and Innovation for Advanced Indonesia (B-733/II.7.5/FR.06/4/2023) provided.

NOVELTY STATEMENT

This current work provides an overview of the effects of trace mineral supplementation using a combination of organic selenium and zinc on the blood profile and antioxidant status in early-lactating dairy cows. These findings imply that dietary selenium and zinc in their organic forms can effectively enhance the antioxidant capacity of cows, potentially alleviating the adverse effects of oxidative stress while not impacting their blood metabolite profiles.

AUTHOR'S CONTRIBUTIONS

Moh Sofi'ul Anam and Ali Agus: Research design, data collection, and manuscript preparation.
Andriyani Astuti, Budi Prasetyo Widyobroto and Gunawan: data collection, interpretation, and reviewing.

CONFLICT OF INTEREST

No conflict of interest.

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