META-ANALYSIS OF MANGANESE SUPPLEMENTATION ON ENHANCEMENT OF PRODUCTIVITY AND EGG QUALITY INDICES IN LAYING HENS

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Abstract. This meta-analysis was evaluated to determine the effect of dietary manganese (Mn) supplementation on productivity and egg quality indices in laying hens. Scopus, ScienceDirect, Google Scholar, PubMed, and Web of Science were searched for published studies on effects of Mn supplementation on feed intake (FI), feed conversion ratio (FCR), egg production and quality in laying hens. The systematic search identified 560 articles, and 12 were used for the meta-analysis. Eligibility criteria for a study to be included in the meta-analysis were information on measured outcomes [FI, FCR, hen day egg production (HDP), egg weight (EW), egg mass (EM), Haugh unit (HU), and eggshell thickness (EST], covariates (form of Mn, dosage, supplementation duration, hen age, and hen strains) and measures of variance. Results were pooled using standardised mean difference (SMD) at 95% confidence interval (CI). Dietary Mn supplementation improved FCR, HDP, EM, and EST in laying hens. Subgroup analysis revealed that the results of the meta-analysis were influenced by covariates studied. Meta-regression showed that aspects of studied covariates (hen age and Mn form) were predictors of EST in laying hens fed Mn-supplemented diets. The result of this study showed the potential of Mn supplementation to improve FCR, HDP, EM and EST in laying hens.

Keywords: *feed intake, feed conversion, egg production, shell quality, publication bias, meta-regression*

Introduction

Mn is a naturally occurring trace element and an essential nutrient required for growth, cartilage formation, bone development, eggshell formation, reproduction, and nutrient absorption in chickens (Wang et al., 2021). Mn serves as a cofactor for several enzymes involved in the formation of mucopolysaccharides and glycoproteins, both of which play a vital role in eggshell formation (Saleh et al., 2020). Studies have shown that it plays an important part in carbohydrate, lipid, and protein metabolism (Li and Yang, 2018; Barrioni et al., 2019). Mn has been shown to activate glycosyl transferases involved in the formation of proteoglycans (Xiao et al., 2014), which are required for shell formation. Mn is supplemented to the laying hen diet daily to meet their nutritional requirements since many feed ingredients are marginally deficient in Mn (Rutherfurd et al., 2012) and are poorly absorbed in the gut (Attia et al., 2010). The National Research Council (NRC, 1994) set the Mn requirements for laying hens at 20 mg/kg feed. This requirement can be met by supplementing Mn to layer diets either as organic (e.g., MnPro, Mn-amino acid, Mn-Gly) or as inorganic (e.g., Mn-oxide, Mn-sulphate).

There is evidence that Mn deficiency in chickens causes ataxia, lameness, perosis, reduced shell strength and quality, and retarded growth (Wang et al., 2013; Wang et al., 2015). In laying hens, Mn deficiency results in the formation of thin-shelled eggs, reduced egg production, and poor hatchability (Gheisari et al., 2011; Olgun, 2017). In a bid to

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enhance productivity, researchers (Xiao et al., 2015; Zhang et al., 2017; Khoshbin et al., 2023; Zarghi et al., 2023) are exceeding the 20 mg/kg Mn set for laying hens by the NRC (1994) because of the low Mn content in maize-soybean meal, which constitutes over 60% of layer diets. However, the results of these studies appear to be inconclusive. Some authors reported that Mn enhanced shell strength and quality (Ochrimenko et al., 1990; Zarghi et al., 2023), increased egg production and quality (Fassani et al., 2000; Xiuli et al., 2002; Zarghi et al., 2023), and others reported decreased egg production (Ochrimenko et al., 1990). Zamani et al. (2005) found that inclusion of Mn at 30 to 120 mg/kg in layer did not influence egg production, FI, and FCR. Therefore, poultry nutritionists, researchers, and poultry farmers need to know the efficacy of Mn supplementation on egg production and quality measures to make appropriate decisions about the use of these products in their management systems.

One likely variation across studies that assessed the impact of Mn supplementation on laying hen performance is that these studies may have lacked adequate sample size and, in turn, the statistical power to detect differences in the production measures. Lack of statistical power can increase the risk of type 2 errors (false negatives), thus missing the true intervention effect (Egger et al., 2001). Other likely sources of heterogeneity in response to Mn supplementation in laying hens may be chicken age, diet composition, layer strain, amount of Mn added to the feed, duration of feeding Mn, and type of Mn that was used. Differences exist between inorganic and organic forms of Mn, as well as their mechanisms of action (Klecker et al., 2002; Xiao et al., 2015; Zarghi et al., 2023).

Meta-analysis, a statistical tool that combines multiple studies addressing a similar research question, has been proposed as a method to pool effect estimates, resolve uncertainty, identify knowledge gaps, and create new insights (Ogbuewu et al., 2024). However, there is scanty information on the meta-analysis of the response of laying hens to Mn supplementation. The objective of this meta-analysis, therefore, was to resolve the conflicting reports concerning the impact of Mn supplementation on laying performance and egg quality indices in laying hens.

Materials and methods

Literature search and eligibility criteria

This study was done by carrying out a comprehensive literature search of published articles on the topic using the PICO (Population, Intervention, Comparators, Outcomes) format as described by Ogbuewu and Mbajiorgu (2022a). PICO stands for Population (laying hens), Intervention (diet supplemented with Mn), Comparators (diets without Mn supplementation), and Outcomes (FI, FCR, HDP, EW, EM, HU, and EST). Studies were retrieved following a search conducted on Scopus, ScienceDirect, Google Scholar, PubMed, and Web of Science databases using Boolean operators (AND/OR). The following keywords: Manganese, laying hens, egg production, egg quality, FI, and FCR were used. The systematic search adhered to the Preferred Reporting Item for Systematic Reviews and Meta-analyses (PRISMA) guidelines. The identified studies were screened for eligibility using standardised criteria as described by Ogbuewu and Mbajiorgu (2022a). For a study to be included in the meta-analysis, it must have assessed the impact of supplemental Mn on at least one of the measured outcomes of interest with their corresponding measures of variance [i.e., standard error (SE) or standard deviation (SD)] or a P-value. The included studies must compare the effect of a diet with and without Mn supplementation on laying hen performance and egg quality. Include study must not blend

Mn with other microminerals. The search yielded 560 publications, of which 12 studies met the eligibility criteria as presented in *Figure 1*.

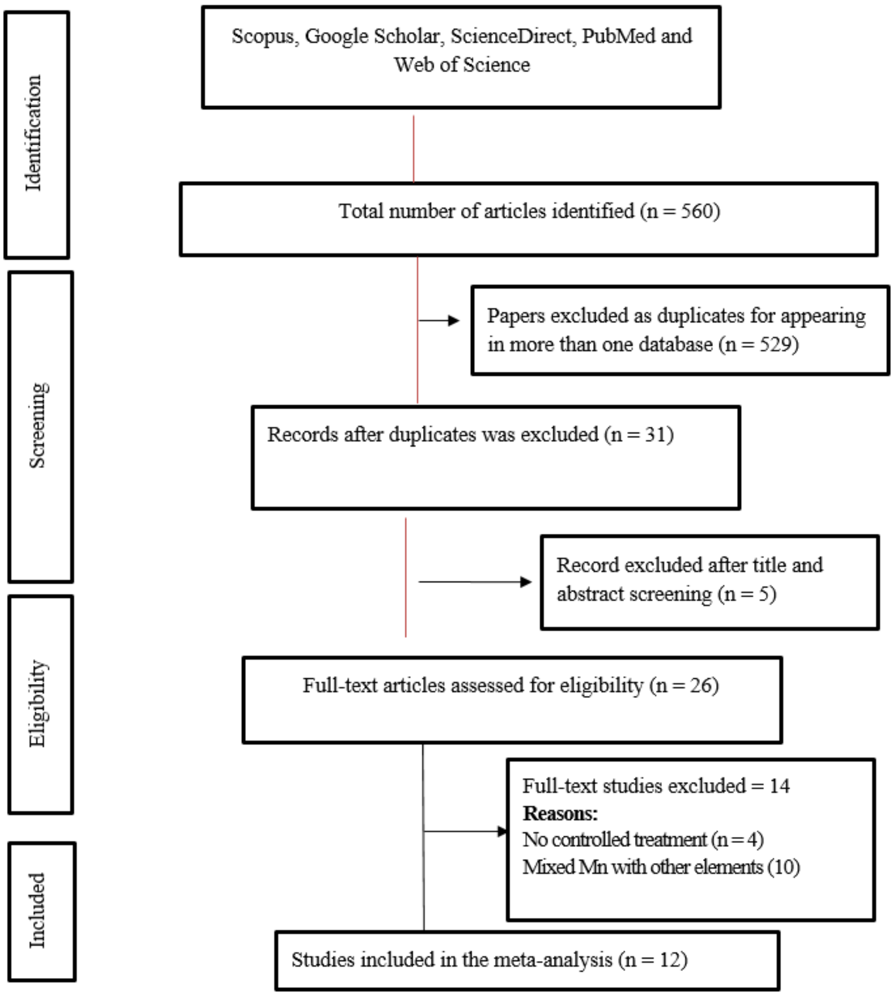


Figure 1. *Study selection flow chart*

Data extraction and analysis

Data were extracted from eligible studies that assessed the impact of supplemental Mn on egg production and egg quality. Also, the following data were extracted from eligible studies for subgroup and meta-regression analyses if the information was reported: study location (country, continent), covariates (form of Mn, dosage, supplementation duration, hen age, and hen strains), and mean of the control and treatment groups with their corresponding SD. WebPlotDigitiser designed and built by Rohatgi (2021) was used to extract data that were presented as graphs.

Data extracted from the 12 eligible studies were analysed using Open Meta-analyst for Ecology and Evolution (OpenMEE) software (Wallace et al., 2016). Data were pooled using random-effect models, and results were expressed as SMD at a 95% confidence interval. SMD was classified as follows: small effect (0.2 |SMD| < 0.5); medium effect (0.5 |SMD| < 0.8) and large effect (|SMD| ≥ 0.8) (Andrade, 2020). Differences in study characteristics (explanatory moderators or covariates) considered a priori to influence the outcomes of the meta-analysis were explored using restricted subgroup and meta-regression analyses. Subgroups with fewer than 3 comparisons were excluded from the analysis due to the low sample size. Heterogeneity between studies was assessed using Q-statistic and quantified using the I^2 - statistic (Higgins and Thompson, 2002). Meta-regression was not conducted in measured outcomes with fewer than 10 studies due to the low sample size (Borenstein et al., 2010). All analyses were considered significant at a 5% probability value.

Results

Overview of studies included in the meta-analysis

The features of the 12 peer-reviewed journal articles used for the meta-analysis are displayed in *Table 1*. The studies used for the analysis were published in eight countries that cut across three continents. Most of the studies were conducted in Asia (n = 8), followed by Europe (n = 3). *Table 1* indicates that the most recent study was published in 2023 and the least in 1994, spanning 29 years. Five out of the 12 studies used for the meta-analysis used inorganic Mn source, 3 used organic Mn source, and 4 used blends of the 2 Mn sources. Hy-Line strain (n = 6) was the most investigated strain, followed by the Lohmann strain (n = 2). The layers used for the meta-analysis were aged from 18 to 83 weeks at the start of the study.

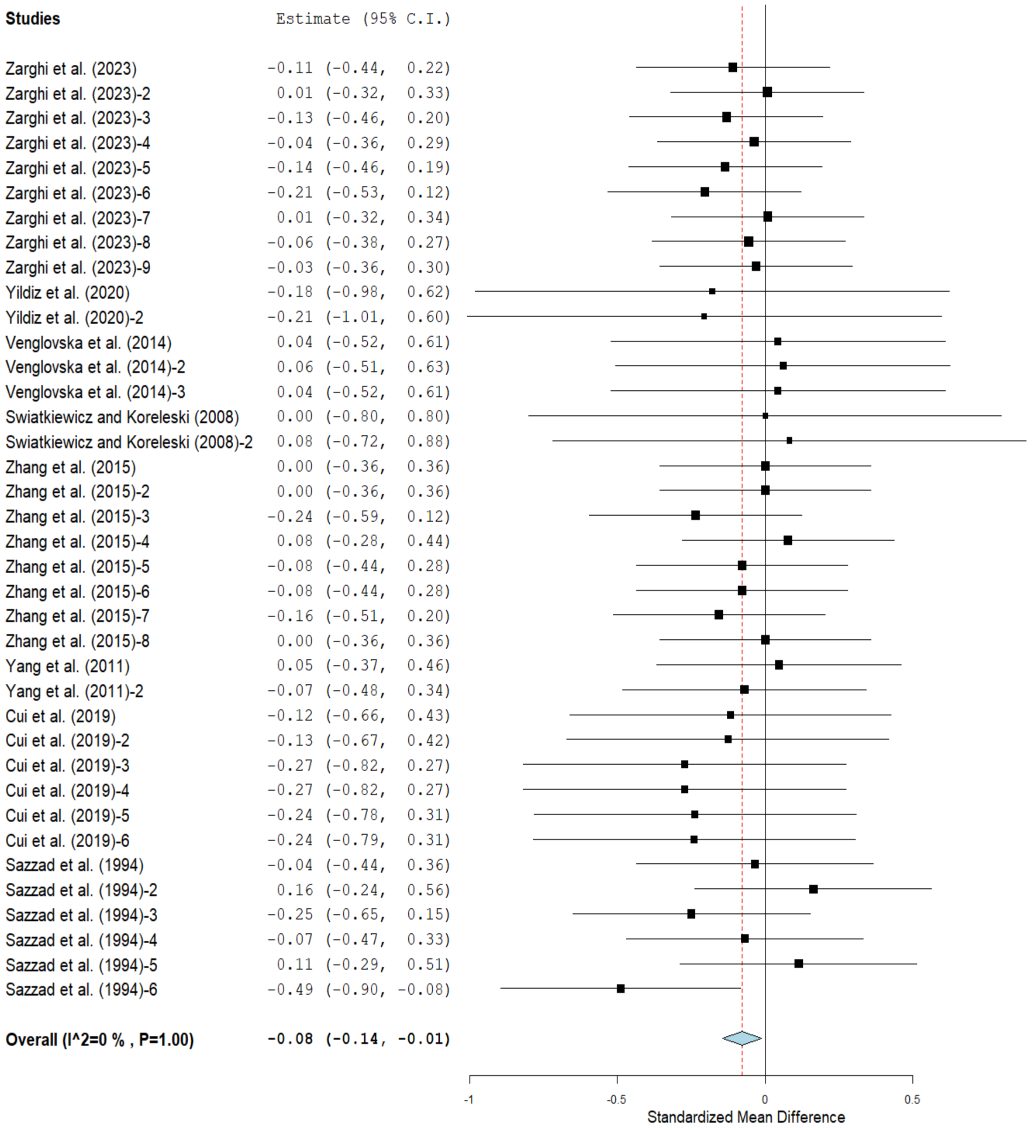
Table 1. *Characteristics of studies used for the analysis*

Study ID	**Country**	**Continent**	**NT**[a]	**Covariates**				
				HS[b]	**HA**[c]	**SD**[d]	**Dosage**[e]	**Mn form**
Zarghi et al. (2023)	Iran	Asia	4	Hy-line	83	12	0, 30, 60, 90	1, 2
Yildiz et al. (2010)	Turkey	Europe	3	Brown Nick	22	20	0, 35, 70	1
Venglovska et al. (2014)	Slovak Republic	Europe	2	Lohmann	20	8	0, 120	1, 2
Swiatkiewicz & Koreleski (2008)	Poland	Europe	3	Hy-line	25	45	0, 50, 100	2
Xiao et al. (2015)	China	Asia	5	Hy-line	50	12	0, 25, 50, 100, 200	1, 2
Zhang et al. (2017)	China	Asia	5	Jing Brown	48	8	0, 40, 80, 120, 160	1, 2
Khoshbin et al. (2023)	Iran	Asia	5	Leghorn	80	12	0, 25, 50, 75, 100	1
Yang et al. (2012)	USA	North America	3	Lohmann	18	10	0, 15, 35	1
Zamani et al. (2005)	Iran	Asia	4	Hy-Line	28	12	0, 30, 60, 90	1
Cui et al. (2019)	China	Asia	7	Hy-Line	23	24	0, 20, 40, 80, 120, 400,800	2
Li et al. (2018)	China	Asia	5	Jinghong-1	53	8	0, 20, 40, 60, 80	2
Sazzad et al. (1994)	India	Asia	4	Hy-Line	23	8	0, 20, 40. 80	1

[a]Number of treatment, [b]Hen strains, [c]Hen age in weeks, [d]Duration of supplementation of Mn in weeks, [e]Dosage of Mn in mg/kg, 1 = Inorganic form of Mn, 2 = Organic form of Mn

Feed intake and FCR

The forest plots showed that Mn supplementation reduced feed intake (SMD = −0.08, 95% CI: −0.14, −0.01; I^2 = 0%; *Figure 2*) and improved FCR (SMD = −0.09, 95% CI: −0.47, −0.17; I^2 = 81%; *Figure 3*) in laying hens. Disaggregation of feed intake by studied covariates (hen age, dosage, supplementation duration of Mn, hen strains, and form of Mn) as presented in *Table 2* revealed that dosage had significant and negative effect on FI in layers fed Mn-supplemented diets. In addition, Hy-Line layers fed Mn supplemented diets had significantly reduced FI compared to the control (SMD = −0.32, 95% CI: −0.18, −0.00).



Studies	Estimate (95% C.I.)
Zarghi et al. (2023)	-0.11 (-0.44, 0.22)
Zarghi et al. (2023)-2	0.01 (-0.32, 0.33)
Zarghi et al. (2023)-3	-0.13 (-0.46, 0.20)
Zarghi et al. (2023)-4	-0.04 (-0.36, 0.29)
Zarghi et al. (2023)-5	-0.14 (-0.46, 0.19)
Zarghi et al. (2023)-6	-0.21 (-0.53, 0.12)
Zarghi et al. (2023)-7	0.01 (-0.32, 0.34)
Zarghi et al. (2023)-8	-0.06 (-0.38, 0.27)
Zarghi et al. (2023)-9	-0.03 (-0.36, 0.30)
Yildiz et al. (2020)	-0.18 (-0.98, 0.62)
Yildiz et al. (2020)-2	-0.21 (-1.01, 0.60)
Venglovska et al. (2014)	0.04 (-0.52, 0.61)
Venglovska et al. (2014)-2	0.06 (-0.51, 0.63)
Venglovska et al. (2014)-3	0.04 (-0.52, 0.61)
Swiatkiewicz and Koreleski (2008)	0.00 (-0.80, 0.80)
Swiatkiewicz and Koreleski (2008)-2	0.08 (-0.72, 0.88)
Zhang et al. (2015)	0.00 (-0.36, 0.36)
Zhang et al. (2015)-2	0.00 (-0.36, 0.36)
Zhang et al. (2015)-3	-0.24 (-0.59, 0.12)
Zhang et al. (2015)-4	0.08 (-0.28, 0.44)
Zhang et al. (2015)-5	-0.08 (-0.44, 0.28)
Zhang et al. (2015)-6	-0.08 (-0.44, 0.28)
Zhang et al. (2015)-7	-0.16 (-0.51, 0.20)
Zhang et al. (2015)-8	0.00 (-0.36, 0.36)
Yang et al. (2011)	0.05 (-0.37, 0.46)
Yang et al. (2011)-2	-0.07 (-0.48, 0.34)
Cui et al. (2019)	-0.12 (-0.66, 0.43)
Cui et al. (2019)-2	-0.13 (-0.67, 0.42)
Cui et al. (2019)-3	-0.27 (-0.82, 0.27)
Cui et al. (2019)-4	-0.27 (-0.82, 0.27)
Cui et al. (2019)-5	-0.24 (-0.78, 0.31)
Cui et al. (2019)-6	-0.24 (-0.79, 0.31)
Sazzad et al. (1994)	-0.04 (-0.44, 0.36)
Sazzad et al. (1994)-2	0.16 (-0.24, 0.56)
Sazzad et al. (1994)-3	-0.25 (-0.65, 0.15)
Sazzad et al. (1994)-4	-0.07 (-0.47, 0.33)
Sazzad et al. (1994)-5	0.11 (-0.29, 0.51)
Sazzad et al. (1994)-6	-0.49 (-0.90, -0.08)
Overall (I^2=0 % , P=1.00)	**-0.08 (-0.14, -0.01)**

Figure 2. *Forest plot of FI of layers on dietary Mn. FI = feed intake; C.I. = confidence interval;* I^2 *= Inconsistency index. The solid vertical line shows no effect (SMD = 0). Plots to the left and right of the no effect line show a decrease and an increase in FI, respectively. Individual square in the plot represents the mean effect size for each study, while the upper and lower 95% CI for the effect size are the horizontal line that joined the squares. The dotted line with the diamond at the base of plot is the mean effect size. Mean effect size is said to be significant when the line of no effect did not touch the diamond at the bottom of the forest plot*

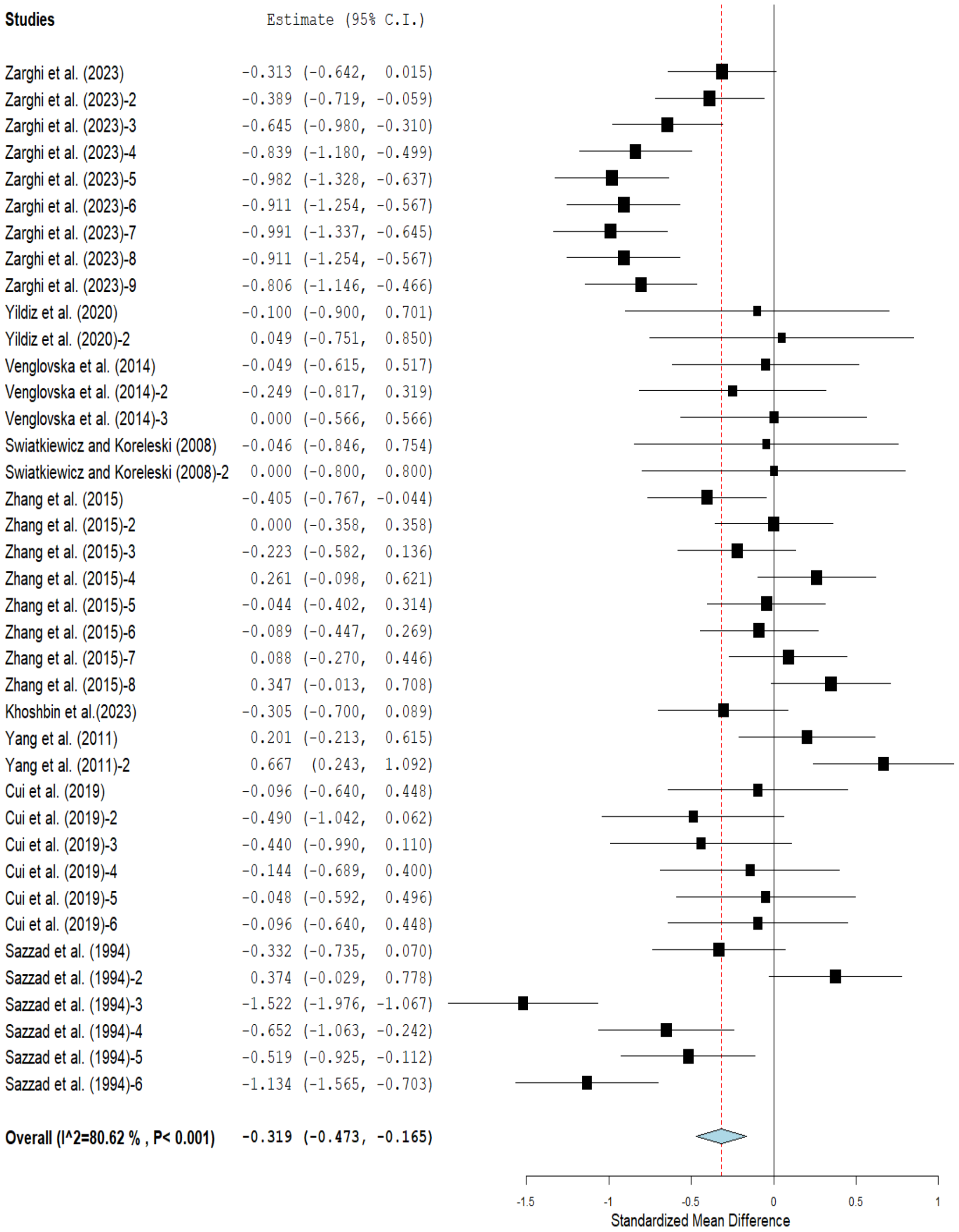


Figure 3. *Forest plot of the effect of Mn on FCR*

The effect of covariates on FCR in laying hens on dietary Mn is illustrated in *Table 3*. Results showed that layers aged > 50 weeks had better FCR than those aged ≤ 50 weeks. Layers fed Mn at ≤ 100 mg/kg for ≤ 12 weeks had improved FCR. Dietary Mn supplementation had significant and negative influence on FCR. Hy-Line layers recorded significantly better FCR (SMD = −0.52, 95% CI: −0.72, −0.32) than the Jing brown layers (SMD = −0.01, 95% CI: −0.18, 0.16). *Table 3* indicates that subgroup analyses did not solve the problem of significant heterogeneity across the studies that evaluated the effect of Mn supplementation on FCR.

Table 2. *Effect of moderators on feed intake in laying hens*

Covariates	Subgroup	n	SMD (95% CI)		*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Hen age (weeks)	≤ 50	29	-0.09	-0.16, 0.04	0.063	0	0.996
	> 50	9	-0.08	-0.19, 0.03	0.168	0	0.992
Dosage (mg/kg)	≤ 100	28	-0.08	-0.15, -0.00	0.049	0	0.999
	> 100	10	-0.09	-0.23, 0.05	0.049	0	0.939
SD (weeks)	≤ 12	28	-0.07	-0.13, 0.01	0.070	0	0.996
	> 12	10	-0.18	-0.37, 0.02	0.071	0	0.999
Hen strains	Hy-Line	20	-0.09	-0.18, -0.00	0.048	0	0.999
	Lohmann	8	-0.06	-0.22, 0.10	0.459	0	0.575
	Jing brown	9	-0.06	-0.19, 0.07	0.364	0	0.954
Mn form	Organic	17	-0.08	-0.19, 0.03	0.147	0	0.955
	Inorganic	21	-0.08	-0.16, 0.01	0.071	0	1.000

SD = supplementation duration of Mn

Table 3. *Effect of moderators on FCR in laying hens*

Covariates	Subgroup	n	SMD (95% CI)		*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Hen age (weeks)	≤ 50	29	-0.16	-0.33, 0.01	0.058	59	0.009
	> 50	10	-0.71	-0.88, -0.54	< 0.001	74	0.000
Dosage (mg/kg)	≤ 100	29	-0.41	-0.59, -0.23	< 0.001	82	0.001
	> 100	10	-0.02	-0.18, 0.13	0.765	14	0.311
SD (weeks)	≤ 12	29	-0.36	-0.55, -0.18	< 0.001	85	0.000
	> 12	10	-0.17	-0.37, 0.02	0.085	0	0.958
Hen strains	Hy-Line	20	-0.52	-0.72, -0.32	< 0.001	77	0.000
	Lohmann	8	-0.22	-0.64, 0.20	0.297	85	0.000
	Jing brown	9	-0.01	-0.18, 0.16	0.926	44	0.087
Mn form	Organic	17	-0.26	-0.47, -0.04	0.022	81	0.000
	Inorganic	22	-0.37	-0.58, -0.15	< 0.001	82	0.000

SD = supplementation duration of Mn

Hen day egg production (HDP) and EM

The results showed that Mn supplementation significantly increased HDP (SMD = 0.27, 95% CI: 0.13, 0.41; *Figure 4*) and EM (SMD = 0.34, 95% CI: 0.17, 0.50; *Figure 5*) with evidence of large heterogeneity (I^2 = 67 - 77%). The subgroup results of HDP by covariates as illustrated in *Table 4* indicate that studies that fed ≤ 100 mg Mn/kg for ≤ 12 weeks to layers aged > 50 weeks had significantly higher HDP than that in studies that fed > 100 mg Mn/kg for > 12 weeks to layers aged ≤ 50 weeks. Hy-Line strain had a higher magnitude of effect size (SMD = 0.55) than the Lohmann strain (SMD = 0.25) and Jing Brown strain (SMD = 0.28).

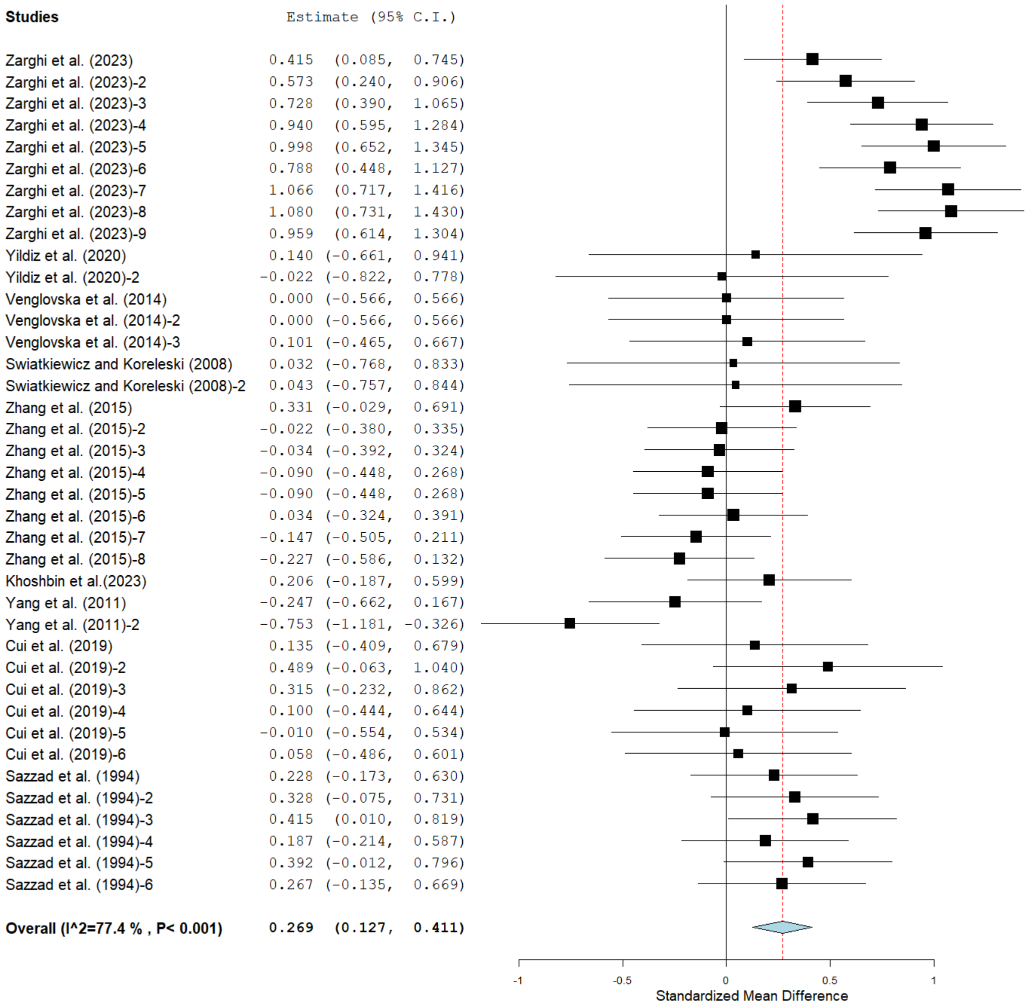


***Figure 4.** Forest plot of the effect of Mn on HDP*

***Table 4.** Impact of moderators on HDP in laying hens*

Covariates	Subgroup	n	SMD (95% CI)		*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Hen age (weeks)	≤ 50	29	0.06	-0.04, 0.15	0.241	61	0.006
	> 50	10	0.78	0.60, 0.96	< 0.001	23	0.129
Dosage (mg/kg)	≤ 100	29	0.37	0.20, 0.53	< 0.001	78	0.000
	> 100	10	-0.06	-0.20, 0.08	0.400	0	0.991
SD (weeks)	≤ 12	29	0.30	0.13, 0.47	< 0.010	83	0.000
	> 12	10	0.15	-0.05, 0.34	0.140	0	0.980
Hen strains	Hy-Line	20	0.55	0.38, 0.71	< 0.001	66	0.000
	Lohmann	8	-0.01	-0.28, 0.27	0.965	64	0.007
	Jing brown	8	-0.03	-0.16, 0.10	0.630	0	0.569
Mn form	Organic	17	0.25	0.03, 0.50	0.048	80	0.000
	Inorganic	22	0.28	0.11, 0.46	0.002	77	0.000

SD = supplementation duration of Mn

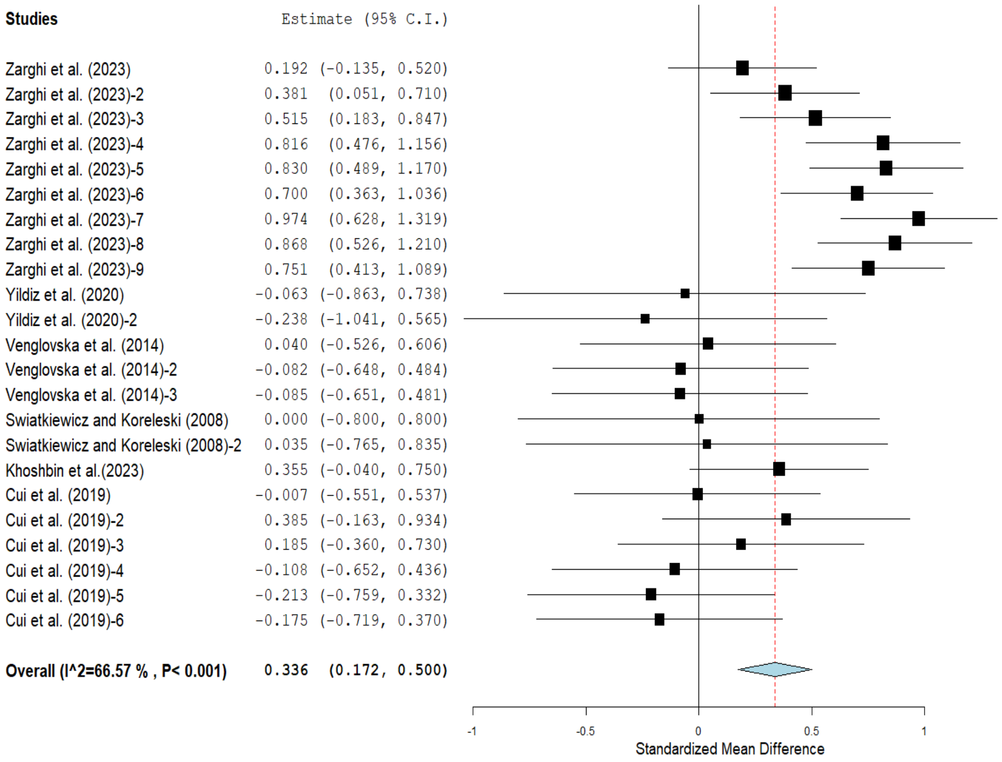


Figure 5. *Forest plot of the effect of Mn on EM*

Subgroup analysis of the influence of covariates on EM in laying hens fed Mn-supplemented diets is presented in *Table 5*. Subgroup analyses by covariates indicate that Mn form had no effect on EM, although the magnitude of effect estimate was lower in layers fed organic Mn than those fed inorganic Mn. Studies that fed ≤ 100 mg Mn/kg for ≤ 12 weeks to layers aged > 50 weeks had significantly higher EM than that fed > 100 mg Mn/kg for > 12 weeks to layers aged ≤ 50 weeks. The Hy-Line strain had higher EM than the Lohmann strain. *Tables 4 and 5* revealed that subgroup analyses did not remove the problem of significant heterogeneity among the studies that evaluated the effect of Mn on HDP and EM.

Table 5. *Effect of studied moderators on EM*

Covariates	Subgroup	n	SMD (95% CI)		*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Hen age (weeks)	≤ 50	13	-0.02	-0.18, 0.15	0.844	0	0.984
	> 50	10	0.64	0.48, 0.80	< 0.001	55	0.017
Dosage (mg/kg)	≤ 100	17	0.49	0.33, 0.65	< 0.001	58	0.001
	> 100	6	-0.11	-0.33, 0.12	0.358	0	0.993
SD (weeks)	≤ 12	13	0.53	0.35, 0.71	< 0.001	66	0.000
	> 12	10	-0.01	-0.20, 0.19	0.938	0	0.922
Hen strains	Hy-Line	17	0.43	0.24, 0.61	< 0.001	68	0.000
	Lohmann	3	-0.04	-0.37, 0.28	0.800	0	0.941
Mn form	Organic	13	0.24	-0.03, 0.51	0.083	73	0.000
	Inorganic	10	0.44	0.25, 0.64	< 0.001	56	0.016

SD = supplementation duration of Mn

Egg weight, HU, and EST

The pooled results revealed that layers on supplemental Mn had significantly lower EW than the control (SMD = −0.06, 95% CI: −0.11, −0.004; I^2 = 0%; *Figure 6*). In comparison with the controls, supplemental Mn had no effect on HU (SMD = −0.02, 95% CI: −0.20, 0.16; *Figure 7*), but significantly increased EST (SMD = 0.21, 95% CI: 0.14, 0.29; *Figure 8*). There was evident of significant heterogeneity across studies that assessed the effect of Mn on HU (I^2 = 78%; $p < 0.001$; *Figure 7*) and EST (I^2 = 45%; $p < 0.001$; *Figure 8*). Hy-line layers fed on supplemental Mn had significantly reduced EW (SMD = −0.12, 95% CI: −0.19, −0.05; *Table 6*) and HU (SMD = −0.22, 95% CI: −0.35, −0.09; *Table 7*) compared to the controls. Layers fed inorganic Mn had significantly lower HU (SMD = −0.27, 95% CI: −0.42, −0.11; Table 6) than the control. Hy-line layers fed Mn-supplemented diets had significantly reduced EW compared to the control (SMD = −0.12, 95% CI: −0.19, −0.05; *Table 7*).

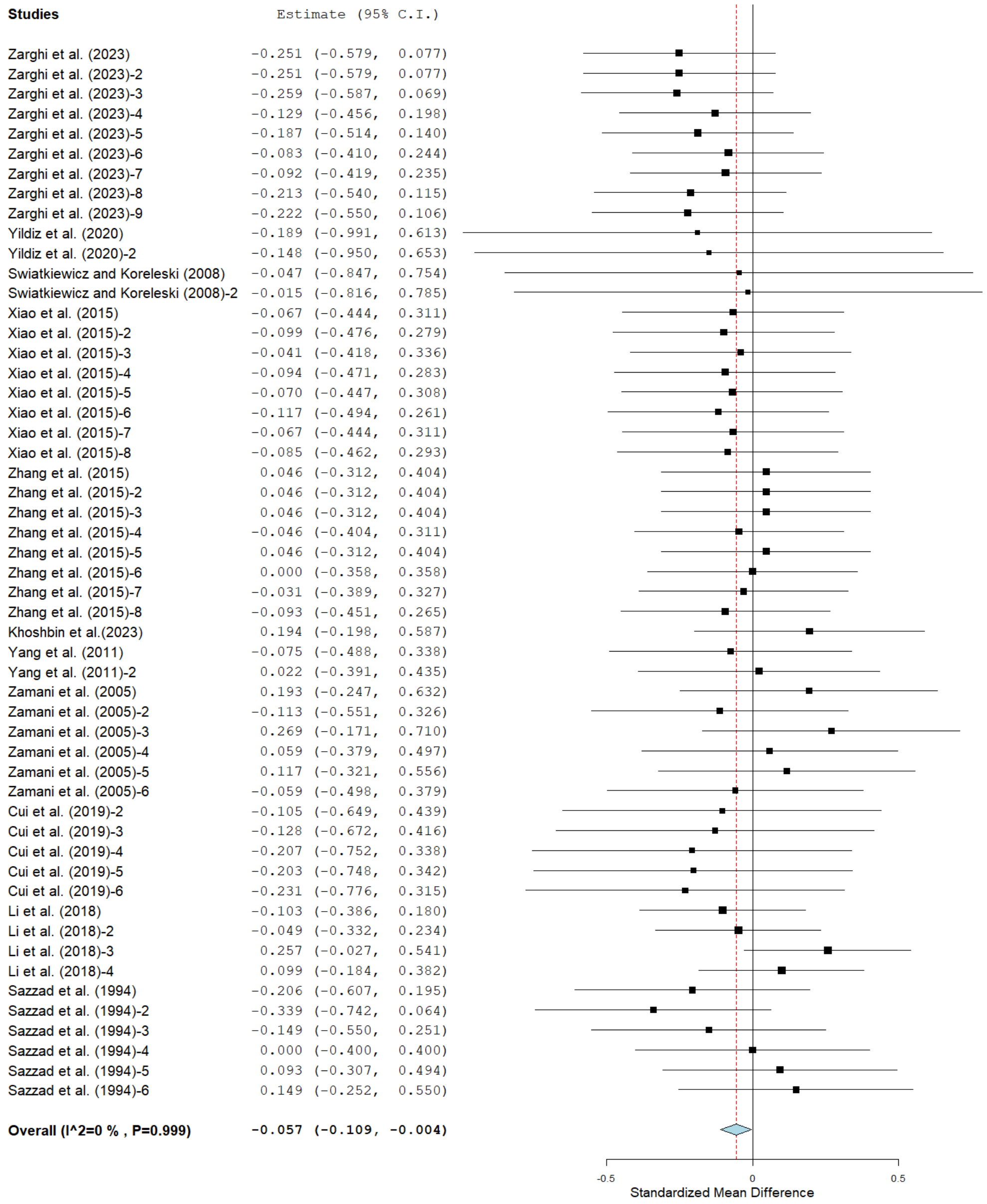


***Figure 6.** Forest plot of the effect of Mn on EW*

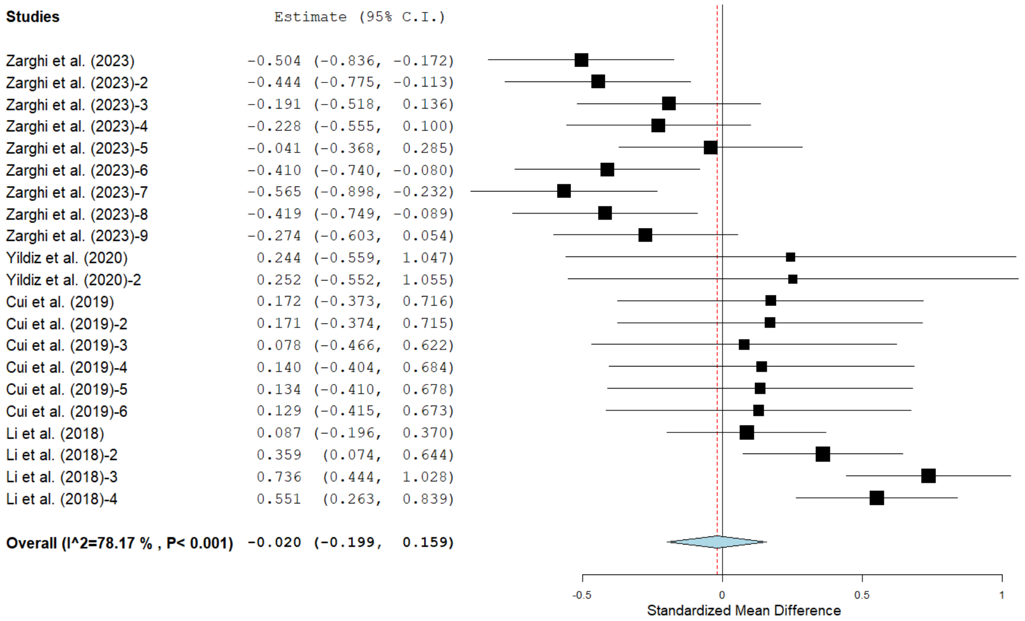


Figure 7. *Forest plot of the effect of Mn on HU*

Table 6. *Effect of studied moderators on EW*

Covariates	**Subgroup**	**n**	**SMD (95% CI)**		***p*-value**	**Heterogeneity**	
						***I*² (%)**	***p*-value**
Hen age (weeks)	≤ 50	40	-0.04	-0.11, 0.03	0.257	0	1.000
	> 50	14	-0.09	-0.17, 0.02	0.055	7	0.378
Dosage (mg/kg)	≤ 100	44	-0.06	-0.12, -0.01	0.048	0	0.994
	> 100	10	-0.05	-0.18, 0.08	0.451	0	0.942
SD (weeks)	≤ 12	45	-0.05	-0.10, 0.04	0.071	0	0.989
	> 12	9	-0.15	-0.36, 0.05	0.146	0	1.000
Hen strains	Hy-Line	33	-0.12	-0.19, -0.05	< 0.001	0	1.000
	Lohmann	5	0.04	-0.14, 0.22	0.670	0	0.952
	Jing brown	8	0.02	-0.13, 0.13	0.978	0	0.999
Mn form	Organic	22	-0.05	-0.13, 0.03	0.188	0	0.979
	Inorganic	32	-0.06	-0.13, 0.01	0.097	0	0.985

SD = supplementation duration of Mn

Table 7. *Effect of studied moderators on HU*

Covariates	**Subgroup**	**n**	**SMD (95% CI)**		***p*-value**	**Heterogeneity**	
						***I*² (%)**	***p*-value**
Hen age (weeks)	≤ 50	8	0.15	-0.06, 0.36	0.150	0	1.000
	> 50	13	-0.10	-0.33, 0.14	0.420	86	0.000
Dosage (mg/kg)	≤ 100	18	-0.04	-0.24, 0.16	0.689	81	0.000
	> 100	3	0.13	-0.18, 0.45	0.402	0	1.000
SD (weeks)	≤ 12	13	-0.10	-0.33, 0.14	0.420	86	0.000
	> 12	8	0.15	-0.06, 0.36	0.150	0	1.000
Hen strains	Hy-Line	15	-0.22	-0.35, -0.09	< 0.001	37	0.071
Mn form	Organic	13	0.10	-0.14, 0.34	0.408	80	0.000
	Inorganic	8	-0.27	-0.42, -0.11	< 0.001	22	0.251

SD = supplementation duration of Mn

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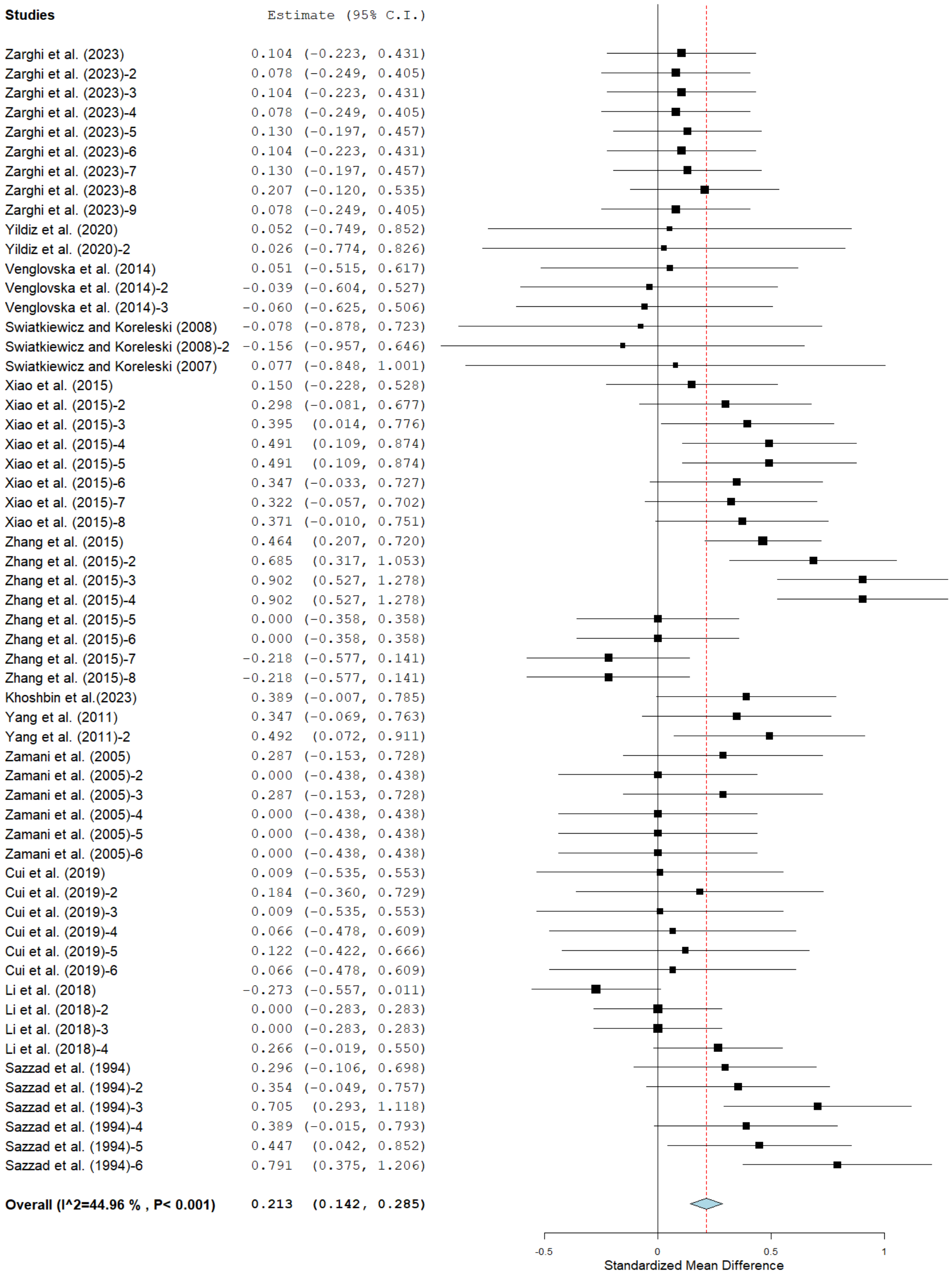

Studies	Estimate (95% C.I.)
Zarghi et al. (2023)	0.104 (−0.223, 0.431)
Zarghi et al. (2023)-2	0.078 (−0.249, 0.405)
Zarghi et al. (2023)-3	0.104 (−0.223, 0.431)
Zarghi et al. (2023)-4	0.078 (−0.249, 0.405)
Zarghi et al. (2023)-5	0.130 (−0.197, 0.457)
Zarghi et al. (2023)-6	0.104 (−0.223, 0.431)
Zarghi et al. (2023)-7	0.130 (−0.197, 0.457)
Zarghi et al. (2023)-8	0.207 (−0.120, 0.535)
Zarghi et al. (2023)-9	0.078 (−0.249, 0.405)
Yildiz et al. (2020)	0.052 (−0.749, 0.852)
Yildiz et al. (2020)-2	0.026 (−0.774, 0.826)
Venglovska et al. (2014)	0.051 (−0.515, 0.617)
Venglovska et al. (2014)-2	−0.039 (−0.604, 0.527)
Venglovska et al. (2014)-3	−0.060 (−0.625, 0.506)
Swiatkiewicz and Koreleski (2008)	−0.078 (−0.878, 0.723)
Swiatkiewicz and Koreleski (2008)-2	−0.156 (−0.957, 0.646)
Swiatkiewicz and Koreleski (2007)	0.077 (−0.848, 1.001)
Xiao et al. (2015)	0.150 (−0.228, 0.528)
Xiao et al. (2015)-2	0.298 (−0.081, 0.677)
Xiao et al. (2015)-3	0.395 (0.014, 0.776)
Xiao et al. (2015)-4	0.491 (0.109, 0.874)
Xiao et al. (2015)-5	0.491 (0.109, 0.874)
Xiao et al. (2015)-6	0.347 (−0.033, 0.727)
Xiao et al. (2015)-7	0.322 (−0.057, 0.702)
Xiao et al. (2015)-8	0.371 (−0.010, 0.751)
Zhang et al. (2015)	0.464 (0.207, 0.720)
Zhang et al. (2015)-2	0.685 (0.317, 1.053)
Zhang et al. (2015)-3	0.902 (0.527, 1.278)
Zhang et al. (2015)-4	0.902 (0.527, 1.278)
Zhang et al. (2015)-5	0.000 (−0.358, 0.358)
Zhang et al. (2015)-6	0.000 (−0.358, 0.358)
Zhang et al. (2015)-7	−0.218 (−0.577, 0.141)
Zhang et al. (2015)-8	−0.218 (−0.577, 0.141)
Khoshbin et al.(2023)	0.389 (−0.007, 0.785)
Yang et al. (2011)	0.347 (−0.069, 0.763)
Yang et al. (2011)-2	0.492 (0.072, 0.911)
Zamani et al. (2005)	0.287 (−0.153, 0.728)
Zamani et al. (2005)-2	0.000 (−0.438, 0.438)
Zamani et al. (2005)-3	0.287 (−0.153, 0.728)
Zamani et al. (2005)-4	0.000 (−0.438, 0.438)
Zamani et al. (2005)-5	0.000 (−0.438, 0.438)
Zamani et al. (2005)-6	0.000 (−0.438, 0.438)
Cui et al. (2019)	0.009 (−0.535, 0.553)
Cui et al. (2019)-2	0.184 (−0.360, 0.729)
Cui et al. (2019)-3	0.009 (−0.535, 0.553)
Cui et al. (2019)-4	0.066 (−0.478, 0.609)
Cui et al. (2019)-5	0.122 (−0.422, 0.666)
Cui et al. (2019)-6	0.066 (−0.478, 0.609)
Li et al. (2018)	−0.273 (−0.557, 0.011)
Li et al. (2018)-2	0.000 (−0.283, 0.283)
Li et al. (2018)-3	0.000 (−0.283, 0.283)
Li et al. (2018)-4	0.266 (−0.019, 0.550)
Sazzad et al. (1994)	0.296 (−0.106, 0.698)
Sazzad et al. (1994)-2	0.354 (−0.049, 0.757)
Sazzad et al. (1994)-3	0.705 (0.293, 1.118)
Sazzad et al. (1994)-4	0.389 (−0.015, 0.793)
Sazzad et al. (1994)-5	0.447 (0.042, 0.852)
Sazzad et al. (1994)-6	0.791 (0.375, 1.206)
Overall (I^2=44.96 % , P< 0.001)	**0.213 (0.142, 0.285)**

Figure 8. *Forest plot of the effect of Mn on EST*

The effect of covariates on EST in laying hens fed Mn-supplemented diets is presented in *Table 8*. Studies that offered ≤ 100 mg Mn/kg for ≤ 12 weeks to layers aged ≤ 50 weeks had better EST than those that offered > 100 mg Mn/kg for > 12 weeks to layers aged > 50 weeks. Hy-Line and Lohmann strains on supplemental Mn recorded higher EST than the controls. Subgroup analyses by covariates indicate that layers fed inorganic Mn had significantly higher EST than those offered organic Mn.

Table 8. *Effect of studied moderators on EST*

Covariates	Subgroup	n	SMD (95% CI)		*p*-value	Heterogeneity	
						I^2 (%)	*p*-value
Hen age (weeks)	≤ 50	42	0.27	0.18, 0.36	< 0.001	45	0.001
	> 50	16	0.09	0.00, 0.17	0.049	0	0.583
Dosage (mg/kg)	≤ 100	45	0.21	0.14, 0.27	< 0.001	28	0.046
	> 100	13	0.23	-0.00, 0.46	0.054	71	0.000
SD (weeks)	≤ 12	47	0.23	0.15, 0.31	< 0.001	54	0.000
	> 12	11	0.05	-0.14, 0.24	0.606	0	1.000
Hen strains	Hy-Line	37	0.20	0.13, 0.26	< 0.001	0	0.917
	Lohmann	8	0.35	0.16, 0.55	< 0.001	29	0.197
	Jing brown	8	0.31	-0.00, 0.63	0.053	85	0.000
Mn form	Organic	26	0.32	-0.01, 0.15	0.075	3	0.418
	Inorganic	32	0.07	0.23, 0.41	< 0.001	44	0.005

SD = supplementation duration of Mn

Meta-regression and publication bias

Meta-regression showed that there was no significant relationship between EW and studied covariates as presented in *Table 9*. In converse, meta-regression found the effect for hen age ($p = 0.016$, $R^2 = 24\%$) and Mn form ($p = 0.002$, $R^2 = 38\%$) as covariates for EST. Publication bias was explored using funnel graphs (*Figures 9–10*). The Rosenberg Nfs were 9 for EW and 920 for EST. The Rosenberg Nfs value for EW was three times higher than the threshold of 300 (5 × n = 58 + 10) required to declare the mean effect size of EW robust.

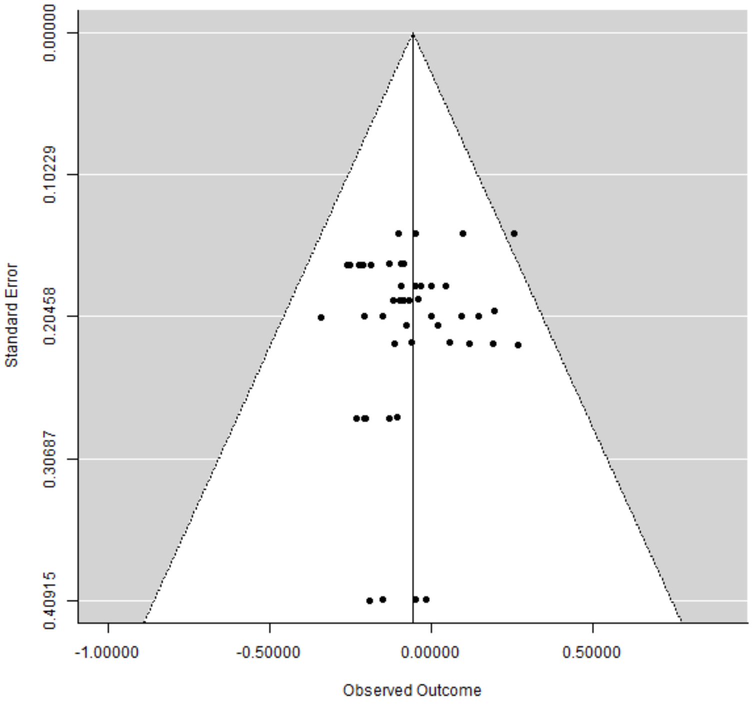

Figure 9. *Funnel plots of the effect of Mn on egg weight in laying hens*

***Table 9.** Relationships between measured outcomes and studied moderators*

Items	Moderators	Q_M	df	*p*-value	R^2 (%)
EW	Hen age	0.705	1	0.401	0
	Hen strains	12.80	8	0.120	0
	Dosage	0.015	1	0.903	0
	Supplementation duration	0.901	1	0.342	0
	Mn form	0.011	1	0.917	0
EST	Hen age	5.76	1	0.016	24
	Hen strains	10.50	8	0.232	15
	Dosage	0.146	1	0.703	0
	Supplementation duration	2.42	1	0.120	2
	Mn form	13.4	1	0.002	38

Q_M coefficient of moderators; *EW* egg weight; *p* probability; *SD* supplementation duration of Mn; *df* degree of freedom; *EST* shell thickness; R^2 the amount of heterogeneity accounted for

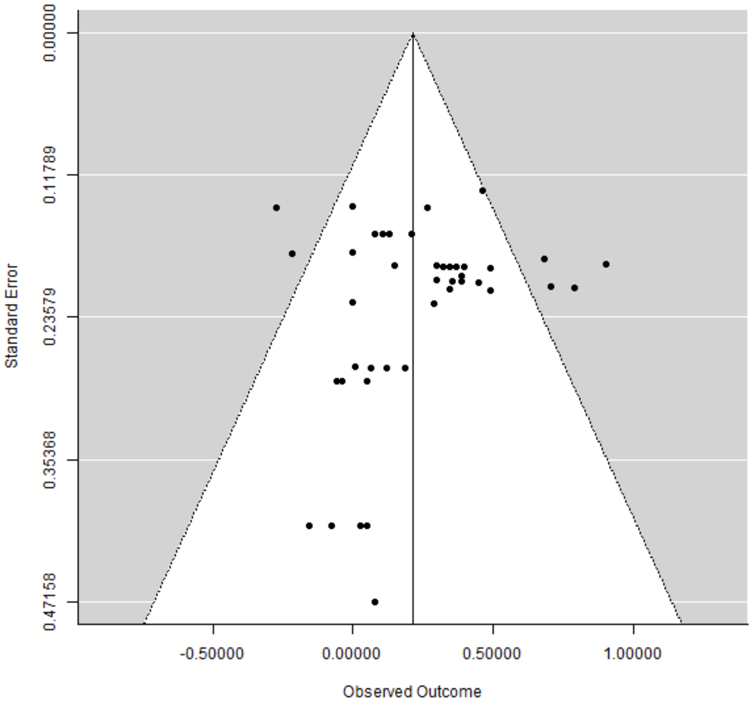

***Figure 10.** Funnel plots of the effect of Mn on eggshell thickness in laying hens*

Discussion

This meta-analysis investigated the effect of supplemental Mn on egg production and quality in laying hens. The results of this study revealed that dietary Mn supplementation had a significant reduction effect on FI in laying hens. Attia et al. (2010) found that Mn supplementation did not affect FI in layers. In converse, Xiao et al. (2015) found reduced FI in laying hens (aged 50 weeks) fed diets supplemented with different levels of organic or inorganic Mn for 12 weeks. The significantly lower FI in laying hens fed Mn-supplemented feed as reported in the current meta-analysis could be due to the role of Mn in energy metabolism (Studer et al., 2022), as reported that chickens eat to meet their energy requirements (Classen, 2017). Low FCR suggests better feed utilisation, while high FCR connotes poor feed utilisation. The pooled analysis results showed that layers on dietary Mn supplementation had improved FCR, which agrees with the findings of other investigators (Zamani et al., 2005; Yildiz et al., 2010). The significantly low FCR in this study implies better FCR in laying hens offered Mn-supplemented diets. This observation could be ascribed to enhanced quality of Mn-supplemented diets. In converse, Yildiz et al. (2011) found comparable FCR in layers offered a basal diet supplemented with varying levels of Mn. This disparity could be attributed to factors such as diet composition, dosage, age, and several others reported to influence FCR in laying hens (Ogbuewu and Mbajiorgu, 2022b).

Studies indicate supplemental Mn did not increase HDP in laying hens (Swiatkiewicz and Koreleski, 2008; Yildiz et al., 2010; Venglovska et al., 2014), which disagrees with the findings of this meta-analysis. Enhanced HDP in layers offered Mn-supplemented diets could be linked to the role of Mn in nutrient metabolism, as confirmed by Li and Yang (2018) and Barrioni et al. (2019). The reports by some authors (Fassani et al., 2000; Mabe et al., 2003; Xiao et al., 2014) that Mn supplementation reduced egg production in laying hens might be attributable to Type II error, which occurs when authors get false negative results due to low sample size and assume that treatment had no effect when it actually has impact.

Pooled effect estimation revealed that supplemental Mn reduced EW in layers but had no effect on HU. These findings differ with the results of Yıldız et al. (2011), who found increased EM in 49-week-old layers fed a ration supplemented with different levels of organic Mn. This variation could be linked to differences in diet composition, age, climate (ambient temperature, lighting, air velocity, and relative humidity), and the amount of Mn included in the diet. Pooled analysis also indicates that Mn supplemented increased EM. The improved EM in this study could be ascribed to the ability of Mn to modulate fat and carbohydrate metabolism in animals (Studer et al., 2022).

Manganese improves eggshell quality by encouraging the synthesis of mucopolysaccharides (Olgun, 2017). The present meta-analysis suggests that dietary Mn supplementation had a positive and significant effect on EM in laying hens. This corroborated the findings of Sazzad et al. (1994), who reported that incorporation of Mn-oxide in layer diets at 20, 40 and 80 mg/kg increased EST. Similarly, Xiao et al. (2014) discovered that Mn supplementation at 25 or 100 mg/kg in the diet of 50-weeks old layers increased EST and breaking strength. In contrast, other researchers found that Mn supplementation did not affect eggshell quality (Mamdooh et al., 2021; Kim et al., 2022; Domel et al., 2024). Other authors observed no differences in EST in laying hen offered varying levels of Mn (Yildiz et al., 2010; Favero et al., 2013; Junchang and Ruangpanit, 2023).

Studies have shown that Mn modulates the production of ovarian steroids by acting as a cofactor for the synthesis of numerous enzymes required for cholesterol synthesis (Goering, 2003). In the present study, subgroup analysis suggested that Mn supplementation influenced EM and HDP in laying hens aged > 50 weeks. The mechanisms by which Mn increased egg production in layers aged > 50 weeks are not known. However, it could be attributed to the role of Mn in the synthesis of cholesterol, which serves as a precursor for the synthesis of ovarian steroids, including testosterone, progestogens, and oestrogens, all needed for optimal reproductive functions (Studer et al., 2022).

The present study revealed that hen age is a limiting factor among studies that assessed the impact of Mn supplementation on EST in laying hens and accounted for 24% of the sources of heterogeneity. Results showed that layers aged ≤ 50 weeks had significantly heavier EST than those aged >50 weeks. This confirmed the earlier report of Domel et al. (2024) that Mn supplementation in laying hen diets improves shell quality. One possible explanation for this is that Mn plays a crucial part in eggshell quality by enhancing the synthesis of mucopolysaccharides (Olgun, 2017). This observation agrees with the earlier results of Xiao et al. (2014), who reported that Mn improved EST in laying hens.

Organic Mn sources have been demonstrated to have better bioavailability than inorganic sources in poultry (Brooks et al., 2012). Meta-regression revealed that Mn source is a significant predictor of the effect of Mn on EST in laying hens and accounted for about 38% of the sources of variation in this meta-analysis. Subgroup analysis also suggests that the magnitude of effect size for EST was higher in laying hens fed diets supplemented with organic Mn sources than those fed diets supplemented with inorganic Mn sources. This finding corroborated the results of Xiao et al. (2014), who demonstrated that layers fed organic Mn sources had improved eggshell quality when compared to those offered inorganic Mn sources.

Publication bias

Publication bias, which is the preference for editors or researchers to publish only studies with significant positive findings over studies with negative findings is a serious issue in meta-analysis as it usually affects the validity of pool results (Thornton and Lee, 2000). In the absence of publication bias, funnel graph should resemble a funnel with wide dispersion of results among smaller studies and a narrower range of results for large studies (LeVois and Layard, 1995). In the present study, funnel plot results indicate the existence of publication bias, and this was anticipated as studies used for analysis were published in different journals. The Roseberg Nfs, which is the number of null unpublished papers articles to be added to the meta-analysis to change the significance (Rosenberg, 2005) is three times higher than the threshold required to declare the results of the present meta-analysis robust. As a result, publication bias was not a serious problem in this meta-analysis since it would require a large number of unpublished null studies to remove significance from the findings of this meta-analysis.

Limitations and strengths of the meta-analysis

Some of the constraints in this meta-analysis are as follows: variations in analytical methods, the age of layers, form of Mn used, quantity of Mn supplemented, feeding duration, and study location may all have an effect on the validity of the findings. This study explored the impacts of Mn supplementation on laying performance and egg quality in laying hens, which may not be used in other livestock species. In spite of these constraints, the study’s strength is its systematic characterisation of uncharacterised studies by pooling results on the topic to increase statistical power, resolve conflicts, identify research gaps, and generate new insights. This work also contributed significantly to our understanding of the influence of Mn supplementation on laying hen productivity, as well as setting guidelines for standardised experimental designs in future trials.

Conclusion and future research directions

The pooled analysis revealed that dietary Mn supplementation improved FCR, HDEP, EM, and EST in laying hens. The pooled analysis suggested that Mn supplementation reduced FI and EW in layers but had no effect on HU. Pooled analysis showed the existence of significant heterogeneity across studies that assessed the impact of Mn on FCR, HDP, EM, HU, and EST. Meta-regression found the effect for age and Mn form as covariates and explained some of the sources of heterogeneity. These findings may serve as a useful guide to poultry nutritionists, feed producers, and policymakers in making decisions on the use of Mn to improve egg production and quality. There is scanty data on the effect of Mn on the absorptive capacity of the small intestines of layers, and future studies should be channeled to this area. The mechanism by which Mn reduces FI in laying hens is not known and should also be investigated. The best supplementation levels of Mn that optimised egg production and quality in laying hens should also be determined, as such information is lacking. This meta-analysis has standardised experimental designs for future experiments on the influence of supplemental Mn on laying performance and egg quality.

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