

# Effects of Dietary Organic Selenium Supplementation on Performance and Antioxidant Enzymes of Broilers under Heat Stress Conditions: A Meta-Analysis

Meta Analysis

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## ABSTRACT

Heat stress is a major cause of growth retardation and one of the most significant stressors that affect poultry, especially in hot parts of the world. This meta-analysis aimed to evaluate the effect of organic selenium supplementation on antioxidant enzymes and the performance of broiler chickens in heat-stress conditions. From 2003 to 2022, the Scopus, PubMed, Web of Science, and Google Scholar databases were searched. A total of 49, 43, 48, 19, and 27 trials were included to evaluate organic Se effects on feed intake (FI), average daily gain (ADG), feed conversion ratio (FCR), superoxide dismutase (SOD), and glutathione peroxidase (GP<sub>x</sub>) respectively. The use of organic Se supplementation had no significant effect on ADG (SMD=-0.009, 95% CI -0.105 to +0.086, I<sup>2</sup>=67.28, P=0.85), FI (SMD=-0.092, 95% CI -0.392 to +0.208, I<sup>2</sup>=58.53, P=0.548) and FCR (SMD=0.003, 95% CI -0.006 to +0.013, I<sup>2</sup>=62.89, P=0.47) in the pooled standardized mean difference random effect model. A low concentration of GP<sub>x</sub> was found in the control group (P=0.000, I<sup>2</sup>=86.32) in comparison to organic Se supplemented broilers. On the other hand, when the random model was applied to GP<sub>x</sub> studies, there was much heterogeneity. The standard mean differences of SOD were significantly higher (P<0.041, 95 CI 0.000 to +0.006, I<sup>2</sup>=0.71) in the treatment receiving Se supplementation. In conclusion, this meta-analysis shows that adding organic Se did not significantly affect on performance, but significantly increased the concentration of SOD and GP<sub>x</sub> in broilers under heat stress.

**KEY WORDS** antioxidant enzymes, broiler chickens, heat stress, performance.

## INTRODUCTION

Today, with the development of the commercial poultry industry, broiler chickens face many stress conditions that have been shown to impact the antioxidative status of poultry negatively (Goo *et al.* 2019). Heat stress is an important welfare concern that can result in significant annual economic losses due to poor performance, weakened immunity, and unhealthy conditions. During heat stress, increased excretion, decreased feed intake, and bioavailability of essential nutrients all contribute to a diminished immune

response and performance (Lara and Rostagno, 2013). On the other hand, oxidation causes cell damage and drip loss in broiler meat. Through free radicals' prevention and lipid peroxidation inhibition, natural antioxidants play essential roles in protecting cells from reactive oxygen species (ROS). Selenium (Se) has been recognized as an essential trace element for all types of animals. The use of dietary supplementation of Se has a positive effect on the growth performance of broiler chickens, antioxidant, and immune status (Liu, 2019). Furthermore, Se plays a crucial role in redox signaling by removing lipid hydroperoxides and

hydrogen peroxide by using glutathione as a final electron donor (Ibrahim *et al.* 2019). The national research council (NRC, 1994) recommended supplementing of broilers diet with low selenium (0.15 mg/kg).

Sochor *et al.* (2012) stated that selenium could be consumed in organic and inorganic forms. The results of the studies clearly showed that organic compounds have a higher tissue retention rate and bioavailability than inorganic compounds (Han *et al.* 2009). However, poultry diets must be supplemented with Se to protect a safety margin against deficiency, maintain better productive performance, and ensure health and high meat quality (Göçmen *et al.* 2016).

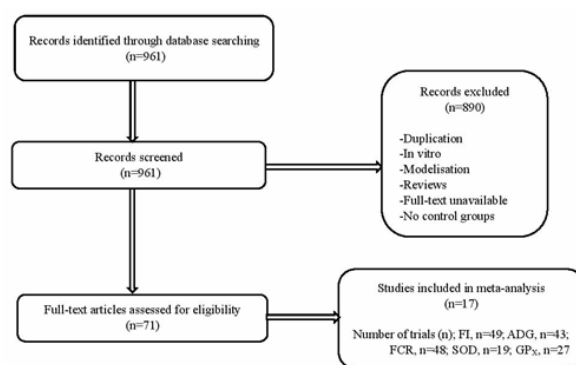
The synthesis of selenocysteine (Se-Cys), an amino acid found in selenoproteins of biological significance to poultry metabolism like glutathione peroxidase, requires Se in avian species (GSH-Px), which plays an essential part in controlling the level of lipid peroxides and hydrogen peroxide which are produced by regular metabolic activity (Zheng *et al.* 2012). Many antioxidant enzymes are part of the antioxidant system, including catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD).

The antioxidant defense of broiler muscle is significantly enhanced by glutathione peroxidase, and selenium supplementation in the diet may reduce tissue susceptibility to lipid peroxidation and improve the oxidative consistency of skeletal muscle. In addition, skeletal myodegeneration, muscle haemorrhages, exudative diathesis (ED), decreased production of eggs, atrophy of the pancreas, liver injury, inhibited growth of bursal, thymic and reduced hatchability are all signs of selenium deficiency in poultry (Gao *et al.* 2012). It is the standard method to supplement feeds with Se in either organic Se (Se-yeast) or an inorganic (such as sodium selenite, blends of SS and soya, a protein hydrolysate) form to meet the needs and the requirements of poultry and prevent Se, deficiency. Se-enriched yeast primarily consists of Se-Met, widely used due to its greater bioavailability (Hua *et al.* 2021). The fact that Se-Met is metabolized as a component of the methionine pool, which results in the formation of a storage depot of Se in the body tissue of animals, is the primary advantage of feeding Se in the form of Se-Met over feeding Se from inorganic sources or other organic Se compounds (Surai *et al.* 2012). There are many studies with different results on the effects of organic and inorganic Se on the performance of broilers. Therefore, we conducted the current meta-analysis to determine the effect of different Se sources provided by organic form on performance for meta-analysis to provide a scientific basis for whether organic Se can replace inorganic Se as a nutritional additive.

## MATERIALS AND METHODS

### Data collection and selection

This meta-analysis only included studies whose results were published in peer-reviewed journals between 2003 and 2022 and was randomized and controlled experiments on broilers without apparent disease. Antibiotics and growth promoters were not used to manage any broiler flocks. Through a literature review, we collected peer-reviewed scientific publications. Scopus, PubMed, Web of Science, and Google Scholar were the four databases used, and the search covered all fields. The following Boolean search string (Figure 1).



**Figure 1** Study selection flow diagram

The selected studies met the following criteria; 1) they were published in English language peer-reviewed Journals, 2) provided the specific Se addition values (organic), 3) used a corn and soybean meal-based diet.

The database and descriptive statistics used in the meta-analysis are presented in Table 1 and Table 2, respectively. When SE was reported, we transformed it to SD by using the formula  $SD = SE \cdot \sqrt{n}$ . Keywords used were poultry, broiler, "heat stress", "selenium supplementation", "organic selenium", "antioxidant enzymes", "feed intake", "feed conversion" and "weight gain". In addition, additional studies were identified from the reference list in the reviewed articles. By using funnel plots, the presence of publication bias was examined. An Egger-based adjusted rank correlation test (Egger *et al.* 1997) and Begg's (Begg and Mazumdar, 1994) were utilized to evaluate publication bias. If at least one of the statistical methods was significant, bias was considered present ( $P < 0.05$ ). The "trim-and-fill" method (Duval and Tweedie, 2000) was used to estimate the quantity and magnitude of missing studies and the unbiased effect size if there was any evidence of publication bias from either the statistical tests or the funnel plot.

**Table 1** Description of studies in the database

Study name	Brides (n)	Period (day)	Strain	Type on organic Se	Dosage (mg)
Gul <i>et al.</i> (2022)	120	1-42	-	Selenomethionine, Se yeast	0.15, 0.22
Chen <i>et al.</i> (2022)	720	1-42	-	Se yeast	0.15, 0.22
Khan <i>et al.</i> (2020)	200	1-42	Ross 308	Se probiotic	0.30
Sun <i>et al.</i> (2021)	324	1-42	Cobb 500	Se yeast, selenomethionine	0.30
Safiullah <i>et al.</i> (2019)	480	1-42	-	Se yeast	0.30, 0.40
Amizare <i>et al.</i> (2017)	120	1-35	CP707	-	0.30
Rao <i>et al.</i> (2016)	200	1-21	Cobb 400	Sel-Plex	0.10, 0.20, 0.30, 0.40
Rao <i>et al.</i> (2013)	240	1-42	-	Min-Plex selenium	0.30
Habibian <i>et al.</i> (2015)	360	1-49	Cobb 500	Selenomethionine	0.50, 1
Boostani <i>et al.</i> (2015)	320	1-42	Cobb 500	Sel-Plex, Alltech Inc.	0.30
Celi <i>et al.</i> (2015)	216	1-42	Ross 308	Selenohomolanthionine, selenomethionine	0.57
Liao <i>et al.</i> (2012)	210	22-42	Arbor Acres	Se yeast, Se protein (AMMS)	0.15, 0.30
Harsini <i>et al.</i> (2012)	240	1-49	Cobb 500	Selenomethionine	0.50, 1
Khajali <i>et al.</i> (2010)	300	1-49	Ross 308	Selenomethionine	0.30
Fan <i>et al.</i> (2009)	144	1-21	Avian	Se yeast	0.10, 0.40
Dlouha <i>et al.</i> (2008)	300	1-42	Ross 308	Se-chlorella	0.30
Kamel <i>et al.</i> (2003)	180	1-28	Arbor Acres	Se yeast	0.46

**Table 2** Data description (means and SD between studies)

Item	Unit	NC	Mean		SD	
			Control	Treatment	Control	Treatment
FI	g/d	43	83.38	82.04	31.57	29.98
ADG	g/d	49	41.79	43.95	19.23	20.19
FCR	g FI/g BW	48	2.13	2.03	0.54	0.48
SOD	mg	9	85.12	98.93	23.59	37.43
GPx	mg	27	90.25	109.97	37.2	42.13

NC: number of comparisons; FI: feed intake; ADG: average daily gain; FCR: feed conversion ratio; SOD: superoxide dismutase; GPx: glutathione peroxidase and BW: body weight.

SD: standard deviation.

Heterogeneity was quantified using the  $I^2$  statistic, which describes the percentage of the total variation across studies that is due to heterogeneity rather than chance (Lean *et al.* 2009). The  $I^2$  statistic was calculated as follows:

$$I^2 = (Q - (k-1)/Q) \times 100$$

Where:

Q:  $I^2$  heterogeneity statistic.

k: number of trials.

A value of heterogeneity greater than 50% can be considered substantial heterogeneity (Appuhamy *et al.* 2013).

### Statistical analysis

Statistical analysis used Comprehensive Meta-Analysis version 2.2 (2011).

Due to continuous variables being analyzed, results are shown as standardized mean differences (SMD) between the selenium treatment and controls with 95% confidence intervals (CIs) using a random effect model. In this model, the actual impact could vary from experiment to experiment, and between experiment variability (true heterogeneity) and sampling error are included (Borenstein *et al.* 2009). Among studies, heterogeneity was evaluated using the DerSimonian and the Laird test (Q-statistic). By inconsistency index, the degree of heterogeneity was quantified (Higgins and Thompson, 2002).

## RESULTS AND DISCUSSION

The effect size (SMD), P-values, standard error (SE), and measures of heterogeneity for each of the response variables (ADG, FI, FCR, SOD, and GPx) are shown in Table 3.

For ADG, FI, FCR, SOD, and GPx, 49, 48, 43, 19, and 27 trials, in total, were analyzed. In the pooled estimate, control groups decreased ADG compared to groups receiving organic Se (SMD=-0.009, 95% CI -0.105 to 0.086; Table 3, Figure 2) in the pooled SMD random effect model. The experimental results had no significant effect on average FI. However, FI decreased (SMD=-.0092, 95% CI -0.392 to +0.208,  $I^2=58.53$ ) in the control group under heat stress (Table 3, Figure 4). Significant difference was not observed between treatments for FCR (SMD=0.003,  $P=0.47$ ,  $I^2=62.89$ , Table 3, Figure 6). Publication bias for ADG occurred for 49 trials as confirmed by Egger's test ( $P=0.00645$ , Figure 3). Funnel plots of the effect sizes (SMD) following selenium supplementation for FI and FCR are indicated in Figure 5 and Figure 7. The concentration of GPx was reduced in the control groups (SDM=-1.110,  $P=0.000$ ) from a total of 27 trials analyzed; when the random model, the heterogeneity was substantially increased ( $I^2=86.32$ , Table 3, Figure 8). According Figure 9, there was of publication bias for study on the GPx enzyme ( $P$ -value for Egger's test was 0.04. The use of organic Se supplementation had a positive effect on SOD, with an effect size of +0.003 and  $I^2=71.08$ , 95% CI 0.000 to 0.006 (Table 3, Figure 10).

Decrease in broiler performance due to high ambient temperature has been well documented, especially at the later stages of growth (Dai *et al.* 2009; Azad *et al.* 2010; Zhang *et al.* 2012). Broilers are sensitive to humidity and high temperature, so broilers need comfortable temperatures for optimal production (18-27 °C) (Kuczynski, 2002; Olanrewaju *et al.* 2010; Aljuobori *et al.* 2016). The present study indicated that using organic Se form did not significantly affect ADG and FI in broiler chickens under heat stress. However, broilers receiving organic Se had higher ADG and FI than the control group. Similar to our results, Khajali *et al.* (2010) and Harsini *et al.* (2012) showed that using organic Se supplement had no effect on performance broilers under heat stress, but there was a reduction in body weight, and feed intake when the chickens were exposed to heat stress. The study's results by Rahimi *et al.* (2011) indicated that using organic Se had no significant effect on performance broilers under heat stress. However, ADG decreased in the control group. Ca and P are mainly involved in the mineralization and development of bone (Proszkowiec-Weglarz and Angel, 2013). Heat stress negatively changes the Ca and P levels in bones, and predisposes hens to tibial bone dyschondroplasia (Hosseini-Vashan *et al.* 2016). The occurrence can be reduced with dietary supplementation of Ca and P (Attia and Hassan, 2017). Earlier studies indicated that different sources of Se improve calcium deposition in the tibia of hens (Attia *et al.* 2010). Se improved the cartilage integrity and reversed the

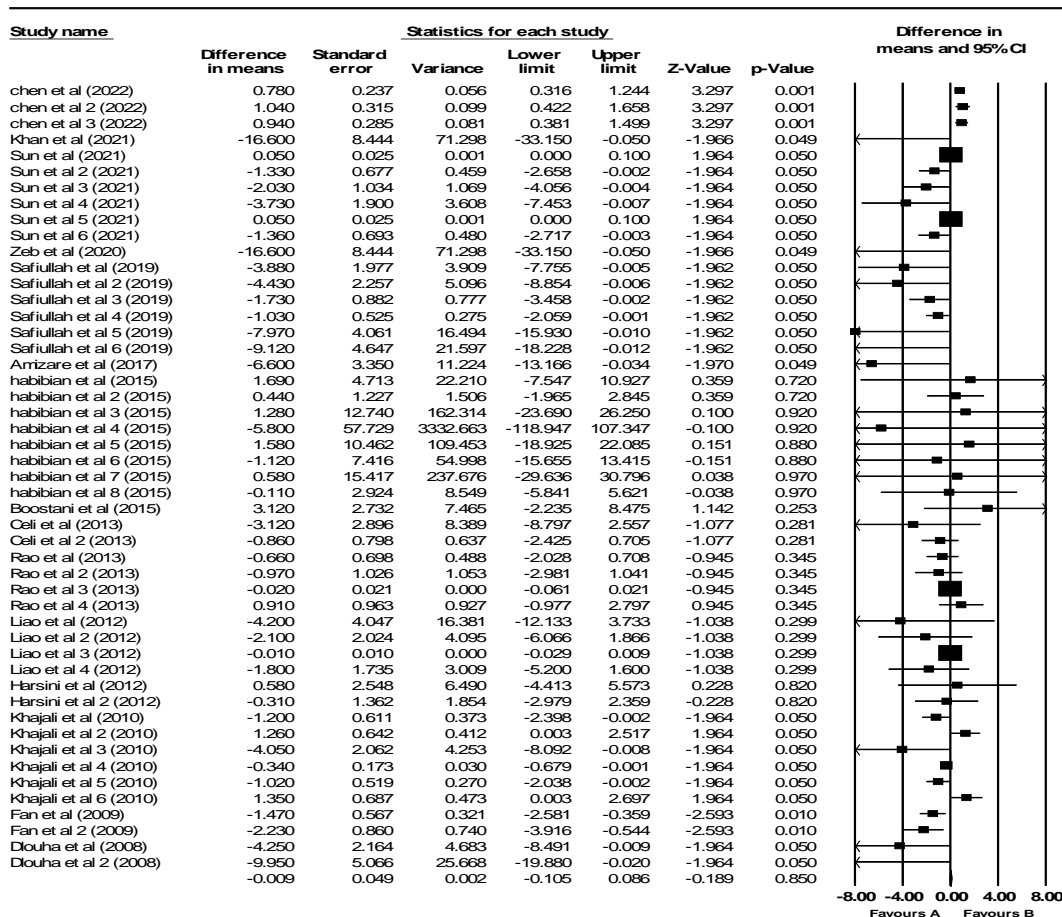
mycotoxin-induced cartilage necrosis (Medeiros, 2016). The entire broiler chicken performance was in agreement with the results of Boostani *et al.* (2015); Rao *et al.* (2013); Payne and Southern (2005); Rahimi *et al.* (2011), and Ryu *et al.* (2005), indicated that ADG, FI, and FCR were not affected by dietary organic selenium. Contrary to our results, Upton *et al.* (2008) showed considerable increases in the body weight of broilers when they were given diets supplemented with 0.2 mg/kg of organic selenium, compared with a control group. The results of the study Calik *et al.* (2022) indicated that using organic selenium caused increased FI and ADG compared with the control group. Furthermore, different results for ADG, FCR and FI were observed with diet's selenium content, bird strain, and housing condition. Organic selenium through the amino acid transport mechanisms, actively absorbed in the small intestine. Glutathione peroxidase is an essential member of the antioxidant selenoprotein family, responsible for removing reactive oxygen species (ROS) (Guillin *et al.* 2019). The low activity of this group of enzymes is related to a few pathologies. Selenium is a structural component of GPx. Selenium supplementation increased GPx activity in all tissues and animal species. The exact relationship between selenium supply and GPx activity depended on the form of selenium (organic and inorganic) used and the tissue being investigated (Bermingham *et al.* 2014). The current study indicated that GPx concentration in the control group significantly decreased compared to the organic selenium group. Some previous studies (Mahan *et al.* 1999; Yoon *et al.* 2007; Wang and Xu, 2008) and the current experiment indicated that GPx activity in the liver expanded as dietary supplemental Se level increased, recommending that dietary Se supplementation could improve the antioxidative status of heat-stressed broilers. The study results by Celi *et al.* (2015) indicated that organic selenium increased concentration GPx in the group receiving organic selenium rather than the control group. So, all these findings support the finding of the present study. In agreement with the present study, Gul *et al.* (2022) indicated that the use of 0.22 mg/kg seleno-methionine in the starter diet of broilers during heat stress caused significantly increasing GPx concentration (14.28 U/L) compared with the control group (12.10 U/L). The antioxidative systems in the body contain numerous antioxidative enzymes, such as SOD and GPx.

In a study, Ahmad *et al.* (2012) showed that the use of dietary selenium yeast and sodium selenite on the oxidative stability of chicken meat caused sodium to increase the oxidative stability of chicken meat in the group receiving selenium enriched yeast. Additionally, other studies showed that the oxidative stability of chicken improved by organic selenium sources compared to inorganic sources (Kuricova *et al.* 2003).

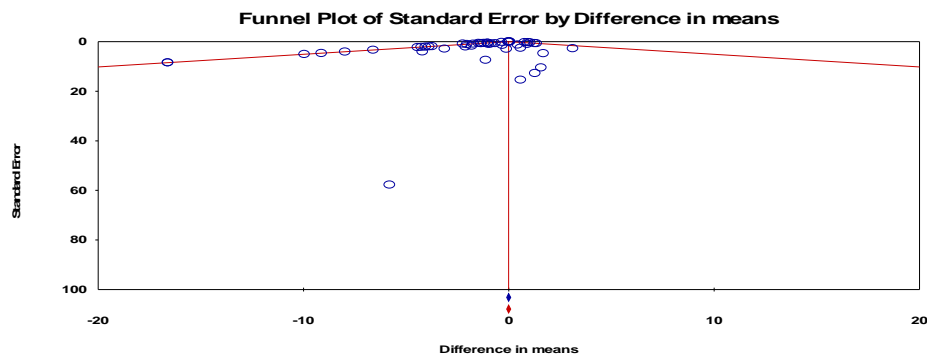
**Table 3** Summary of the effect size (SMD) between organic Se supplementation on performance and GP<sub>x</sub> and SOD enzymes of broiler chickens under heat stress, calculated according to fixed and random effects models

Variable	SMD	SE	P-value	Heterogeneity		
				I <sup>2</sup>	Q <sup>2</sup>	P-value
<b>ADG</b>						
Fixed effects models	-0.001	0.008	0.949	67.28	146.7	0.000
Random effects models	-0.009	0.049	0.850	-	-	-
<b>FI</b>						
Fixed effects models	0.074	0.055	0.18	58.53	101.27	0.000
Random effects models	-0.092	0.159	0.548	-	-	-
<b>FCR</b>						
Fixed effects models	-0.000	0.001	0.843	62.89	126.06	0.000
Random effects models	0.003	0.005	0.47	-	-	-
<b>SOD</b>						
Fixed effects models	0.003	0.000	0.000	71.08	62.26	0.000
Random effects models	0.003	0.002	0.041	-	-	-
<b>GP<sub>x</sub></b>						
Fixed effects models	-0.098	0.032	0.002	86.32	190.73	0.000
Random effects models	-1.11	0.259	0.000	-	-	-

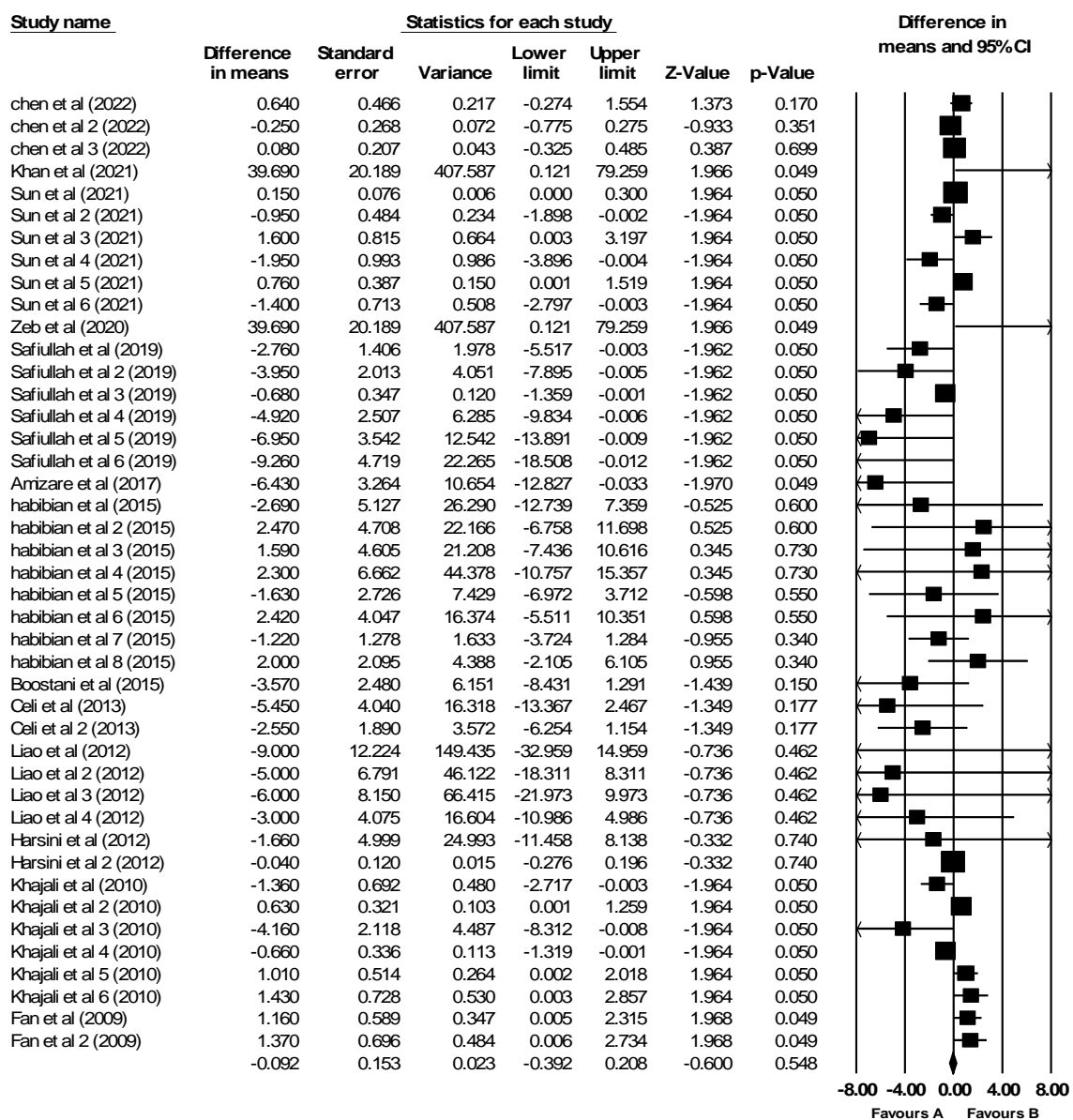
ADG: average daily gain; FI: feed intake; FCR: feed conversion ratio; SOD: superoxide dismutase and GP<sub>x</sub>: glutathione peroxidase. I<sup>2</sup>: measure of heterogeneity of random model (RM). SMD: standardized mean difference and SE: standard error mean.



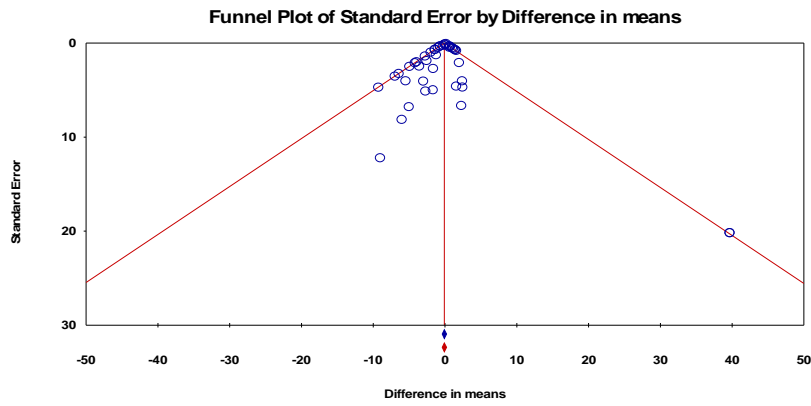
**Figure 2** Forest plots for ADG using random-effects model. A: control group and B: treatment supplemented with organic Se. The size of the squares illustrated the weight of each study relative to the mean effect size. Black horizontal line is indicative of confidence interval for each study; Diamond located at the bottom of plot represents the summary of results



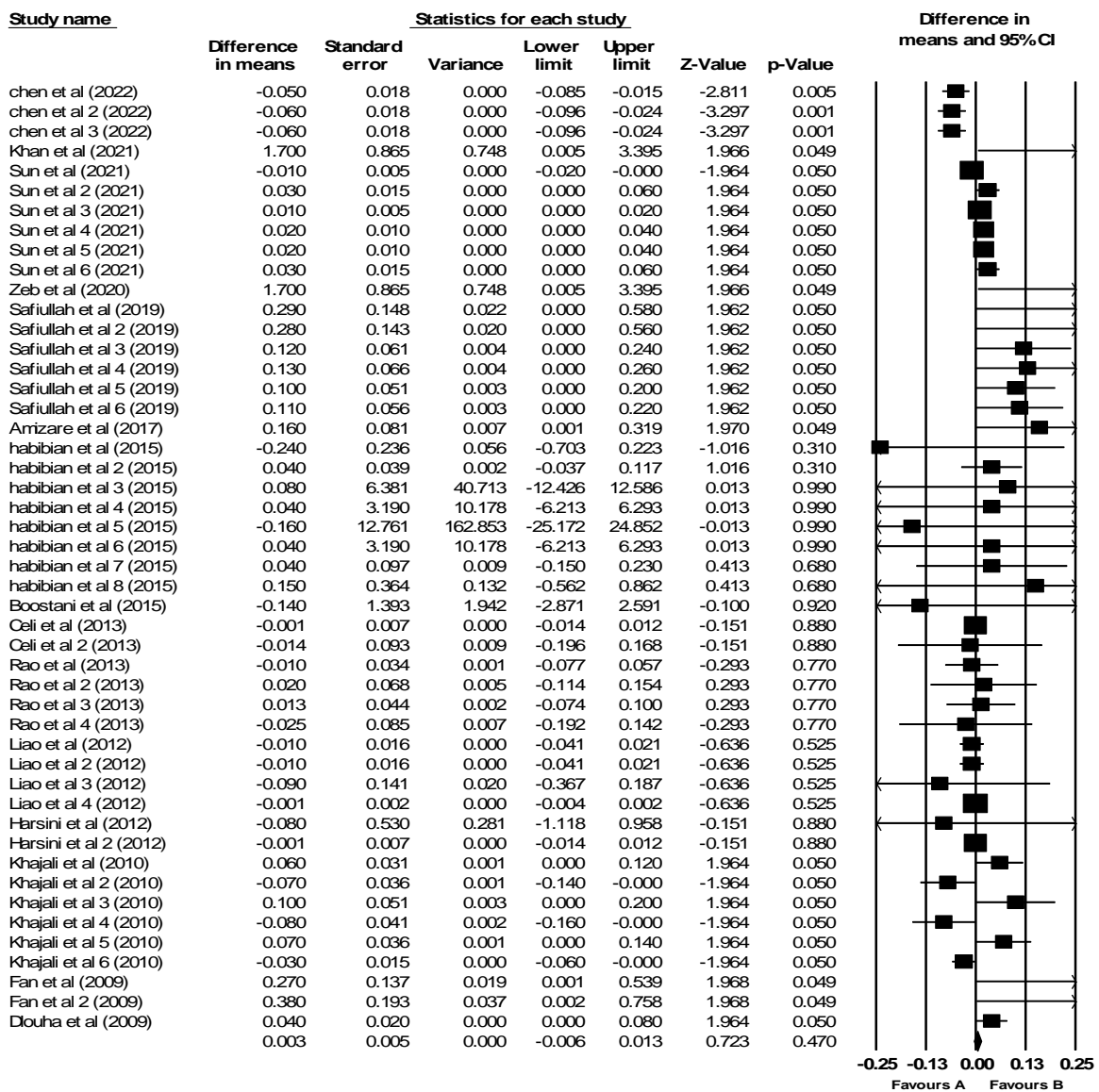
**Figure 3** Funnel plot representing the effect of organic Se supplementation on ADG. Empty circles indicate observed values, and full circles are possible missing values



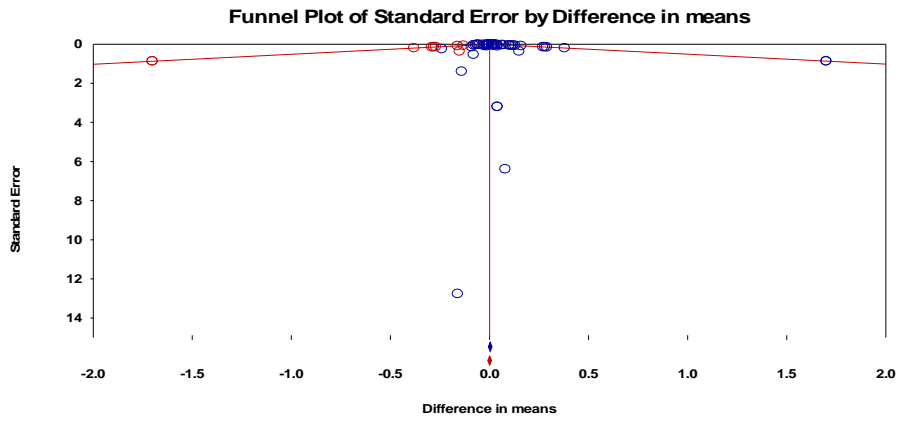
**Figure 4** Forest plots for FI using random-effects model. A: control group and B: treatment supplemented with organic Se. The size of the squares illustrated the weight of each study relative to the mean effect size. Black horizontal line is indicative of confidence interval for each study; Diamond located at the bottom of plot represents summary of the results



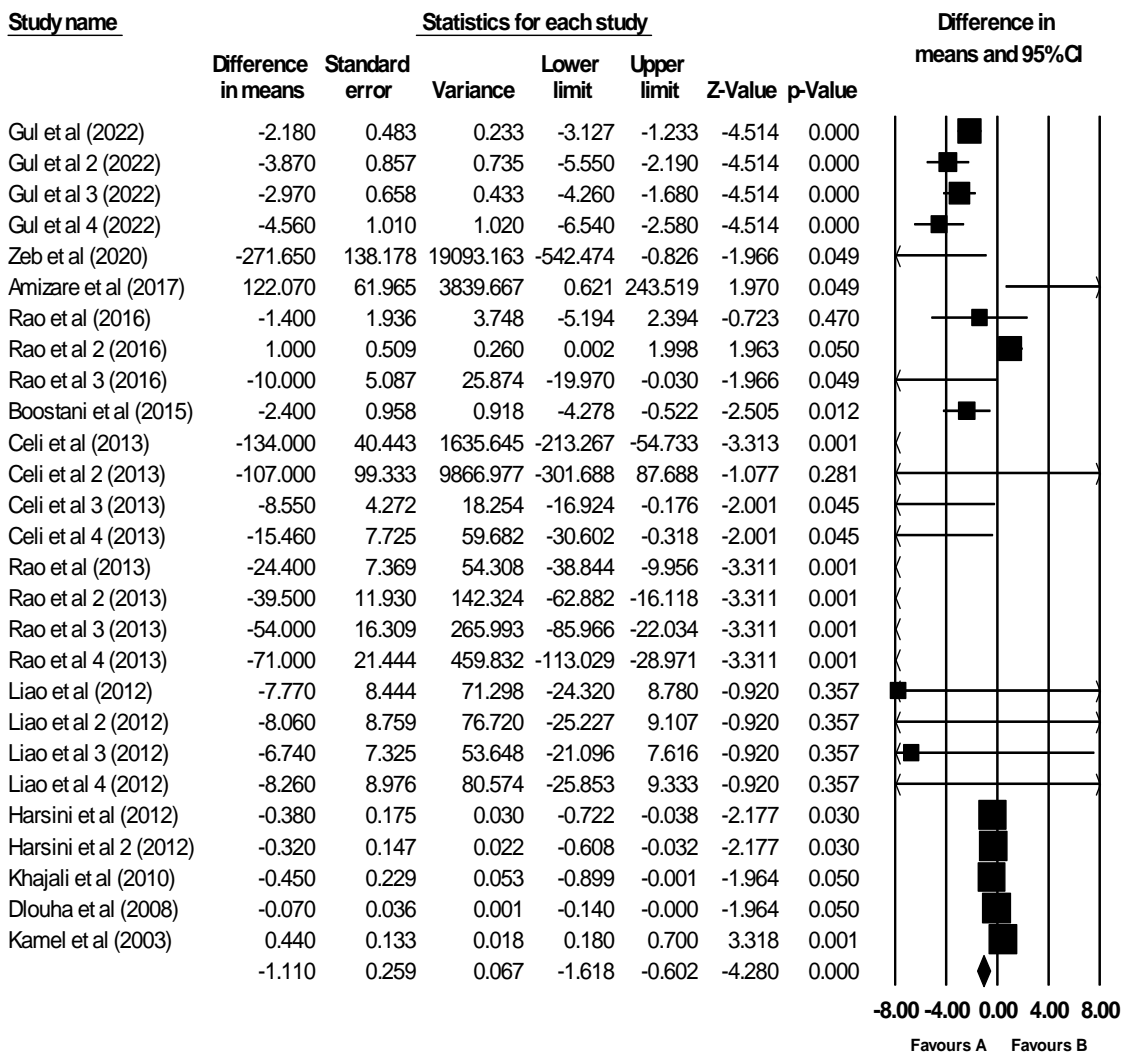
**Figure 5** Funnel plot representing the effect of organic Se supplementation on FI. Empty circles indicate observed values, and full circles are possible missing values



**Figure 6** Forest plots for FCR using random-effects model. A: control group and B: treatment supplemented with organic Se. The size of the squares illustrated the weight of each study relative to the mean effect size. Black horizontal line is indicative of confidence interval for each study; Diamond located at the bottom of plot represents summary of the results

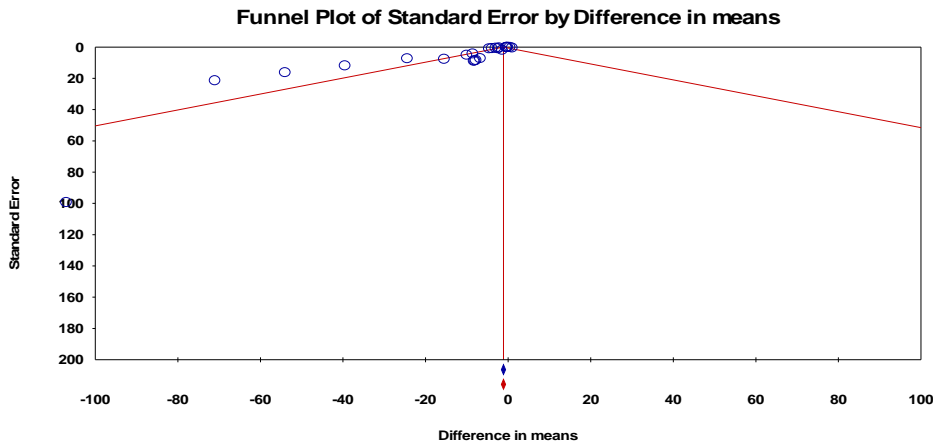


**Figure 7** Funnel plot representing the effect of organic Se supplementation on FCR. Empty circles indicate observed values, and full circles are possible missing values

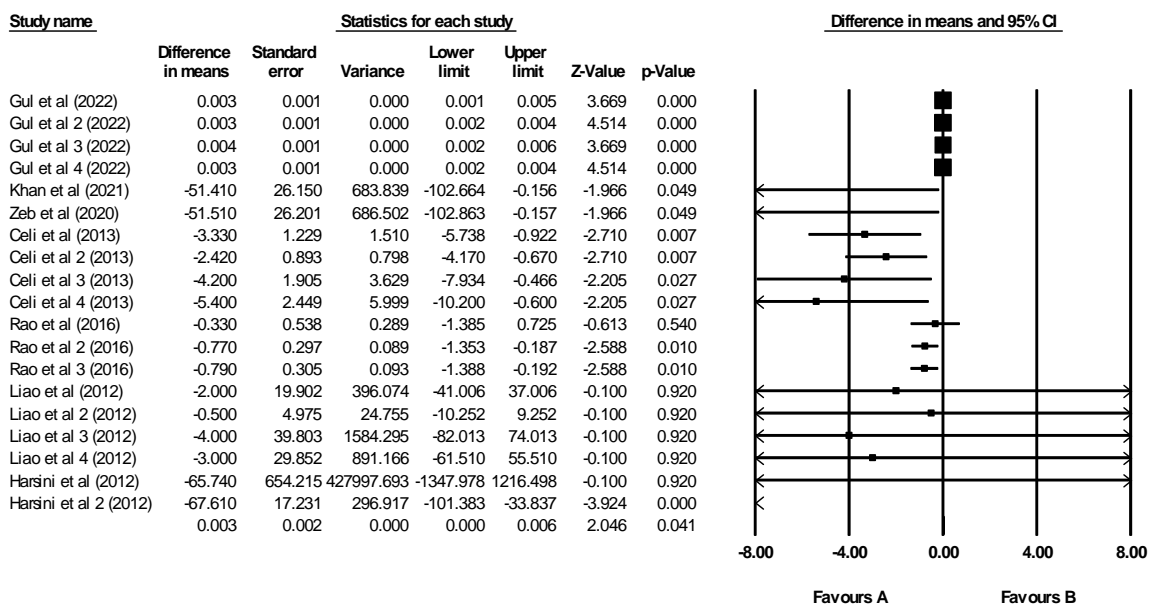


**Figure 8** Forest plots for GPX using random-effects model. A: control group and B: treatment supplemented with organic Se. The size of the squares illustrated the weight of each study relative to the mean effect size. Black horizontal line is indicative of confidence interval for each study; Diamond located at the bottom of plot represents summary of the results

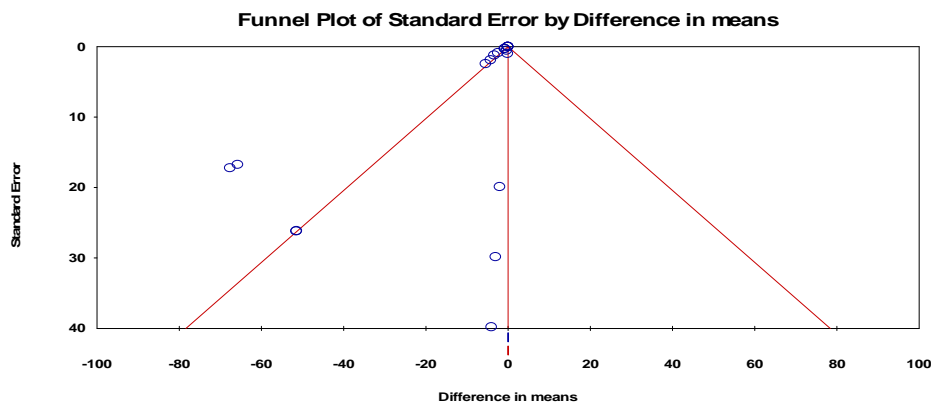




**Figure 9** Funnel plot representing the effect of organic Se supplementation on GPx. Empty circles indicate observed values, and full circles are possible missing values



**Figure 10** Forest plots for SOD using random-effects model. A: control group and B: treatment supplemented with organic Se. The size of the squares illustrated the weight of each study relative to the mean effect size. Black horizontal line is indicative of confidence interval for each study; Diamond located at the bottom of plot represents summary of the results



**Figure 11** Funnel plot representing the effect of organic Se supplementation on SOD. Empty circles indicate observed values, and full circles are possible missing values

In the present study, using organic selenium increased SOD concentration in broiler chickens compared to the control group. Different experimental studies have produced similar findings, concluding that the organic form of selenium is the best source for increasing SOD activities in chicken tissues (Hu *et al.* 2012; Suchý *et al.* 2014). In the study, Gul *et al.* (2022) use of organic selenium form caused decreased SOD compared to the control group. Furthermore, Se yeast increased liver SOD activity compared to control groups in condition heat stress; however, there were no significant differences between groups.

## CONCLUSION

The differences between studies are removed by meta-analysis, which can make the corrected data comparable, creating more objective and convincing data. Although there is an inconsistent report from different authors concerning the feeding of organic Se form. Through the 17 studies included in the present meta-analysis, we found that organic Se supplementation has no effect on FI, ADG, and FCR in broilers under heat stress. The present meta-analysis showed that several factors including the type of chicken, type of organic Se supplementation, and analytical method used affect the performance broilers under heat stress. However, the concentration of GPx and SOD was significantly lower in broilers exposed to heat stress.

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