Veterinary Integrative Sciences 2025; 23(2): e2025044-1-14. DOI: 10.12982/VIS.2025.044

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)

Research article

Effect of dietary zinc supplementation on production performance, milk quality, immunity, and blood plasma of dairy cows: A meta-analysis

R.I Kusuma, I. Wijayanti*, Y. Retnani, A. Jayanegara and S.T Risyahadi

Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis, Kampus IPB Dramaga Bogor 16680, Indonesia

Abstract

A meta-analysis was conducted to evaluate the effects of Zinc supplementation on production performance, milk quality, immunity, and blood plasma of dairy cows. This study analyzed 25 related articles with 117 data points using a mixed model method with SAS web. The results showed that Zinc supplementation level increased ($P<0.05$) milk yield and decreased somatic cell count (SCC). Zinc supplementation level also linearly increased ($P<0.05$) IgM, IgG, and blood zinc. However, it does not have any effect on milk quality and blood biochemistry. In comparison to the control, both organic and inorganic Zinc sources increased ($P<0.05$) IgG, blood zinc, and decreased ($P<0.05$) SCC respectively. There was an interaction ($P<0.05$) observed between Zinc level and source on IgM and blood Zinc concentrations. Organic Zinc produced a higher effect ($P<0.05$) in increasing IgM and blood Zinc concentration than the inorganic source. It can be concluded that the provision of levels and sources of organic Zinc has a more favorable effect on dairy cows than inorganic Zinc.

Keywords: Dairy cows, Inorganic zinc, Meta-analysis, Organic zinc

***Corresponding author**: Indah Wijayanti, Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis, Kampus IPB Dramaga Bogor 16680, Indonesia. E-mail: wijayanti@apps.ipb.ac.id.

Article history; *received manuscript: 15 May 2024,*
revised manuscript: 20 June 2024,
accepted manuscript: 2 August 2024,
published online: 19 August 2024,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

The transition period in dairy cows refers to the critical time around calving when the cow transitions from late pregnancy to early lactation. This period typically a critical physiological stage, which spans three weeks before calving and three weeks after calving (De et al., 2014). Many metabolic and infectious diseases tend to occur during this period. Various diseases, such as milk fever, fatty liver syndrome, displaced abomasum, and ketosis, as well as reproductive disorders, including retained placenta, dystocia, uterine infections, and mammary gland infections (mastitis and udder edema), have been reported. (Sundrum, 2015; Tufarelli et al., 2024). The health of dairy animals during the transition period significantly affects their future productivity and reproductive ability. According to a study conducted by Bais and Singh (2018), dairy cows often experience impaired health during the transition period, leading to a loss of 4.54-9.07 kg of peak milk production, which is equivalent to 907.18-1814.37 kg of unused milk production in the world.

By increasing immunity, zinc supplementation throughout the transition period is essential to prevent viral and metabolic illnesses (Singh et al., 2020). Approximately 300 enzyme systems that are helpful for the body's metabolism, production of carbohydrates, and other biochemical processes relies on zinc to function (Kambe et al., 2015; Costa et al., 2023). The growth and function of immune cells, including monocytes, natural killer (NK) cells, macrophages, neutrophils, T cells, and B cells, are influenced by zinc (Ma et al., 2020). Zinc supplementation has been shown in studies by Chang et al. (2020) and Wang et al. (2021) to enhance milk production, blood levels of immunoglobulin, cytokinin, and lactation cows SCC. The sources of zinc usually used in dairy cows feed are either inorganic (e.g., ZnO, $ZnSO_4$, or $ZnCl_2$) or organic zinc (e.g., Zn proteinate, Zn amino acids, Zn picolinate, or Zn methionine. In a study conducted by Chandra et al. (2014), it was reported that administering 60 $mg.kg^{-1}$ $ZnSO_4$ increased milk production. However, another study by Lanni et al. (2019) found no substantial effect on milk production and lactation performance after administering 40-65 $mg.kg^{-1}$ ZnO. It is important to note that various studies have yielded conflicting results, potentially due to differences in zinc sources and concentrations. A meta-analysis can help resolve these discrepancies by combining data from previous research to provide more conclusive findings (Sauvant et al., 2008). Therefore, this study aimed to evaluate the effects of zinc levels and sources on production, milk quality, immunity, and blood plasma in dairy cows by integrating related studies using a meta-analysis method.

MATERIALS AND METHODS

Development Database

A search for potential articles was conducted in Scopus using the keywords "dairy," "supplement," and "zinc" to collect a database of studies. The journal screening results are shown in Figure 1. A total of 249 potential articles were obtained from 2005 to 2023, and after screening abstracts, 95 articles were obtained. The full-text review resulted in 65 articles, of which 25 met the inclusion criteria. Journals selected for database compilation had the following criteria: 1) the article was published in English, 2) the experiment was conducted on dairy cows, 3) the source and level of Zinc (mg.kg-1) were reported, and 4) there was a control treatment (not added), organic, and inorganic Zinc. The database criteria observed included journals with milk production parameters (kg.day-1), Dry Matter Intake (DMI) ($kg.day^{-1}$), milk fat (%), milk protein (%), Solid Non-Fat (%), milk lactose (%), SCC ($10^3 cells.ml^{-1}$), IgA ($\mu g.ml^{-1}$), IgG ($mg.ml^{-1}$), IgM ($mg.ml^{-1}$), total cholesterol ($mg.dl^{-1}$), glucose ($mg.dl^{-1}$), albumin ($mg.dl^{-1}$), total blood protein ($mg.ml^{-1}$), and blood zinc (mg.l^{-1}). The final study selection results showed that 25 journals with 117 data points can be submitted to the database (Table 1). In this study, the Zinc level used was 0–500 mg.kg-1 DM. When entering data into a database, the related variables were transformed into the same uniform units, which simplified the analysis.

Table 1 Articles used in the meta-analysis of dietary zinc supplementation in dairy cows

No	Article	Type of dairy cow	Feed	Source Zn	Level (mg.kg^{-1})
1	Aggarwal dan Chandra (2018)	Friesian Holstein	Corn	Zinc sulfate	0;60
2	Alhussein et al. (2021)	Karan-Fries	Wheat straw	Zinc sulfate	0;60
3	Azizzadeh et al. (2005)	Friesian Holstein	Corn	Zinc sulfate	0;50;100
4	Bakhzhizadeh et al. (2019)	Friesian Holstein	Corn silage	Zinc glycin, zinc nitrate, zinc oksida	0;60
5	Bordignon et al. (2019)	Friesian Holstein	Hay	Zinc sulfate	0;80
6	Cai et al. (2021)	Friesian Holstein	Corn silage	Zinc methionine, zinc oksida	0;40
7	Chandra et al. (2014)	Friesian Holstein	Oat hay	Zinc sulfate	0;60
8	Chandra et al. (2018)	Sahiwal Cows	Wheat silage	Zinc sulfate	0;60
9	Chang et al. (2020)	Friesian Holstein	Corn	Zinc methionine, zinc sulfate	0;80
10	Chen et al. (2020)	Friesian Holstein	Oat hay	Zinc methionine	0;20;40;60
11	De et al. (2014)	Karan-Fries	Corn	Zinc sulfate	0;80
12	Kinal et al. (2011)	Friesian Holstein	Corn	Zinc methionine	0;30
13	Lanni et al. (2020)	Friesian Holstein	Corn silage	Zinc oksida	0;60
14	Liu et al. (2023) a	Friesian Holstein	N/A	Zinc methionine, zinc proteinate	0;80
15	Liu et al. (2023) b	Friesian Holstein	N/A	Zinc oksida, zinc proteinate	0;12;24;36
16	Ma et al. (2020)	Friesian Holstein	N/A	Zinc methionine, zinc oksida	0;80
17	Pandey et al. (2022)	Friesian Holstein	Wheat straw	Zinc sulfate	0;32
18	Singh et al. (2019)	Friesian Holstein	forage	Zinc sulfate	0;80
19	Sobhanirad et al. (2010)	Friesian Holstein	Corn silage	Zinc methionine, zinc sulfate	0;500
20	Sobhanirad dan Naserian (2012)	Friesian Holstein	Soybean flour	Zinc methionine, zinc sulfate	0;500
21	Vanegas et al. (2007)	California cows	TMR	Zinc sulfat	0;64
22	Wang et al. (2013)	Friesian Holstein	Corn silage	Zinc sulfat	0;40
23	Wang et al. (2021)	Friesian Holstein	Corn silage	Zinc sulfat	0;10;20;30
24	Wiking et al. (2008)	Danish Holstein	Silage	Zinc oksida	0;39
25	Wo et al. (2022)	Friesian Holstein	N/A	Zinc methionine, zinc proteinate	0;80

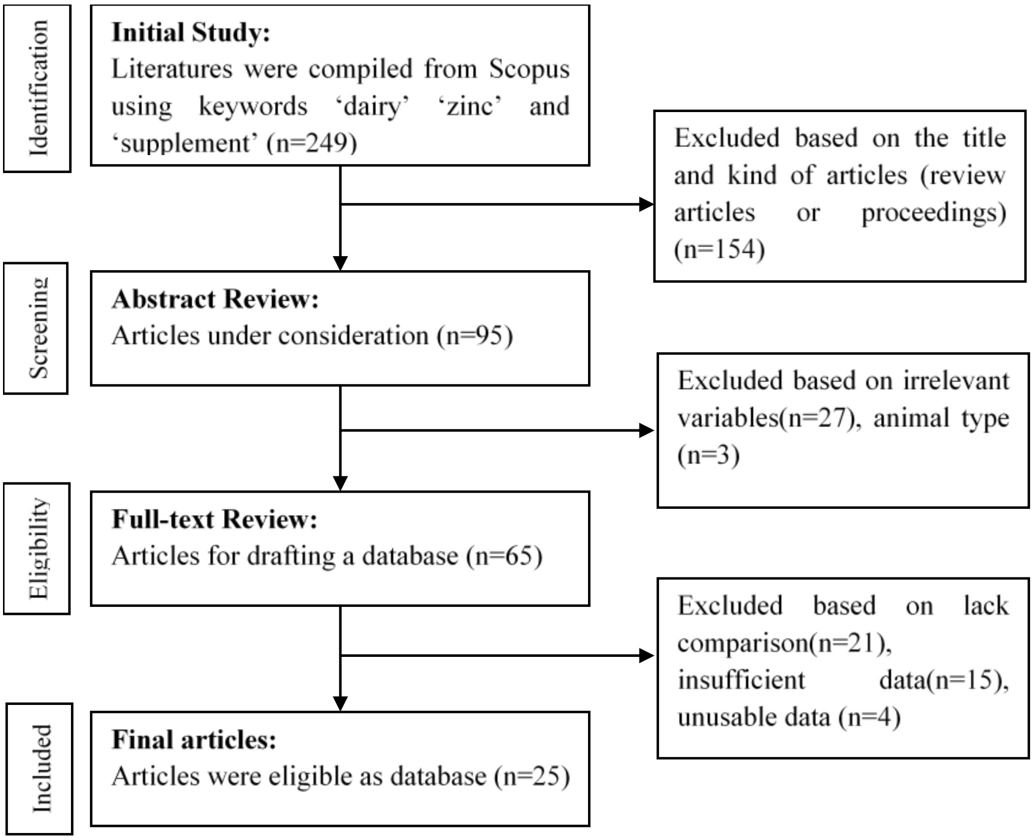

Figure 1 Diagram flow for study selection in the meta-analysis of dietary zinc supplementation in dairy cows.

Analysis of Data

This meta-analysis used a mixed-model methodology, as described by Sauvant et al. (2008). Subsequently, statistical analysis was performed using the PROC MIXED method in SAS software. During the analysis, the studies were considered random effects, while the level and source of Zinc were considered fixed effects. The levels of Zinc were used as continuous predictor variables using the following equation:

$$Y_{ij} = A0 + A1X_{ij} + A2X2_{ij} + P_i + a_iX_{ij} + e_{ij}$$

Where Y_{ij}: Response variable (dependent), i: the group or block effect, j: the treatment effect, A0: Total Intercept from all experiments, A1: Linear regression constant Y on X, A2: Quadratic regression constant Y on X, X_{ij}: Value of continuous forecaster variable (level of Zn supplementation), P_i: Random effect from study i, a_i: Random effect from study i on regression constant Y on X, e_{ij}: Study error. This study's statistical model relied on p-values. A variable was deemed significant if the p-value was less than 0.05, and if the p-value included 0.05 and 0.1, the effect tended to be significant.

Data analysis of the effect of Zinc sources on the response variable was conducted using the mixed model method with the following equation:

$$Y_{ijk} = \mu + P_i + \tau_j + A1X_{ij} + a_iX_{ij} + A_jX_{ij} + e_{ijk}$$

where Y_{ijk}: Response variable (dependent), i: the group or block effect, j: the treatment effect, k: the individual observation within i-th group and j-th treatment, μ: Overall mean, τ_j: Fixed effect of j levels of factors τ, A1: Overall regression constant of Y on X, X_{ij}: Value of continuous forecaster variable, P_i: Random effect from the study, a_i: Random effect from the study on the regression constant Y on X, e_{ijk}: Study error

An analysis was conducted on sources related to Zinc using Analysis of Variance (ANOVA) and continued with the HSD Test if there were significant differences. In addition, statistical analysis was performed using the PROC MIXED method in SAS software.

Data analysis of the interaction effect between the level and Zinc source was carried out using the following equation:

$$Y_{ij} = \mu + p_i + \tau_j + p\tau_{ij} + A0 + A1X_{ij} + A2X2_{ij} + a_iX_{ij} + e_{ij}$$

where Y_{ij}: dependent variable, i: the group or block effect, j: the treatment effect, μ: general mean, pi: random result of the i-th study, τ_j: fixed result of the j-th level of factor τ, $p\tau_{ij}$: random interaction among the i-th study and jth level of factor τ, A0: general Intercept across all treatment, B1: Linear regression constant of Y on X (fixed effect), X_{ij}: Value of continuous forecaster variable (Zinc) continuous predictor variable (level of Zinc supplementation), b_i: Random effect of study on regression constant Y on X in study I, e_{ij}: Unsolved residual error.

RESULTS

The effects of Zinc level or source on dairy cows are presented in Tables 2 and 3, respectively. Zinc levels significantly ($P<0.05$) quadratically increased milk yield and decreased SCC as shown in Figure 2 and 3. The level of Zinc that provided the highest milk production was 36.74 $mg.kg^{-1}$, and the lowest level of zinc that reduced SCC was 112.54 $mg.kg^{-1}$. Zinc supplementation linearly increased ($P<0.05$) IgM, IgG, and blood Zinc levels. However, it did not affect milk quality (protein, fat, lactose, and total solids) or blood biochemistry (total protein, cholesterol, albumin, and glucose).

Compared with the control, both organic and inorganic zinc sources increased ($P<0.05$) IgG and blood Zinc levels and decreased ($P<0.05$) SCC. There were no significant differences in production, milk quality (protein, fat, lactose, and total solids), IgA, IgM, and blood plasma (total protein, albumin, glucose, and total cholesterol) with the inclusion of sources of Zinc. The results of the interaction between Zinc level and source are presented in Table 4, as well as Figure 3 and 4. There was an interaction ($P<0.05$) between Zinc level and source on IgM and blood zinc concentrations (Figure 5). Organic Zinc produced a higher effect ($P<0.05$) in increasing IgM and blood Zinc concentration than the inorganic source.

Table 2 Results of regression analysis of the influence of Zn levels on production performance, milk quality, immunity, and blood plasma

Parameter	Unit	N	M	Parameter estimates				Model Estimate
				Intercept	SE Intercept	Slope	SE Slope	P-Value
Production Performance								
Milk Production	Kg.day^{-1}	22	Q	34.9	3.81	0.136	0.042	<0.01
						-0.002	0.008.10^{-1}	0.012
DMI	Kg.day^{-1}	29	L	17.3	3.82	0.011	0.006	0.113
Milk Quality								
Protein	%	39	L	4.71	1.39	-0.007.10^{-2}	0.001	0.703
Fat	%	39	L	3.97	0.30	0.009.10^{-2}	0.005.10^{-1}	0.874
Lactose	%	37	L	4.47	0.29	0.004.10^{-1}	0.003.10^{-1}	0.209
Total Solid	%	13	L	17.2	4.69	-0.007.10^{-1}	0.003	0.844
SCC	10^{3}cells.ml^{-1}	26	Q	2691	326	0.018	0.006	0.015
						-10.762	3.363	<0.01
Immunity								
IgA	μg.ml^{-1}	24	L	49.6	21.1	0.065	0.038	0.105
IgM	mg.ml^{-1}	24	L	3.92	0.49	0.010	0.046	0.031
IgG	mg.ml^{-1}	28	L	14.0	2.63	0.024	0.078	<0.01
Blood Plasma								
TP	mg.ml^{-1}	32	L	75.6	13.9	0.008	0.014	0.545
ALB	mg.ml^{-1}	29	L	37.4	4.13	-0.009.10^{-1}	0.009	0.921
GLU	mg.dl^{-1}	29	L	83.5	19.8	-0.001	0.021	0.959
TC	mg.dl^{-1}	12	L	195	97.8	0.006	0.053	0.909
Blood zinc	mg.l^{-1}	46	L	1.24	0.24	0.005	0.001	<0.01

$P \leq 0.01$: very significant, $P \leq 0.05$: significant, $P > 0.05$: not significant, SCC (Somatic Cell Count), DMI (Dry Matter Intake), IgA: Immunoglobulin A, IgM: Immunoglobulin M, IgG: Immunoglobulin G, TP: Total Protein: ALB: Albumin, GLU: Glucose, TC: Total cholesterol n: amount of data, M: Model, L : linear, Q: quadratic, Intercept: average value of the response parameter when the zinc level is equal to zero, SE Intercept: standard error of Intercept, Slope: value of the slope of the line, SE Slope: standard error of slope.

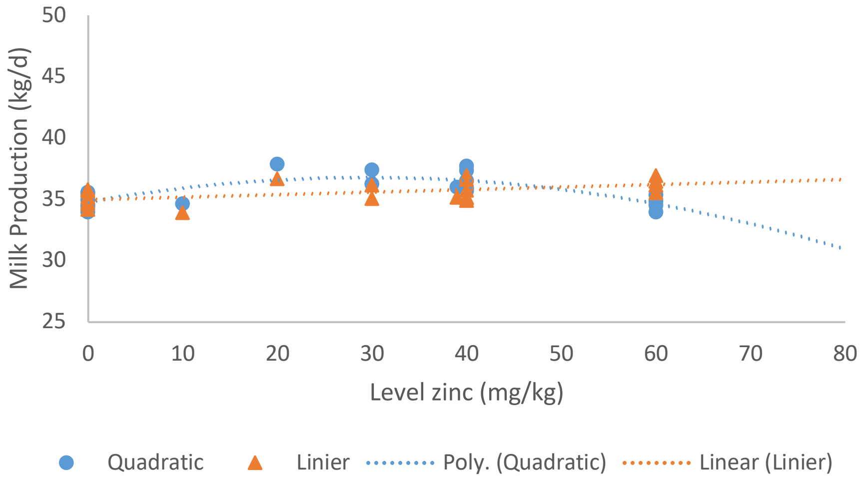

Figure 2 Linear and quadratic graphs of zinc levels on milk production.

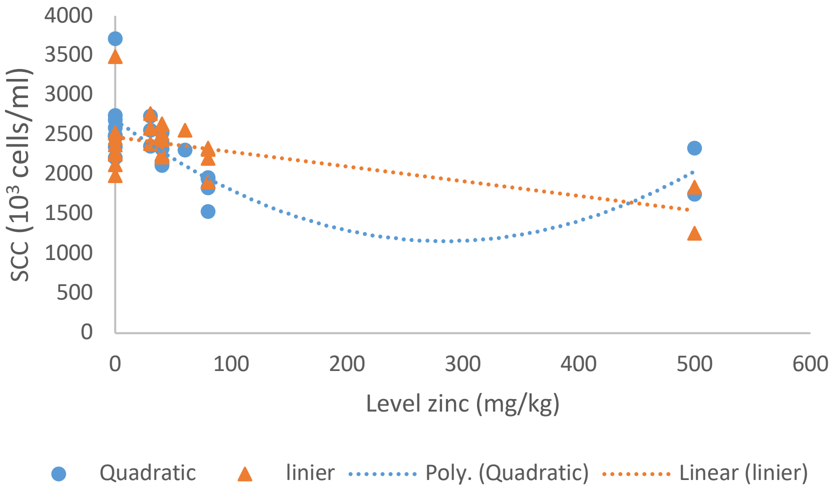

Figure 3 Linear and Quadratic graph of zinc levels on SCC.

Table 3 Results of statistical analysis of the influence of Zn sources on production performance, milk quality, immunity, and blood plasma

Parameter	Unit	N	Zinc Form			P-Value
			Control	Organic	Inorganic	
Average dose	mg/kg		0	57.0	50.2	
Production Performance						
Milk Production	Kg.day^{-1}	22	34.8	36.0	35.9	0.099
DMI	Kg.day^{-1}	29	17.2	17.9	17.6	0.330
Milk Quality						
Protein	%	39	4.74	4.60	4.64	0.917
Fat	%	39	3.92	4.12	3.95	0.297
Lactose	%	37	4.47	4.54	4.50	0.495
Total Solid	%	13	17.1	17.6	16.7	0.865
SCC	10^3cells.ml^{-1}	26	2547^a	2321^{ab}	2116^b	0.026
Immunity						
IgA	µg.ml^{-1}	24	49.5	53.1	53.6	0.400
IgM	mg.ml^{-1}	24	3.85	4.39	5.03	0.057
IgG	mg.ml^{-1}	28	13.9^a	15.1^b	16.1^b	0.011
Blood Plasma						
TP	mg.ml^{-1}	32	73.3	77.0	78.0	0.189
ALB	mg.ml^{-1}	29	37.0	38.1	37.3	0.947
GLU	mg.dl^{-1}	29	84.3	83.7	82.9	0.943
TC	mg.dl^{-1}	12	198	201	191	0.887
Blood zinc	mg.l^{-1}	46	1.24^a	1.65^b	1.44^b	<0.01

P≤0.01: very significant, P≤0.05: significant, P >0.05: not significant kan SCC (*Somatic Cell Count*), DMI (*Dry Matter Intake*), IgA: Immunoglobulin A, IgM: Immunoglobulin M, IgG: Immunoglobulin G, TP: Total Protein: ALB: Albumin, GLU: Glucose, TC: Total cholesterol

Table 4 Interaction of zinc levels and sources on production performance, milk quality, immunity, and blood plasma

Parameter	Unit	Level	Source	L x S
Production performance				
Milk Production	$Kg.day^{-1}$	<0.01	ns	ns
DMI	$Kg.day^{-1}$	ns	ns	ns
Milk Quality				
Protein	%	ns	ns	ns
Fat	%	ns	ns	ns
Lactose	%	ns	ns	ns
Total solid	%	ns	ns	ns
SCC	10^3 $cells.ml^{-1}$	0.01	0.02	ns
Immunity				
IgA	$\mu g.ml^{-1}$	ns	ns	ns
IgM	$mg.ml^{-1}$	0.03	ns	0.01
IgG	$mg.ml^{-1}$	<0.01	0.01	Ns
Blood Plasma				
TP	$mg.ml^{-1}$	ns	ns	ns
ALB	$mg.dl^{-1}$	ns	ns	ns
GLU	$mg.dl^{-1}$	ns	ns	ns
TC	$mg.l^{-1}$	ns	ns	na
Blood zinc	$mg.ml^{-1}$	<0.01	<0.01	0.04

P≤0.01: very significant, P≤0.05: significant, P >0.05: not significant, SCC (Somatic Cell Count), DMI (Dry Matter Intake), IgA: Immunoglobulin A, IgM: Immunoglobulin M, IgG: Immunoglobulin G, TP: Total Protein: ALB: Albumin, GLU: Glucose, TC: Total cholesterol, ns: not significant

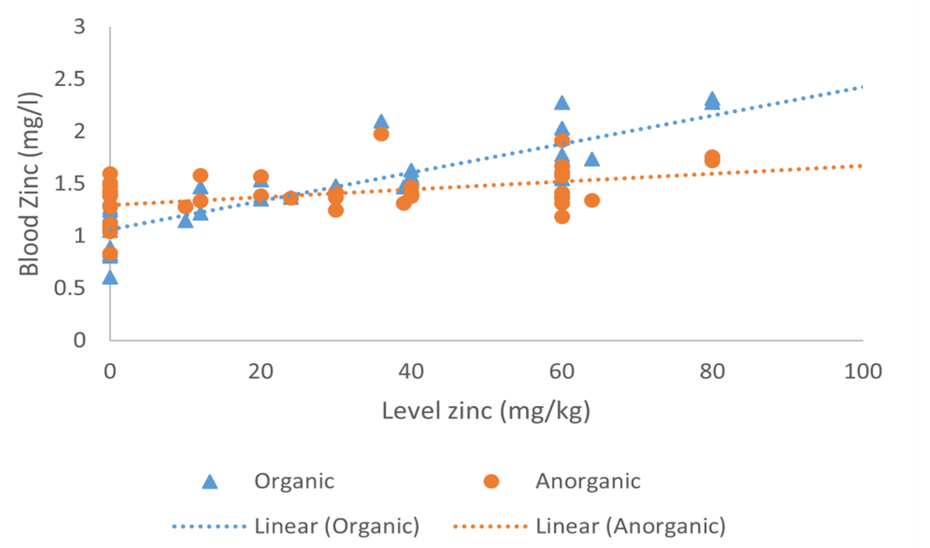

Figure 4 Interaction between zinc level and source on zinc concentration

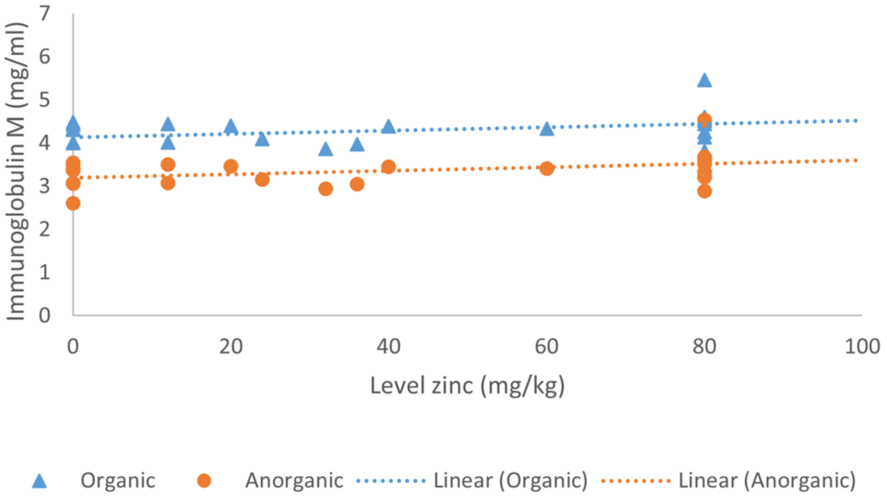

Figure 5 Interaction between zinc level and source on source on IgM.

DISCUSSION

Effect of Zn Levels on Production Performance, Milk Quality, Immunity, and Blood Plasma of Dairy Cows

In this study, Zinc supplementation did not affect milk quality, although this statement is based on previous investigations that found no effect of Zinc supplementation on milk quality in dairy cows (Wang et al., 2021). This shows that Zinc supplementation did not have any adverse effect on milk quality. However, Chen et al. (2023) research showed that a prolonged zinc addition can raise the milk's protein and fat content. The response to intramammary infection (IMI) in mammary glands is measured by SCC (Schwarz et al., 2020). This means that SCC can be used to identify mastitis indicators. Increasing the supply of zinc to the mammary glands can enhance immune function, leading to a reduction in somatic cell release in milk (Lanni et al., 2019). Zinc is crucial for keratin development, as the keratin layer in the nipple canal can trap and prevent bacteria from entering the mammary glands (Singh et al., 2020).

According to Chen et al. (2020), cow's immunity can be increased significantly by ncreasing zinc levels. The study found that zinc boosts the replication and proliferation of T and B lymphocytes, leading to the formation of immunoglobulin cells. These cells form when B lymphocytes are activated by recognizing antigens or interacting with helper T cells. The activated B lymphocytes then proliferate and differentiate into plasma cells with the help of Zip7 and Zip10 transporters, which use zinc. These plasma cells produce and secrete antibodies that support the immune system in dairy cows.

Interactions with helper T cells also influence B-lymphocyte activation. According to Kumar et al. (2021), Zinc can induce T-lymphocyte proliferation by producing interleukin-2 (IL-2). The production of IL-2 is very important for the creation of T lymphocytes, including cytotoxic T lymphocytes and helper T lymphocytes, and for proposing the S phase of the cell cycle. The high production of helper T cells increases the activation of B lymphocytes to produce immunoglobulins. Therefore, the function of Zinc in the formation of immune cells is very important, and the supplementation in the feed can increase immunoglobulins.

Zinc supplementation did not significantly alter blood plasma levels of total protein, albumin, glucose, or total cholesterol. However, the duration of zinc administration influences the increase in blood metabolite levels (Bakhshizadeh et al., 2019). Zinc plays a role in enzymatic processes and cellular functions, but blood metabolites are primarily influenced by enzyme activity and specific metabolic pathways. Therefore, zinc supplementation does not directly impact blood metabolite levels (Chandra et al., 2019).

Zinc supplementation in dairy cows significantly increases blood zinc concentrations. A study by Chandra et al. (2019) found that Zinc supplementation during the dry period and early lactation increases plasma Zinc concentrations in Sahiwal cows. This rise in blood zinc levels can be used to evaluate the overall zinc status in the body.

Effect of Zn Source on Production Performance, Milk Quality, Immunity, and Blood Plasma of Dairy Cows

A previous study found that organic zinc supplementation can lead to the highest milk production. This is attributed to the high chelating power of organic zinc amino acids, which reduces antagonism compared to inorganic forms (Neto et al., 2020; Zhang et al., 2022). This study is in line with the investigation carried out by Bakhsizadeh et al. (2019), which found that the administration of Zinc oxide and glycine had the same effect on DMI. During the first three weeks of lactation, DMI value increased approximately by 1.5-2.5 kg.week^{-1} due to increased feed intake.

According to the results, organic and inorganic zinc supplementation can reduce SCC. Bakhshizadeh et al. (2019) reported that Zinc glycine and nitrate can lower somatic cell counts (SCC) compared to control feed. The study indicated that inorganic zinc nitrate has antimicrobial properties, which are influenced by the size of zinc particles. The effectiveness of these antimicrobial properties is associated with the specific surface area, volume proportion, and particle size (Nayeri et al., 2014)

Interleukin-2 (IL-2) and interleukin-6 (IL-6) concentrations in the blood increases when various Zinc supplements are obtained (Chen et al., 2020). Active B-lymphocytes transform into plasma cells, which produce immunoglobulins. IgG is a type of B cell-based humoral immunity. Zinc can influence the expression of genes essential for IgG production and secretion. This influence often occurs through the modulation of transcription factors like Nuclear Factor kappa B (NF-κB) and Signal Transducer and Activator of Transcription (STAT) that regulate these genes (Jackson et al., 2018). Moreover, zinc can reduce the production of pro-inflammatory cytokines, such as TNF-α, which can negatively affect B cells' ability to produce IgG (Yusuf et al., 2019).

Supplementing dairy cows with Zinc sources significantly increased zinc concentrations in their blood. Organic Zinc, known for its high bioavailability, allows dairy cows to utilize it more effectively, thereby improving their health, as evidenced by elevated levels of organic Zinc in their bloodstream (Nayeri et al., 2014).

Effect of Interaction Levels and Sources Zn on Production Performance, Milk Quality, Immunity, and Blood Plasma of Dairy Cows

This study examined the interaction between zinc levels and sources, focusing on IgM levels and blood zinc concentrations. Organically sourced higher zinc levels were found to effectively enhance IgM and blood zinc concentrations. The increased blood zinc levels associated with organic sources are attributed to their enhanced stability and better absorption in the gastrointestinal tract, leading to greater zinc bioavailability (Chen et al., 2020). However, the optimal Zinc concentration in ruminant blood ranges from 0.8-1.2 mg.l^{-1}. The high concentrations in the blood can cause various toxic effects including immune system disorders, digestion, and growth. A well-absorbed optimal amount of zinc in the body can help to produce immunoglobulins. Nagalakshmi et al. (2018) detected a better immune response to organic zinc supplementation in sheep and dairy cows. Supplementation with 60 ppm zinc propionate produced the same response in performance and immunity with 80 ppm zinc sulfate (Nagalakshmi et al., 2018).

CONCLUSIONS

In conclusion, Zinc supplementation had a positive impact on milk production and immunity, although it had a negative impact on SCC. Organic and inorganic sources of Zinc have the same effects on SCC, IgG, and blood Zn levels. There was an interaction between the level and source of zinc in IgM and the blood zinc levels. Organic zinc levels had a greater influence on IgM and Zn concentrations in the blood.

ACKNOWLEDGEMENTS

We express our heartfelt appreciation to all those who have played a crucial role in the completion of this research paper. We are particularly grateful to the Directorate of Strategic Studies and Academic Reputation (DKASRA) IPB University for providing the necessary resources and publication fees. We would also like to acknowledge our study participants for their invaluable cooperation and willingness to share their experiences. Their contribution has greatly enriched the quality of our findings.

AUTHOR CONTRIBUTIONS

Conception and design of the study: S. T Risyahadi, Y. Retnani, I. Wijayanti, A.Jayanegara, R.I Kusuma.
Acquisition of data: I. Wijayanti, R.I Kusuma, S.T Risyahadi.
Analysis and/or interpretation of data: R.I Kusuma, A. Jayanegara.
Drafting the manuscript: R.I Kusuma, I. Wijayanti, Y. Retnani.
Critical review/revision: A. Jayanegara, I. Wijayanti, Y.Retnani

CONFLICT OF INTEREST

There were no conflicts of interest related to the discussed material, including financial, personal, or other relationships with individuals or organizations.

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How to cite this article;

R.I Kusuma, I. Wijayanti, Y. Retnani, A. Jayanegara and S.T Risyahadi. Effect of dietary zinc supplementation on production performance, milk quality, immunity, and blood plasma of dairy cows: A meta-analysis. Veterinary Integrative Sciences. 2025; 23(2): e2025044-1-14.